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Handbook of Enzymes

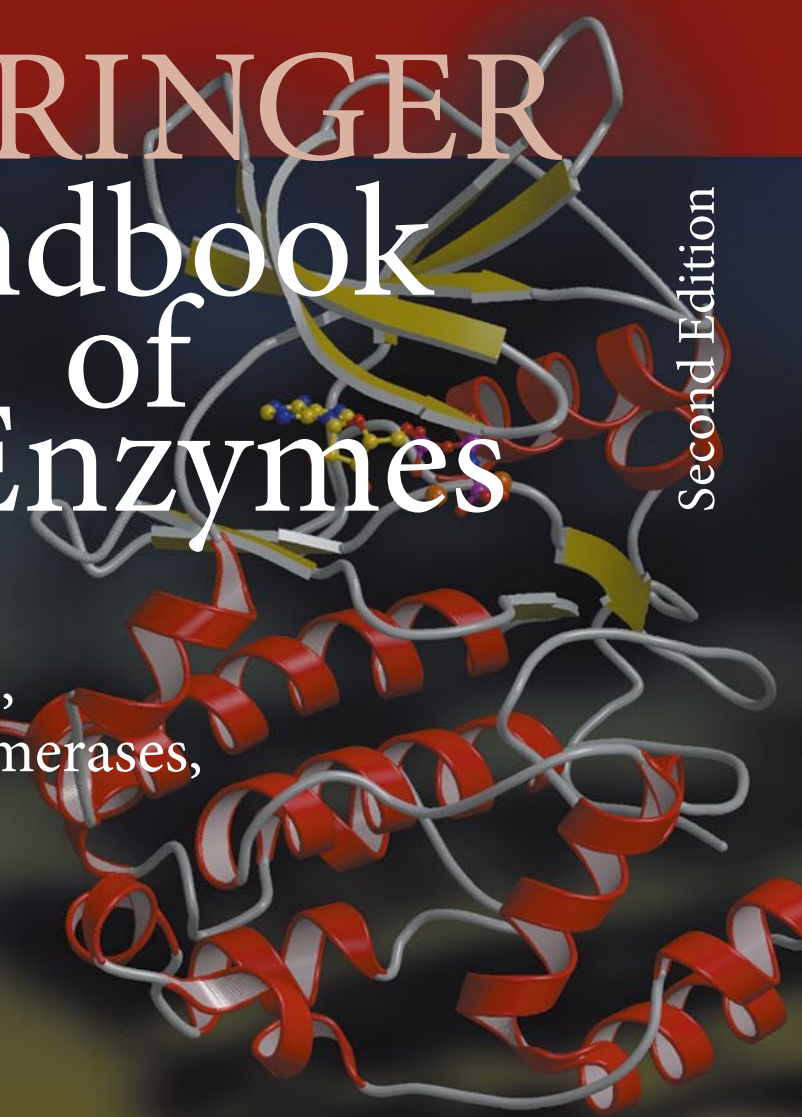
Second Edition

SUPPLEMENT VOLUME S10

CLASS 3.4–6

Hydrolases, Lyases, Isomerases, Ligases

EC 3.4–6



Springer

Springer Handbook of Enzymes
Supplement Volume S10

Dietmar Schomburg and
Ida Schomburg (Eds.)

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Class 3.4–6
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coedited by Antje Chang

Second Edition

Springer

Professor Dietmar Schomburg
e-mail: d.schomburg@tu-bs.de

Dr. Ida Schomburg
e-mail: i.schomburg@tu-bs.de

Dr. Antje Chang
e-mail: a.chang@tu-bs.de

Technical University Braunschweig
Bioinformatics & Systems Biology
Langer Kamp 19b
38106 Braunschweig
Germany

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Preface

Today, as the full information about the genome is becoming available for a rapidly increasing number of organisms and transcriptome and proteome analyses are beginning to provide us with a much wider image of protein regulation and function, it is obvious that there are limitations to our ability to access functional data for the gene products – the proteins and, in particular, for enzymes. Those data are inherently very difficult to collect, interpret and standardize as they are widely distributed among journals from different fields and are often subject to experimental conditions. Nevertheless a systematic collection is essential for our interpretation of genome information and more so for applications of this knowledge in the fields of medicine, agriculture, etc. Progress on enzyme immobilisation, enzyme production, enzyme inhibition, coenzyme regeneration and enzyme engineering has opened up fascinating new fields for the potential application of enzymes in a wide range of different areas.

The development of the enzyme data information system BRENDA was started in 1987 at the German National Research Centre for Biotechnology in Braunschweig (GBF), continued at the University of Cologne from 1996 to 2007, and then returned to Braunschweig, to the Technical University, Institute of Bioinformatics & Systems Biology. The present book "Springer Handbook of Enzymes" represents the printed version of this data bank. The information system has been developed into a full metabolic database.

The enzymes in this Handbook are arranged according to the Enzyme Commission list of enzymes. Some 5,000 "different" enzymes are covered. Frequently enzymes with very different properties are included under the same EC-number. Although we intend to give a representative overview on the characteristics and variability of each enzyme, the Handbook is not a compendium. The reader will have to go to the primary literature for more detailed information. Naturally it is not possible to cover all the numerous literature references for each enzyme (for some enzymes up to 40,000) if the data representation is to be concise as is intended.

It should be mentioned here that the data have been extracted from the literature and critically evaluated by qualified scientists. On the other hand, the original authors' nomenclature for enzyme forms and subunits is retained. In order to keep the tables concise, redundant information is avoided as far as possible (e.g. if K_m values are measured in the presence of an obvious cosubstrate, only the name of the cosubstrate is given in parentheses as a commentary without reference to its specific role).

The authors are grateful to the following biologists and chemists for invaluable help in the compilation of data: Cornelia Munaretto and Dr. Antje Chang.

Braunschweig
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Dietmar Schomburg, Ida Schomburg

List of Abbreviations

A	adenine
Ac	acetyl
ADP	adenosine 5'-diphosphate
Ala	alanine
All	allose
Alt	altrose
AMP	adenosine 5'-monophosphate
Ara	arabinose
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine 5'-triphosphate
Bicine	N,N'-bis(2-hydroxyethyl)glycine
C	cytosine
cal	calorie
CDP	cytidine 5'-diphosphate
CDTA	trans-1,2-diaminocyclohexane-N,N,N,N-tetraacetic acid
CMP	cytidine 5'-monophosphate
CoA	coenzyme A
CTP	cytidine 5'-triphosphate
Cys	cysteine
d	deoxy-
D-	(and L-) prefixes indicating configuration
DFP	diisopropyl fluorophosphate
DNA	deoxyribonucleic acid
DPN	diphosphopyridinium nucleotide (now NAD^+)
DTNB	5,5'-dithiobis(2-nitrobenzoate)
DTT	dithiothreitol (i.e. Cleland's reagent)
EC	number of enzyme in Enzyme Commission's system
E. coli	Escherichia coli
EDTA	ethylene diaminetetraacetate
EGTA	ethylene glycol bis(-aminoethyl ether) tetraacetate
ER	endoplasmic reticulum
Et	ethyl
EXAFS	extended X-ray absorption fine structure
FAD	flavin-adenine dinucleotide
FMN	flavin mononucleotide (riboflavin 5'-monophosphate)
Fru	fructose
Fuc	fucose
G	guanine
Gal	galactose

GDP	guanosine 5'-diphosphate
Glc	glucose
GlcN	glucosamine
GlcNAc	N-acetylglucosamine
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GMP	guanosine 5'-monophosphate
GSH	glutathione
GSSG	oxidized glutathione
GTP	guanosine 5'-triphosphate
Gul	gulose
h	hour
H4	tetrahydro
HEPES	4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid
His	histidine
HPLC	high performance liquid chromatography
Hyl	hydroxylysine
Hyp	hydroxyproline
IAA	iodoacetamide
IC 50	50% inhibitory concentration
Ig	immunoglobulin
Ile	isoleucine
Ido	idose
IDP	inosine 5'-diphosphate
IMP	inosine 5'-monophosphate
ITP	inosine 5'-triphosphate
K_m	Michaelis constant
L-	(and D-) prefixes indicating configuration
Leu	leucine
Lys	lysine
Lyx	lyxose
M	mol/l
mM	millimol/l
m-	*meta*-
Man	mannose
MES	2-(N-morpholino)ethane sulfonate
Met	methionine
min	minute
MOPS	3-(N-morpholino)propane sulfonate
Mur	muramic acid
MW	molecular weight
NAD^+	nicotinamide-adenine dinucleotide
NADH	reduced NAD
$NADP^+$	NAD phosphate
NADPH	reduced NADP
NAD(P)H	indicates either NADH or NADPH

NBS	N-bromosuccinimide
NDP	nucleoside 5'-diphosphate
NEM	N-ethylmaleimide
Neu	neuraminic acid
NMN	nicotinamide mononucleotide
NMP	nucleoside 5'-monophosphate
NTP	nucleoside 5'-triphosphate
o-	*ortho*-
Orn	ornithine
p-	*para*-
PBS	phosphate-buffered saline
PCMB	*p*-chloromercuribenzoate
PEP	phosphoenolpyruvate
pH	-log10[H^+]
Ph	phenyl
Phe	phenylalanine
PHMB	*p*-hydroxymercuribenzoate
PIXE	proton-induced X-ray emission
PMSF	phenylmethane-sulfonylfluoride
p-NPP	*p*-nitrophenyl phosphate
Pro	proline
Q_{10}	factor for the change in reaction rate for a 10°C temperature increase
Rha	rhamnose
Rib	ribose
RNA	ribonucleic acid
mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	transfer RNA
Sar	N-methylglycine (sarcosine)
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Ser	serine
T	thymine
t_H	time for half-completion of reaction
Tal	talose
TDP	thymidine 5'-diphosphate
TEA	triethanolamine
Thr	threonine
TLCK	N^{α}-*p*-tosyl-L-lysine chloromethyl ketone
T_m	melting temperature
TMP	thymidine 5'-monophosphate
Tos-	tosyl- (*p*-toluenesulfonyl-)
TPN	triphosphopyridinium nucleotide (now $NADP^+$)
Tris	tris(hydroxymethyl)-aminomethane
Trp	tryptophan
TTP	thymidine 5'-triphosphate
Tyr	tyrosine
U	uridine

U/mg	μmol/(mg*min)
UDP	uridine 5'-diphosphate
UMP	uridine 5'-monophosphate
UTP	uridine 5'-triphosphate
Val	valine
Xaa	symbol for an amino acid of unknown constitution in peptide formula
XAS	X-ray absorption spectroscopy
Xyl	xylose

Index of Recommended Enzyme Names

Description of Data Fields

All information except the nomenclature of the enzymes (which is based on the recommendations of the Nomenclature Committee of IUBMB (**I**nternational **U**nion of **B**iochemistry and **M**olecular **B**iology) and IUPAC (**I**nternational **U**nion of **P**ure and **A**pplied **C**hemistry) is extracted from original literature (or reviews for very well characterized enzymes). The quality and reliability of the data depends on the method of determination, and for older literature on the techniques available at that time. This is especially true for the fields *Molecular Weight* and *Subunits*.
The general structure of the fields is: **Information – Organism – Commentary – Literature**
The information can be found in the form of numerical values (temperature, pH, K_m etc.) or as text (cofactors, inhibitors etc.).
Sometimes data are classified as *Additional Information*. Here you may find data that cannot be recalculated to the units required for a field or also general information being valid for all values. For example, for *Inhibitors*, *Additional Information* may contain a list of compounds that are not inhibitory.
The detailed structure and contents of each field is described below. If one of these fields is missing for a particular enzyme, this means that for this field, no data are available.

1 Nomenclature

EC number
The number is as given by the IUBMB, classes of enzymes and subclasses defined according to the reaction catalyzed.

Systematic name
This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Recommended name
This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Synonyms
Synonyms which are found in other databases or in the literature, abbreviations, names of commercially available products. If identical names are frequently used for different enzymes, these will be mentioned here, cross references are given. If another EC number has been included in this entry, it is mentioned here.

CAS registry number
The majority of enzymes have a single chemical abstract (CAS) number. Some have no number at all, some have two or more numbers. Sometimes

two enzymes share a common number. When this occurs, it is mentioned in the commentary.

2 Source Organism

In this data field the organism in which the enzymes has been detected are listed. The systematic names according to the NCBI Taxonomy are preferred. If the scientific name is missing, the synonym or the names from the respective literature references are used. In addition, organism are listed for which a specific protein sequence or nucleotide sequence has been allocated. The accession number and the respective data source, e.g, UNIPROT is given in the commentary.

3 Reaction and Specificity

Catalyzed reaction
The reaction as defined by the IUBMB. The commentary gives information on the mechanism, the stereochemistry, or on thermodynamic data of the reaction.

Reaction type
According to the enzyme class a type can be attributed. These can be oxidation, reduction, elimination, addition, or a name (e.g. Knorr reaction)

Natural substrates and products
These are substrates and products which are metabolized in vivo. A natural substrate is only given if it is mentioned in the literature. The commentary gives information on the pathways for which this enzyme is important. If the enzyme is induced by a specific compound or growth conditions, this will be included in the commentary. In *Additional information* you will find comments on the metabolic role, sometimes only assumptions can be found in the references or the natural substrates are unknown.
In the listings, each natural substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included only if the respective authors were able to demonstrate the formation of the specific product. If only the disappearance of the substrate was observed, the product is included without organisms of references. In cases with unclear product formation only a **?** as a dummy is given.

Substrates and products
All natural or synthetic substrates are listed (not in stoichiometric quantities). The commentary gives information on the reversibility of the reaction, on isomers accepted as substrates and it compares the efficiency of substrates. If a specific substrate is accepted by only one of several isozymes, this will be stated here.

The field *Additional Information* summarizes compounds that are not accepted as substrates or general comments which are valid for all substrates. In the listings, each substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included if the respective authors demonstrated the formation of the specific product. If only the disappearance of the substrate was observed, the product will be included without organisms or references. In cases with unclear product formation only a **?** as a dummy is given.

Inhibitors

Compounds found to be inhibitory are listed. The commentary may explain experimental conditions, the concentration yielding a specific degree of inhibition or the inhibition constant. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Cofactors, prosthetic groups

This field contains cofactors which participate in the reaction but are not bound to the enzyme, and prosthetic groups being tightly bound. The commentary explains the function or, if known, the stereochemistry, or whether the cofactor can be replaced by a similar compound with higher or lower efficiency.

Activating Compounds

This field lists compounds with a positive effect on the activity. The enzyme may be inactive in the absence of certain compounds or may require activating molecules like sulfhydryl compounds, chelating agents, or lipids. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Metals, ions

This field lists all metals or ions that have activating effects. The commentary explains the role each of the cited metal has, being either bound e.g. as Fe-S centers or being required in solution. If an ion plays a dual role, activating at a certain concentration but inhibiting at a higher or lower concentration, this will be given in the commentary.

Turnover number (s^{-1})

The k_{cat} is given in the unit s^{-1}. The commentary lists the names of the substrates, sometimes with information on the reaction conditions or the type of reaction if the enzyme is capable of catalyzing different reactions with a single substrate. For cases where it is impossible to give the turnover number in the defined unit (e.g., substrates without a defined molecular weight, or an undefined amount of protein) this is summarized in *Additional Information*.

Specific activity (U/mg)

The unit is micromol/minute/milligram of protein. The commentary may contain information on specific assay conditions or if another than the natur-

al substrate was used in the assay. Entries in *Additional Information* are included if the units of the activity are missing in the literature or are not calculable to the obligatory unit. Information on literature with a detailed description of the assay method may also be found.

K_m-Value (mM)
The unit is mM. Each value is connected to a substrate name. The commentary gives, if available, information on specific reaction condition, isozymes or presence of activators. The references for values which cannot be expressed in mM (e.g. for macromolecular, not precisely defined substrates) are given in *Additional Information*. In this field we also cite literature with detailed kinetic analyses.

K_i-Value (mM)
The unit of the inhibition constant is mM. Each value is connected to an inhibitor name. The commentary gives, if available, the type of inhibition (e.g. competitive, non-competitive) and the reaction conditions (pH-value and the temperature). Values which cannot be expressed in the requested unit and references for detailed inhibition studies are summerized under *Additional information*.

pH-Optimum
The value is given to one decimal place. The commentary may contain information on specific assay conditions, such as temperature, presence of activators or if this optimum is valid for only one of several isozymes. If the enzyme has a second optimum, this will be mentioned here.

pH-Range
Mostly given as a range e.g. 4.0–7.0 with an added commentary explaining the activity in this range. Sometimes, not a range but a single value indicating the upper or lower limit of enzyme activity is given. In this case, the commentary is obligatory.

pI-Value
The isoelectric point (IEP) of an enzyme is the pH-value at which the protein molecule has no net electric charge, carrying the equal number of positively and negatively ions. In the commentary the method of determination is given, if it is provided by the literature.

Temperature optimum (°C)
Sometimes, if no temperature optimum is found in the literature, the temperature of the assay is given instead. This is always mentioned in the commentary.

Temperature range (°C)
This is the range over which the enzyme is active. The commentary may give the percentage of activity at the outer limits. Also commentaries on specific assay conditions, additives etc.

4 Enzyme Structure

Molecular weight
This field gives the molecular weight of the holoenzyme. For monomeric enzymes it is identical to the value given for subunits. As the accuracy depends on the method of determination this is given in the commentary if provided in the literature. Some enzymes are only active as multienzyme complexes for which the names and/or EC numbers of all participating enzymes are given in the commentary.

Subunits
The tertiary structure of the active species is described. The enzyme can be active as a monomer a dimer, trimer and so on. The stoichiometry of subunit composition is given. Some enzymes can be active in more than one state of complexation with differing effectivities. The analytical method is included.

Posttranslational modifications
The main entries in this field may be proteolytic modification, or side-chain modification, or no modification. The commentary will give details of the modifications e.g.:
- proteolytic modification <1> (<1>, propeptide Name) [1];
- side-chain modification <2> (<2>, N-glycosylated, 12% mannose) [2];
- no modification [3]

5 Isolation / Preparation / Mutation / Application

Source / tissue
For multicellular organisms, the tissue used for isolation of the enzyme or the tissue in which the enzyme is present is given. Cell-lines may also be a source of enzymes.

Localization
The subcellular localization is described. Typical entries are: cytoplasm, nucleus, extracellular, membrane.

Purification
The field consists of an organism and a reference. Only references with a detailed description of the purification procedure are cited.

Renaturation
Commentary on denaturant or renaturation procedure.

Crystallization
The literature is cited which describes the procedure of crystallization, or the X-ray structure.

Cloning
Lists of organisms and references, sometimes a commentary about expression or gene structure.

Engineering
The properties of modified proteins are described.

Application
Actual or possible applications in the fields of pharmacology, medicine, synthesis, analysis, agriculture, nutrition are described.

6 Stability

pH-Stability
This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Temperature stability
This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Oxidation stability
Stability in the presence of oxidizing agents, e.g. O_2, H_2O_2, especially important for enzymes which are only active under anaerobic conditions.

Organic solvent stability
The stability in the presence of organic solvents is described.

General stability information
This field summarizes general information on stability, e.g., increased stability of immobilized enzymes, stabilization by SH-reagents, detergents, glycerol or albumins etc.

Storage stability
Storage conditions and reported stability or loss of activity during storage.

References
Authors, Title, Journal, Volume, Pages, Year.

aminopeptidase S 3.4.11.24

1 Nomenclature

EC number
3.4.11.24

Recommended name
aminopeptidase S

Synonyms
AP <3,6> [2,7]
APCo-II <2> [19,24]
AmpS <1> [21]
M28.003 <3,5,6> (<3,5,6> Merops-ID [1,2,3,4,5,6,7,8,9,10,11]) [1,2,3,4,5,6,7,8,9,10,11]
S9 aminopeptidase <9> [27,29]
S9AP-St <9> [27,29]
SAP <3> [23]
SGAP <3,6,7> [1,2,9,10,11,12,13,16,18,20,22,25,26,30]
SGAPase <3> [8]
SSAP <8> [15,16]
Streptomyces aminopeptidase <4> [17]
Streptomyces dinuclear aminopeptidase <4> [17]
Streptomyces griseus aminopeptidase <3> [10,12,13,20]
Streptomyces griseus leucine aminopeptidase <7> [22]
aminolysin-S <9> [28]
aminopeptidase S <1> [21]
aminopeptidase yscCo-II <2> [19,24]
bacterial leucine aminopeptidase <3> [14]
dinuclear aminopeptidase <3> [23,25,30]
dizinc aminopeptidase <3> [25]
double-zinc aminopeptidase <3> [18,26]
leucine aminopeptidase <7,8> [15,22]
transaminopeptidase <9> [28]

CAS registry number
124404-20-2
9031-94-1

D. Schomburg and I. Schomburg (eds.), *Class 3.4–6 Hydrolases, Lyases, Isomerases, Ligases: EC 3.4–6*, Springer Handbook of Enzymes S10, DOI 10.1007/978-3-642-36260-6_1,

2 Source Organism

<1> *Staphylococcus aureus* [21]
<2> *Saccharomyces cerevisiae* [19,24]
<3> *Streptomyces griseus* [1,3,5,7,8,9,10,11,12,13,14,16,18,20,23,25,26,30]
<4> *Streptomyces sp.* [17]
<5> *Sulfolobus solfataricus* [6]
<6> *Streptomyces griseus* (UNIPROT accession number: P80561) [2,4]
<7> *Streptomyces griseus* (UNIPROT accession number: Q5WA30) [22]
<8> *Streptomyces septatus* (UNIPROT accession number: Q75V72) [15,16]
<9> *Streptomyces thermocyaneoviolaceus* [27,28,29]

3 Reaction and Specificity

Catalyzed reaction

release of an N-terminal amino acid with a preference for large hydrophobic amino-terminus residues (<3> catalytic mechanism, Asp160, Met161, Gly201, Arg202, and Phe219 are involved, active site structure, modeling of enzyme-substrate complex [1]; <3> catalytic mechanism, high preference towards large hydrophobic amino terminus residues, active site structure, Glu131 is involved in the catalytic mechanism, enzyme-substrate and enzyme-product complex modeling [11]; <6> hydrolyses peptide bonds formed by terminal hydrophobic amino acids such as leucine, methionine, and phenylalanine [4]; <3> M161 is involved in substrate binding at the active site cleft [9]; <3> preference for hydrophobic residues at the ultimate and the penultimate positions, D-amino acids at these positions reduce the activity, activity is not restricted by the length of substrate chains [7]; <3> catalytic pathway and reaction mechanism, catalytic residues are Glu131 and Tyr246, Tyr246 is involved in stabilization of the reaction transition state intermediate, also residue Glu151 is involved [18]; <3> raction mechanism, catalytic residues are Glu131 and Tyr246, residues Arg202 and Asp160 stabilize the reaction intermediate together with Glu131 [12]; <3> structure and reaction mechanism [20]; <3> the enzyme prefers large hydrophobic aminoterminal residues, Glu131, Asp160, and Arg202 are involved in binding of the N-terminal substrate amino acid, substrate binding and reaction mechanism, tetrahedral reaction intermediate, modelling of the enzyme-substrate and enzyme-product complexes [13]; <3> catalytic reaction mechanism, a single proton transfer is involved in catalysis at pH 8.0, whereas two proton transfers are implicated at pH 6.5, involvement of a zinc-bound hydroxide as the reaction nucleophile, Tyr246 polarizes the carbonyl carbon and stabilizes the transition state, enzyme-substrate interaction, overview [26])

Reaction type

hydrolysis
hydrolysis of peptide bond

Natural substrates and products

- **S** peptide + H_2O <3,5,6> (Reversibility: ?) [1,2,3,4,5,6,7,8,9,10,11]
- **P** ? <3,5,6> [1,2,3,4,5,6,7,8,9,10,11]
- **S** Additional information <3> (<3> aminopeptidases are involved in peptides processing and degradation, and are important in uptake of nutrients, regulation, overview [14]) (Reversibility: ?) [14]
- **P** ?

Substrates and products

- **S** 4-nitrophenyl phenylphosphonate + H_2O <4> (Reversibility: ?) [17]
- **P** 4-nitrophenol + phenylphosphonate
- **S** 4-nitrophenyl-L-leucine + H_2O <3> (Reversibility: ?) [20]
- **P** 4-nitrophenol + L-leucine
- **S** Ala-4-nitroanilide + H_2O <3> (Reversibility: ?) [25]
- **P** Ala + 4-nitroaniline
- **S** Arg-4-nitroanilide + H_2O <2> (<2> 105.2% of the activity with Lys-4-nitroanilide [24]) (Reversibility: ?) [19,24]
- **P** Arg + 4-nitroaniline
- **S** D-Arg-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** L-Pro-D-Arg-OMe + benzyl alcohol
- **S** D-Arg-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** c(L-Pro-D-Arg) + benzyl alcohol + methanol
- **S** D-Leu-OBzl + D-Leu-OBzl <9> (Reversibility: ?) [27]
- **P** D-Leu-D-Leu-OBzl + benzyl alcohol
- **S** D-Leu-OMe + D-Leu-OMe <9> (Reversibility: ?) [27]
- **P** D-Leu-D-Leu-OMe + MeOH
- **S** D-Leu-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** D-Pro-D-Leu-OMe + benzyl alcohol
- **S** D-Leu-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** L-Pro-D-Leu-OMe + benzyl alcohol
- **S** D-Phe-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** D-Pro-D-Phe-OMe + benzyl alcohol
- **S** D-Phe-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** L-Pro-D-Phe-OMe + benzyl alcohol
- **S** D-Trp-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** D-Pro-D-Trp-OMe + benzyl alcohol
- **S** D-Trp-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** L-Pro-D-Trp-OMe + benzyl alcohol
- **S** D-Tyr-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** D-Pro-D-Tyr-OMe + benzyl alcohol
- **S** D-Tyr-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** L-Pro-D-Tyr-OMe + benzyl alcohol
- **S** D-Val-OBzl + D-Val-OBzl <9> (Reversibility: ?) [27]
- **P** D-Val-D-Val-OBzl + benzyl alcohol
- **S** D-Val-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
- **P** L-Pro-D-Val-OMe + benzyl alcohol

S Glu-4-nitroanilide + H_2O <2> (<2> 5.4% of the activity with Lys-4-nitroanilide [24]) (Reversibility: ?) [24]
P Glu + 4-nitroaniline
S Gly-4-nitroanilide + H_2O <3> (Reversibility: ?) [25]
P Gly + 4-nitroaniline
S Gly-Leu-Gly + H_2O <3> (Reversibility: ?) [7]
P ? <3> [7]
S hemoglobin + H_2O <3> (<3> human hemoglobin [20]) (Reversibility: ?) [20]
P ?
S L-Ala-4-nitroanilide + H_2O <8> (<8> very low activity [16]) (Reversibility: ?) [16]
P L-Ala + 4-nitroaniline
S L-Ala-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Ala-OMe + benzyl alcohol
S L-Arg-4-nitroanilide + H_2O <8> (<8> low activity [16]) (Reversibility: ?) [16]
P L-Arg + 4-nitroaniline
S L-Arg-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Arg-OMe + benzyl alcohol
S L-Arg-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P c(L-Pro-L-Arg) + benzyl alcohol + methanol
S L-Arg-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Arg-OMe + benzyl alcohol
S L-Asn-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Asn-OMe + benzyl alcohol
S L-Asp-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Asp-OMe + benzyl alcohol
S L-Glu-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Glu-OMe + benzyl alcohol
S L-His-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
P D-Pro-L-His-OMe + benzyl alcohol
S L-His-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
P c(D-Pro-L-His) + benzyl alcohol + methanol
S L-His-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-His-OMe + benzyl alcohol
S L-His-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P c(L-Pro-L-His) + benzyl alcohol + methanol
S L-His-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-His-OMe + benzyl alcohol
S L-Ile-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
P D-Pro-L-Ile-OMe + benzyl alcohol
S L-Ile-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Ile-OMe + benzyl alcohol
S L-Ile-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Ile-OMe + benzyl alcohol
S L-Leu-4-nitroanilide + H_2O <7> (Reversibility: ?) [22]

P L-leucine + 4-nitroaniline
S L-Leu-4-nitroanilide + H_2O <3,8> (<3,8> best substrate [16]; <8> preferred substrate of the wild-type enzyme [15]) (Reversibility: ?) [15,16]
P L-Leu + 4-nitroaniline
S L-Leu-NH_2 + H_2O <8> (Reversibility: ?) [15]
P L-Leu + NH_3
S L-Leu-O-methyl ester + H_2O <8> (Reversibility: ?) [15]
P L-Leu + methanol
S L-Leu-OEt + D-Pro-OBzl <9> (Reversibility: ?) [27]
P D-Pro-L-Leu-OEt + benzyl alcohol
S L-Leu-OEt + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Leu-OEt + benzyl alcohol
S L-Leu-OEt + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Leu-OEt + benzyl alcohol
S L-Leu-OEt + β-Ala-OBzl + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Leu-β-Ala-OBzl + benzyl alcohol + ethanol
S L-Lys-4-nitroanilide + H_2O <3,8> (<3,8> low activity [16]) (Reversibility: ?) [16]
P L-Lys + 4-nitroaniline
S L-Lys-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Lys-OMe + benzyl alcohol
S L-Lys-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Lys-OMe + benzyl alcohol
S L-Met-4-nitroanilide + H_2O <3,8> (<3,8> low activity [16]) (Reversibility: ?) [16]
P L-Met + 4-nitroaniline
S L-Met-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
P D-Pro-L-Met-OMe + benzyl alcohol
S L-Met-OMe + L-Met-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Met-L-Met-OMe + benzyl alcohol + methanol
S L-Met-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Met-OMe + benzyl alcohol
S L-Met-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Met-OMe + benzyl alcohol
S L-Met-OMe + β-Ala-OBzl + β-Ala-OBzl <9> (Reversibility: ?) [29]
P (β-Ala)2-L-Met-OMe + benzyl alcohol +
S L-Met-OMe + β-Ala-OBzl + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Met-β-Ala-OBzl + benzyl alcohol + methanol
S L-Phe-4-nitroanilide + H_2O <3,8> (<3,8> low activity [16]) (Reversibility: ?) [15,16]
P L-Phe + 4-nitroaniline
S L-Phe-NH_2 + H_2O <8> (Reversibility: ?) [15]
P L-Phe + NH_3
S L-Phe-O-methyl ester + H_2O <8> (Reversibility: ?) [15]
P L-Phe + methanol
S L-Phe-OEt + D-Pro-OBzl <9> (Reversibility: ?) [27]
P D-Pro-L-Phe-OEt + benzyl alcohol

S L-Phe-OEt + L-Phe-OEt + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Phe-L-Phe-OMe + benzyl alcohol + ethanol
S L-Phe-OEt + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Phe-OEt + benzyl alcohol
S L-Phe-OEt + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Phe-OEt + benzyl alcohol
S L-Phe-OEt + β-Ala-OBzl + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Phe-β-Ala-OBzl + benzyl alcohol + ethanol
S L-Pro-4-nitroanilide + H_2O <8> (<8> very low activity [16]) (Reversibility: ?) [16]
P L-Pro + 4-nitroaniline
S L-Pro-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P L-Pro-β-Ala-OBzl + methanol
S L-Pro-OMe + β-Ala-OBzl + β-Ala-OBzl <9> (Reversibility: ?) [29]
P L-Pro-$(\beta\text{-Ala})_2$-OBzl + methanol + benzyl alcohol
S L-Ser-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Ser-OMe + benzyl alcohol
S L-Thr-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Thr-OMe + benzyl alcohol
S L-Thr-OMe + L-Thr-OMe <9> (Reversibility: ?) [27]
P L-Thr-L-Thr-OMe + methanol
S L-Thr-OMe + L-Thr-OMe <9> (Reversibility: ?) [29]
P $(\text{L-Thr})_2$-OMe + methanol
S L-Thr-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Thr-OMe + benzyl alcohol
S L-Trp-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
P D-Pro-L-Trp-OMe + benzyl alcohol
S L-Trp-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Trp-OMe + benzyl alcohol
S L-Trp-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Trp-OMe + benzyl alcohol
S L-Tyr-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
P D-Pro-L-Tyr-OMe + benzyl alcohol
S L-Tyr-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Tyr-OMe + benzyl alcohol
S L-Tyr-OMe + L-Tyr-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Tyr-L-Tyr-OMe + benzyl alcohol + methanol
S L-Tyr-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Tyr-OMe + benzyl alcohol
S L-Tyr-OMe + β-Ala-OBzl + β-Ala-OBzl <9> (Reversibility: ?) [29]
P $(\beta\text{-Ala})_2$-L-Tyr-OMe + benzyl alcohol
S L-Val-OBzl + L-Val-OBzl <9> (Reversibility: ?) [27]
P L-Val-L-Val-OBzl + benzyl alcohol
S L-Val-OMe + D-Pro-OBzl <9> (Reversibility: ?) [27]
P D-Pro-L-Val-OMe + benzyl alcohol
S L-Val-OMe + L-Pro-OBzl <9> (Reversibility: ?) [27]
P L-Pro-L-Val-OMe + benzyl alcohol

S L-Val-OMe + β-Ala-OBzl <9> (Reversibility: ?) [29]
P β-Ala-L-Val-OMe + benzyl alcohol
S L-Val-OMe + β-Ala-OBzl + β-Ala-OBzl <9> (Reversibility: ?) [29]
P (β-Ala)2-L-Val-OMe + benzyl alcohol
S L-alanine-4-nitroanilide + H_2O <9> (Reversibility: ?) [28]
P L-Ala + 4-nitroaniline
S L-alanine-4-nitroanilide + H_2O <3> (<3> good substrate [3]) (Reversibility: ?) [3,5]
P L-alanine + 4-nitroaniline <3> [3,5]
S L-leucine-4-nitroanilide + H_2O <3,5,6> (<3> best of the amino acid 4-nitroanilide substrates [3,5]) (Reversibility: ?) [2,3,5,6,7,10]
P L-leucine + 4-nitroaniline <3,5,6> [2,3,5,6,7,10]
S L-lysine-4-nitroanilide + H_2O <3> (<3> good substrate [3]) (Reversibility: ?) [3]
P L-lysine + 4-nitroaniline <3> [3]
S L-methionine-4-nitroanilide + H_2O <3> (<3> very good substrate [3]) (Reversibility: ?) [3]
P L-methionine + 4-nitroaniline <3> [3]
S L-phenylalanine ethyl ester + L-phenylalanine + H_2O <9> (<9> assay at 50°C [28]) (Reversibility: ?) [28]
P L-Phe-L-Phe + cyclo(L-phenylalanine-L-phenylalanine) + L-Phe-L-Phe ethyl ester
S L-phenylalanine-4-nitroanilide + H_2O <9> (Reversibility: ?) [28]
P L-Phe + 4-nitroaniline
S L-phenylalanine-4-nitroanilide + H_2O <3> (<3> very good substrate [3]) (Reversibility: ?) [3]
P L-phenylalanine + 4-nitroaniline <3> [3]
S L-proline-4-nitroanilide + H_2O <3> (<3> good substrate [3]) (Reversibility: ?) [3]
P L-proline + 4-nitroaniline <3> [3]
S L-proline-*p*-nitroanilide + H_2O <9> (Reversibility: ?) [28]
P L-Pro + 4-nitroaniline
S L-valine-4-nitroanilide + H_2O <3> (<3> good substrate [3]) (Reversibility: ?) [3,5]
P L-valine + 4-nitroaniline <3> [3,5]
S Leu-4-nitroanilide + H_2O <2,3> (<2> 12.2% of the activity with Lys-4-nitroanilide [24]; <3> assay at pH 8.0, 30°C [30]) (Reversibility: ?) [24,25,30]
P Leu + 4-nitroaniline
S Leu-4-nitroaniline + H_2O <3> (Reversibility: ?) [26]
P Leu + 4-nitroaniline
S Leu-Gly-Gly + H_2O <3> (Reversibility: ?) [7]
P Leu + Gly-Gly <3> [7]
S Lys-4-nitroanilide + H_2O <2,3> (Reversibility: ?) [19,24,25]
P Lys + 4-nitroaniline
S Met-4-nitroanilide + H_2O <3> (Reversibility: ?) [25]
P Met + 4-nitroaniline

S Met-enkephalin + H_2O <1> (<1> substrate is a pentapeptide with sequence Tyr-Gly-Gly-Phe-Met, stepwise degradation from the N-terminus [21]) (Reversibility: ?) [21]
P ?
S Pro-4-nitroanilide + H_2O <2> (<2> 11.7% of the activity with Lys-4-nitroanilide [24]) (Reversibility: ?) [24]
P Pro + 4-nitroaniline
S Val-4-nitroanilide + H_2O <3> (Reversibility: ?) [25]
P Val + 4-nitroaniline
S β-Ala-OBzl + β-Ala-OBzl <9> (Reversibility: ?) [29]
P (β-Ala)$_2$-OBzl + benzyl alcohol
S bis(4-nitrophenyl) phosphate + H_2O <4> (Reversibility: ?) [17]
P 4-nitrophenol + phosphate
S bis(4-nitrophenyl) phosphate + H_2O <3> (<3> assay at pH 8.0, 30°C [30]) (Reversibility: ?) [30]
P ?
S bis(p-nitrophenyl)phosphate + H_2O <3> (Reversibility: ?) [25]
P ?
S casein + H_2O <3> (Reversibility: ?) [10]
P ?
S human hemoglobin α-chain + H_2O <3> (<3> hydrolysis of the first few residues to proline at the 4th position [7]) (Reversibility: ?) [7]
P ? <3> [7]
S human hemoglobin β-chain + H_2O <3> (<3> hydrolysis of the first few residues to proline at the 5th position [7]) (Reversibility: ?) [7]
P ? <3> [7]
S peptide + H_2O <3,5,6> (<3> substrate specificity [7]; <3> high preference towards large hydrophobic amino terminus residues [11]) (Reversibility: ?) [1,2,3,4,5,6,7,8,9,10,11]
P ? <3,5,6> [1,2,3,4,5,6,7,8,9,10,11]
S Additional information <3,8> (<3> no activity with Gly-Pro-4-nitroanilide and Ala-Pro-4-nitroanilide, no acitivity with Xaa-Pro N-terminal sequences, glycine-4-nitroanilide and acidic amino acid 4-nitroanilide are very poor substrates [3]; <3> substrates with blocked amino groups are partially hydrolyzed, poor activity with Val-Gly, Val-Leu, and Trp-Leu, Gly-Gly-Gly and D-leucine-D-leucine are no substrates [7]; <3> aminopeptidases are involved in peptides processing and degradation, and are important in uptake of nutrients, regulation, overview [14]; <8> no activity with L-Glu-4-nitroanilide, D-Phe-4-nitroanilide, and D-Leu-4-nitroanilide [16]; <3> no activity with L-Glu-4-nitroanilide, L-Arg-4-nitroanilide, D-Phe-4-nitroanilide, and D-Leu-4-nitroanilide, L-Ala-4-nitroanilide and L-Pro-4-nitroanilide are poor substrates [16]; <8> substrate specificities of recombinant wild-type and F221 mutant enzymes, overview [15]; <3> the enzyme is active on a wide variety of peptides substrates, no activity with D-Leu-D-Leu, no prolidase activity, but release of N-terminal prolyl residues [20]; <3> the enzyme shows broad substrate specificity preferring N-terminal Leu or Met and Phe, but is not able to hydrolyse peptide

substrates bonds with formed by acidic amino acids in the P1 position or proline in the P1 or P1 position [14]; <3> the enzyme is active toward various peptides with different N-terminal side chains and specific toward hydrophobic ones, the enzyme also exhibits a significant activity toward the hydrolysis of the phosphodiester bis(*p*-nitrophenyl)phosphate, overview, active site structure involves the three auxiliary amino acid side chains of Tyr246, Glu131, and Arg202 that are involved in catalysis, modeling of substrate binding using the crystal structure of the enzyme, overview, the activity shows proton inventory and viscosity dependence, overview [25]; <3> the thermostable enzyme prefers large hydrophobic N-terminal residues in its peptide and protein substrates, a single proton transfer is involved in catalysis at pH 8.0, whereas two proton transfers are implicated at pH 6.5 [26]) (Reversibility: ?) [3,7,14,15,16,20,25,26]

P ? <3> [3,7]

Inhibitors

1,10-phenanthroline <2,3,6> (<2> 1 mM, complete inhibition [19]; <2> 1 mM, 91% inhibition [24]; <6> 50% inhibition at pI 4.9 [2]) [2,3,19,24]
4-iodo-L-phenylalanine <3> (<3> weak inhibition, binds at the active site via the two zinc ions displacing the metal ions, binding structure analysis [12]) [12]
Ca^{2+} <3,8> (<8> slight inhibition at 1 mM [16]) [16]
Cd^{2+} <3,8> [16]
chloroquine <2> (<2> 5 mM, 79% inhibition [24]) [24]
Cr^{2+} <2> [19]
$CrCl_2$ <2> (<2> 1 mM, 83% inhibition in presence of 1 mM $CoCl_2$ [24]) [24]
Cu^{2+} <2,3,8> [16,19]
$CuCl_2$ <2> (<2> 1 mM, 96% inhibition in presence of 1 mM $CoCl_2$ [24]) [24]
D-phenylalanine <3> [5]
EDTA <2,3,5,6> (<2> 5 mM, 90% inhibition [24]; <5> aminopeptidase I and II [6]; <6> complete inhibition, activity cannot be completely restored by addition of 1 mM $CaCl_2$ alone but together with 0.0001 mM $ZnCl_2$ by 90% [2]; <3> complete inhibition at 10 mM, reactivation by divalent metal ions [20]) [2,6,20,24]
EGTA <6> (<6> complete inhibition, activity can be completely restored by addition of 1 mM $CaCl_2$ [2]) [2]
Fe^{2+} <3,8> [16]
$HgCl_2$ <2> (<2> 5 mM, 98% inhibition in presence of 1 mM $CoCl_2$ [24]) [24]
L-alanine <3> [5]
L-arginine <3> [5]
L-aspartate <3> [5]
L-histidine <7> [22]
L-leucine <3> (<3> binding mechanism and structure [11]; <3> very low product inhibition [16]) [5,11,16]
L-leucine chloromethyl ketone <3> [5]
L-methionine <3> (<3> binding mechanism and structure [11]) [5,11]
L-phenylalanine <3> (<3> binding mechanism and structure [11]) [5,11]

L-phenylalanine chloromethyl ketone <3> [5]
L-serine <7> [22]
L-tryptophan <3> (<3> weak inhibition, binds at the active site via the two zinc ions displacing the metal ions, binding structure analysis [12]) [12]
Leu-hydroxamate <2> (<2> 1 mM, 73% inhibition [24]) [24]
Lys-hydroxamate <2> (<2> 1 mM, 80% inhibition [19]; <2> 1 mM, 81% inhibition [24]) [19,24]
Mg^{2+} <3,8> (<8> slight inhibition at 0.1-1 mM [16]) [16]
$MgSO_4$ <2> (<2> 1 mM, 24% inhibition in presence of 1 mM $CoCl_2$ [24]) [24]
Mn^{2+} <3,8> [16]
$MnSO_4$ <2> (<2> 1 mM, 26% inhibition in presence of 1 mM $CoCl_2$ [24]) [24]
Ni^{2+} <2,3,8> [16,19]
$NiSO_4$ <2> (<2> 1 mM, 40% inhibition in presence of 1 mM $CoCl_2$ [24]) [24]
PMSF <2> (<2> 1 mM, complete inhibition [19]) [19]
Zn^{2+} <2,3,8> [16,19]
$ZnCl_2$ <2> (<2> 1 mM, 70% inhibition in presence of 1 mM $CoCl_2$ [24]) [24]
amastatin <3,8> [5,16]
antipain <2> (<2> 0.01 mg/ml, 93% inhibition [24]) [24]
bestatin <2,3,5,8> (<5> aminopeptidase I and II [6]; <2> 0.004 mg/ml, 94% inhibition [24]; <2> 0.004 mg/ml, complete inhibition [19]) [5,6,16,19,24]
diisopropylphosphofluoridate <3> (<3> pretreatment, complete inhibition [7]) [7]
fluoride <3> (<3> noncompetitive inhibitor at pH 5.9-8.0, fluoride ion interacts equally with the free enzyme as with the enzyme-substrate complex [26]; <3> uncompetitive inhibitor toward peptide hydrolysis [25]) [25,26,30]
free amino acids <3> (<3> competitive inhibition, highest inhibition by L-histidine [20]) [20]
leucine <3> (<3> weak inhibitor, binding structure, overview [13]; <3> product inhibition occurs in peptide hydrolysis [25]) [13,25]
leupeptin <2> (<2> 0.01 mg/ml, 51% inhibition [24]) [24]
methionine <3> (<3> weak inhibitor, binding structure, overview [13]) [13]
nitrilotriacetic acid <2> (<2> 5 mM, 95% inhibition [24]) [24]
o-phenanthroline <5> (<5> aminopeptidase I and II [6]) [6]
p-(chloromercuri)benzene sulfonate <5> (<5> aminopeptidase II [6]) [6]
p-hydroxymercuribenzoate <2> (<2> 5 mM, 90% inhibition [24]) [24]
phenylalanine <3> (<3> weak inhibitor, binding structure, overview [13]) [13]
phenylmethylsulfonyl fluoride <2> (<2> 1 mM, 95% inhibition [24]) [24]
phosphate <3> (<3> noncompetitive inhibitor with peptide substrates, the enzyme-substrate-inhibitor ternary complex is inactive, but phosphate is a competitive inhibitor toward bis(*p*-nitrophenyl)phosphate hydrolysis, with K_i ranging from 2.31 to 315 mM at pH 5.0-9.0 [25]) [25,26]
tosyl-lysine chloromethyl ketone <2> (<2> 2 mM, 80% inhibition [19]; <2> 5 mM, 94% inhibition [24]) [19,24]
tosyl-phenylalanine chloromethyl ketone <5> (<5> slight inhibition [6]) [6]
Additional information <1,3,5,8> (<5> aminopeptidase II is not affected by PMSF, pepstatin, tosyl-lysine chloromethyl ketone, tosyl-phenylalanine chlor-

omethyl ketone, leupeptin, and phosphoramidon, aminopeptidase I is not affected by PMSF, *p*-(chloromercuri)benzene sulfonate, pepstatin, tosyl-lysine chloromethyl ketoneleupeptin, and phosphoramidon [6]; <3> nonchelating 1,7-phenanthroline has little effect on the activity [3]; <1> the enzyme activity is completely abolished by metal chelating agents, but can be restored by addition of zinc or cobalt [21]; <8> very low product inhibition by L-leucine [16]) [3,6,16,21]

Activating compounds

Additional information <5> (<5> aminopeptidase II is not affected by PMSF, pepstatin, tosyl-lysine chloromethyl ketone, tosyl-phenylalanine chloromethyl ketone, leupeptin, and phosphoramidon, aminopeptidase I is not affected by PMSF, *p*-(chloromercuri)benzene sulfonate, pepstatin, tosyl-lysine chloromethyl ketoneleupeptin, and phosphoramidon [6]) [6]

Metals, ions

Ca^{2+} <3,6,7> (<3> activates [25,26]; <6> activation [4]; <6> activates about 6fold, non-cooperative binding [2]; <3> activates, binds to the enzyme, activation level with different enzyme substrates, overview [3]; <3> activates, enhances stability of the enzyme, modulates the enzyme activity and affinity towards substrates and inhibitors in a structure-dependent manner, binding structure [5]; <3> location of binding site is in about 25 A distance from the zinc binding site, determination of structural environment [9]; <7> activates and stabilizes, influences substrate specificity, Asp173 and Asp174 are key residues in Ca^{2+} binding and important for enzyme activity, residues Asp3, Ile4, Asp262, and Asp266 are also involved in calcium binding but are important for enzyme stabilization, overview, binding capacity of recombinant wild-type and mutant enzymes, overview [22]; <3> activates the enzyme with some substrates, about 3fold with L-Leu-4-nitroanilide, and affects substrate specificity, e.g. decreases the activity with Lys-4-nitroanilide [16]; <3> activates, has complex effects on the enzyme, stabilizes the enzyme [20]; <3> affects metal binding, inhibition, and entropy of activation of the enzyme [23]; <3> modulating the enzyme activity [13]) [2,3,4,5,9,13,16,18,20,22,23,25,26]

Cd^{2+} <3,4> (<4> dinuclear metal-enzyme derivative, structure [17]; <3> metal binding modelling using titration, kinetic, and thermodynamic data, dinuclear metal enzyme, sequential binding to two metal binding sites, affected by Ca^{2+} [23]) [17,23]

Co^{2+} <1,2,3,4> (<3> activates [20]; <2> activity is strictly dependent on, maximal activity at 0.5 mM $CoCl_2$ [24]; <4> dinuclear metal-enzyme derivative, structure [17]; <2> metalloenzyme containing Co^{2+} in its structure [19]; <3> slightly activating at 0.1-1 mM [16]; <1> the enzyme contains two metal ions with high occupancy and a third metal ion with low occupancy at the active site of the enzyme molecule, Glu319 and a water molecule are bridging, Glu253, His348, His381, Tyr355, and Asp383 are involved in metal binding, structure analysis [21]) [16,17,19,20,21,24]

Mn^{2+} <4> (<4> dinuclear metal-enzyme derivative, structure [17]) [17]

Ni^{2+} <4> (<4> dinuclear metal-enzyme derivative, structure [17]) [17]

Zn^{2+} <1,3,6,7> (<3> 2 mol zinc per mol of enzyme, binding structure [1,5]; <3> 2 mol zinc per mol of enzyme, tightly bound at the active site in a distance of 3.6 A of each other, determination of structural environment [9]; <6> 2 mol/mol of protein, tightly bound, zinc coordination amino acid residues are conserved in similar enzymes [4]; <3> double-zinc exopeptidase [11]; <3,6> metalloprotease, dependent on [2,3]; <3> zinc-metallo-exoprotease [10]; <1> the enzyme contains two metal ions with high occupancy and a third metal ion with low occupancy at the active site of the enzyme molecule, Glu319 and a water molecule are bridging, Glu253, His348, His381, Tyr355, and Asp383 are involved in metal binding, structure analysis [21]; <3> the enzyme is a double-zinc aminopeptidase [18]; <7> the enzyme is a double-zinc aminopeptidase, metal ions are bound at the active site [22]; <3> the enzyme is a double-zinc aminopeptidase, the metal ions are bound at the active site, binding structure analysis [12]; <3> the enzyme is a double-zinc exopeptidase, binding structure, overview [13]; <3> the enzyme is a zinc-metalloenzyme containing 2 mol zinc per mol of enzyme, stabilizes the enzyme [20]; <3> dizinc enzyme [25]; <3> double-zinc aminopeptidase [26]) [1,2,3,4,5,9,10,11,12,13,18,20,21,22,25,26]
Additional information <1,3,4,8> (<3> substitution of the Zn^{2+} ion by Mn^{2+} or Co^{2+} results in altered substrate specificity, e.g. the Co^{2+} containing enzyme highly prefers L-alanine-4-nitroanilide [3]; <8> Ca^{2+} and Co^{2+} do not affect the enzymes substrate specificity at 0.1-1 mM [16]; <1> determination of metal ion identity [21]; <3> the enzyme depends on metals [20]; <4> the enzyme possesses dinuclear metal cluster [17]) [3,16,17,20,21]

Turnover number (s^{-1})

0.0033 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Ni^{2+}-enzyme [17]) [17]
0.0064 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Ni-heterodinuclear aminopeptidase [30]) [30]
0.01 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Mn^{2+}-enzyme [17]) [17]
0.01 <3,4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Ni^{2+}-enzyme [17]; <3> in presence of Ni-Ni-homo-dinuclear aminopeptidase [30]) [17,30]
0.014 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Zn^{2+}-enzyme [17]) [17]
0.016 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Cd-heterodinuclear aminopeptidase [30]) [30]
0.017 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Cd^{2+}-enzyme [17]) [17]
0.022 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Co^{2+}-enzyme [17]) [17]
0.043 <3,4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Cd^{2+}-enzyme [17]; <3> in presence of Cd-Cd-homo-dinuclear aminopeptidase [30]) [17,30]
0.0433 <3> (Leu-4-nitroanilide, <3> in presence of Ni-Ni-homo-dinuclear aminopeptidase [30]) [30]

0.0505 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Ni-hetero-dinuclear aminopeptidase [30]) [30]
0.081 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Mn-homo-dinuclear aminopeptidase [30]) [30]
0.087 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Co-hetero-dinuclear aminopeptidase [30]) [30]
0.1 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Zn-hetero-dinuclear aminopeptidase [30]) [30]
0.13 <3> (L-valine-4-nitroanilide, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
0.191 <3> (L-Val-4-nitroanilide, <3> pH 8.0, 30°C [3]) [3]
0.21 <4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Mn^{2+}-enzyme [17]) [17]
0.26 <3> (L-valine-4-nitroanilide, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
0.28 <3> (Val-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
0.42 <3> (Bis(*p*-nitrophenyl)phosphate, <3> pH 8.0, 30°C [25]) [25]
0.45 <3,4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Zn^{2+}-enzyme [17]; <3> in presence of Zn-Zn-homo-dinuclear aminopeptidase [30]) [17,30]
0.47 <3> (L-alanine-4-nitroanilide, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
0.49 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant mutant E196A, in presence of Ca^{2+} [22]) [22]
0.55 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant mutant E196A, in absence of Ca^{2+} [22]) [22]
0.64 <8> (L-Phe-O-methyl ester, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
0.74 <3,4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Co^{2+}-enzyme [17]; <3> in presence of Co-Co-homo-dinuclear aminopeptidase [30]) [17,30]
0.97 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant wild-type enzyme, in presence of Ca^{2+} [22]) [22]
1.08 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant mutant D3A/D262G, in presence of Ca^{2+} [22]) [22]
1.1 <3> (Gly-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
1.31 <3> (L-leucine-4-nitroanilide, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
1.5 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Cd-hetero-dinuclear aminopeptidase [30]) [30]
1.68 <3> (Leu-4-nitroanilide, <3> in presence of Cd-Cd-homo-dinuclear aminopeptidase [30]) [30]
1.71 <3> (L-Ala-4-nitroanilide, <3> pH 8.0, 30°C [3]) [3]
1.81 <3> (L-alanine-4-nitroanilide, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
2.12 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant wild-type enzyme, in absence of Ca^{2+} [22]) [22]
2.71 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant mutant D3A/D262G, in absence of Ca^{2+} [22]) [22]
2.8 <3> (Lys-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]

2.94 <3> (L-Ala-4-nitroanilide, <3> pH 8.0, 30°C [3]) [3]
2.94 <3> (L-alanine-4-nitroanilide, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
3-6 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant mutant D3A/D262G, in absence of Ca^{2+} [22]) [22]
4.72 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant chimeric mutant, in presence of Ca^{2+} [22]) [22]
4.9 <3> (Ala-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
5.2 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant chimeric mutant, in absence of Ca^{2+} [22]) [22]
10.7 <8> (L-Phe-NH_2, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
17.6 <8> (L-Phe-4-nitroanilide, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
19.3 <8> (L-Phe-4-nitroanilide, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
27.5 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Mn-homo-dinuclear aminopeptidase [30]) [30]
31.7 <8> (L-Leu-NH_2, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]
34 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]
37.4 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Co-hetero-dinuclear aminopeptidase [30]) [30]
41 <3> (Leu-4-nitroanilide, <3> in presence of Co-Co-homo-dinuclear aminopeptidase [30]) [30]
41.5 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant enzyme, in absence of Ca^{2+} [16]) [16]
42.1 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant enzyme, in presence of Ca^{2+} [16]) [16]
43.3 <3> (Met-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
43.8 <8> (L-Phe-NH_2, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
54.3 <8> (L-Phe-O-methyl ester, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
64.2 <8> (L-Leu-O-methyl ester, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
65.9 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
75 <3> (L-leucine-4-nitroanilide, <3> pH 8.0, 22°C, acetylated aminopeptidases 2 [7]) [7]
75.3 <3> (L-Leu-4-nitroanilide, <3> pH 8.0, 37°C, recombinant enzyme, in absence of Ca^{2+} [16]) [16]
92.8 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Zn-hetero-dinuclear aminopeptidase [30]) [30]
101 <3> (Leu-4-nitroanilide, <3> in presence of Zn-Zn-homo-dinuclear aminopeptidase [30]) [30]

104 <8> (L-Leu-NH_2, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
108 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
111 <3> (L-leucine-4-nitroanilide, <3> pH 8.0, 22°C, native aminopeptidases 2 [7]) [7]
115 <8> (L-Phe-NH_2, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]
116 <3> (L-leucine-4-nitroanilide, <3> pH 8.0, 22°C, acetylated aminopeptidases 1 [7]) [7]
142 <8> (L-Leu-O-methyl ester, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
153 <3> (L-leucine-4-nitroanilide, <3> pH 8.0, 22°C, native aminopeptidases 1 [7]) [7]
155 <8> (L-Leu-NH_2, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
169 <8> (L-Leu-O-methyl ester, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
223 <3> (L-Leu-4-nitroanilide, <3> pH 8.0, 37°C, recombinant enzyme, in presence of Ca^{2+} [16]) [16]
224 <8> (L-Phe-O-methyl ester, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]
229 <8> (L-Phe-4-nitroanilide, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]
391 <3> (L-Leu-4-nitroanilide, <3> pH 8.0, 30°C [3]) [3]
657 <3> (Leu-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]

Specific activity (U/mg)

0.0016 <4> (<4> Ni^{2+}-enzyme, substrate 4-nitrophenyl phenylphosphonate [17]; <4> Ni^{2+}-enzyme, substrate bis(4-nitrophenyl) phosphate [17]) [17]
0.0017 <4> (<4> Zn^{2+}-enzyme, substrate 4-nitrophenyl phenylphosphonate [17]) [17]
0.0033 <4> (<4> Mn^{2+}-enzyme, substrate 4-nitrophenyl phenylphosphonate [17]) [17]
0.0048 <4> (<4> Co^{2+}-enzyme, substrate 4-nitrophenyl phenylphosphonate [17]) [17]
0.0078 <4> (<4> Cd^{2+}-enzyme, substrate bis(4-nitrophenyl) phosphate [17]) [17]
0.0097 <4> (<4> Cd^{2+}-enzyme, substrate 4-nitrophenyl phenylphosphonate [17]) [17]
0.02 <9> (<9> substrate β-Ala-4-nitroanilide [29]) [29]
0.031 <4> (<4> Mn^{2+}-enzyme, substrate bis(4-nitrophenyl) phosphate [17]) [17]
0.103 <5> (<5> crude cell extract, cells grown on glucose, exponential phase [6]) [6]
0.116 <5> (<5> crude cell extract, cells grown on yeast extract, exponential phase [6]) [6]

0.122 <5> (<5> crude cell extract, cells grown on glucose, stationary phase [6]) [6]
0.136 <4> (<4> Co^{2+}-enzyme, substrate bis(4-nitrophenyl) phosphate [17]) [17]
0.139 <5> (<5> crude cell extract, cells grown on yeast extract, stationary phase [6]) [6]
0.158 <4> (<4> Zn^{2+}-enzyme, substrate bis(4-nitrophenyl) phosphate [17]) [17]
4.05 <2> [24]
5.13 <9> (<9> substrate L-Pro-4-nitroanilide [29]) [29]
38.4 <9> (<9> substrate L-Phe-4-nitroanilide [29]) [29]
306 <6> (<6> purified API [2]) [2]
317 <6> (<6> purified APII [2]) [2]
461 <6> (<6> purified API in presence of 1 mM $CaCl_2$ [2]) [2]
625.1 <3> (<3> purified enzyme [10]; <3> purified recombinant enzyme [10]) [10]
830 <3> (<3> purified recombinant wild-type enzyme [18]) [18]
1244 <9> (<9> substrate L-Ala-4-nitroanilide [29]) [29]
Additional information <3,7,8> (<7> recombinant wild-type and mutant enzymes, overview [22]) [5,15,22,25]

K_m-Value (mM)

0.00229 <3> (Leu-4-nitroanilide, <3> in presence of Ni-Ni-homo-dinuclear aminopeptidase [30]) [30]
0.00242 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Ni-hetero-dinuclear aminopeptidase [30]) [30]
0.093 <3> (Leu-4-nitroanilide, <3> in presence of Co-Co-homo-dinuclear aminopeptidase [30]) [30]
0.15 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Co-hetero-dinuclear aminopeptidase [30]) [30]
0.16 <2> (Arg-4-nitroanilide, <2> 37°C, 0.5 mM $CoCl_2$ [24]) [19,24]
0.16 <2> (Lys-4-nitroanilide, <2> 37°C, 0.5 mM $CoCl_2$ [24]) [19,24]
0.18 <3> (Val-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
0.19 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Cd-hetero-dinuclear aminopeptidase [30]) [30]
0.213 <3> (Leu-4-nitroanilide, <3> in presence of Cd-Cd-homo-dinuclear aminopeptidase [30]) [30]
0.25 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
0.33 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant enzyme, in absence of Ca^{2+} [16]) [16]
0.34 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant enzyme, in presence of Ca^{2+} [16]) [16]
0.34 <8> (L-Phe-4-nitroanilide, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
0.36 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]

0.44 <8> (L-Phe-4-nitroanilide, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]
0.45 <3> (Leu-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
0.53 <3> (L-Val-4-nitroanilide, <3> pH 8.0, 30°C [3]) [3]
0.55 <3> (L-leucine-4-nitroanilide, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
0.58 <3> (L-Leu-4-nitroanilide, <3> pH 8.0, 30°C [3]) [3]
0.58 <3> (Met-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
0.65 <3> (L-Leu-4-nitroanilide, <3> pH 8.0, 37°C, recombinant enzyme, in presence of Ca^{2+} [16]) [16]
0.67 <3> (L-leucine-4-nitroanilide, <3> pH 8.0, 22°C, native aminopeptidases 1 and 2 [7]) [7]
0.68 <8> (L-Leu-4-nitroanilide, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]
0.72 <3> (L-leucine-4-nitroanilide, <3> pH 8.0, 22°C, acetylated aminopeptidases 1 and 2 [7]) [7]
0.79 <3> (L-valine-4-nitroanilide, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
0.88 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Mn-homo-dinuclear aminopeptidase [30]) [30]
1.4 <3> (Gly-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
1.85 <8> (L-Phe-4-nitroanilide, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
2.15 <3> (L-valine-4-nitroanilide, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
2.3 <3> (L-Leu-4-nitroanilide, <3> pH 8.0, 37°C, recombinant enzyme, in absence of Ca^{2+} [16]) [16]
2.4 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Cd^{2+}-enzyme [17]) [17]
2.54 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant mutant D3A/D262G, in absence of Ca^{2+} [22]) [22]
2.61 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant wild-type enzyme, in absence of Ca^{2+} [22]) [22]
2.97 <3> (L-leucine-4-nitroanilide, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
3 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Ni^{2+}-enzyme [17]) [17]
3.27 <3> (Leu-4-nitroanilide, <3> in presence of Zn-Zn-homo-dinuclear aminopeptidase [30]) [30]
3.31 <3> (Leu-4-nitroanilide, <3> in presence of Mn-Zn-hetero-dinuclear aminopeptidase [30]) [30]
3.4 <3> (bis(*p*-nitrophenyl)phosphate, <3> pH 8.0, 30°C [25]) [25]
3.47 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant mutant E196A, in presence of Ca^{2+} [22]) [22]
3.79 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant mutant E196A, in absence of Ca^{2+} [22]) [22]

3.8 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Zn-hetero-dinuclear aminopeptidase [30]) [30]
3.84 <3> (L-alanine-4-nitroanilide, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
3.9 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Co-hetero-dinuclear aminopeptidase [30]) [30]
3.91 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant chimeric mutant, in presence of Ca^{2+} [22]) [22]
4.02 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant chimeric mutant, in absence of Ca^{2+} [22]; <7> pH 8.0, 37°C, recombinant wild-type enzyme, in presence of Ca^{2+} [22]) [22]
4.07 <7> (L-Leu-4-nitroanilide, <7> pH 8.0, 37°C, recombinant mutant D3A/D262G, in presence of Ca^{2+} [22]) [22]
4.08 <3> (L-Ala-4-nitroanilide, <3> pH 8.0, 30°C [3]) [3]
4.5 <3,4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Zn^{2+}-enzyme [17]; <3> in presence of Zn-Zn-homo-dinuclear aminopeptidase [30]) [17,30]
4.81 <3> (L-alanine-4-nitroanilide, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
4.9 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Mn^{2+}-enzyme [17]) [17]
5 <3> (Lys-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
6.9 <8> (L-Phe-O-methyl ester, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
6.98 <8> (L-Phe-NH_2, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
7.68 <8> (L-Phe-NH_2, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]
7.8 <3> (Ala-4-nitroanilide, <3> pH 8.0, 30°C [25]) [25]
7.9 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Co^{2+}-enzyme [17]) [17]
8.31 <8> (L-Leu-NH_2, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
9.5 <3,4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Co^{2+}-enzyme [17]; <3> in presence of Co-Co-homo-dinuclear aminopeptidase [30]) [17,30]
9.7 <3,4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Cd^{2+}-enzyme [17]; <3> in presence of Cd-Cd-homo-dinuclear aminopeptidase [30]) [17,30]
10.6 <3,4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Ni^{2+}-enzyme [17]; <3> in presence of Ni-Ni-homo-dinuclear aminopeptidase [30]) [17,30]
11 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Cd-hetero-dinuclear aminopeptidase [30]) [30]
12 <4> (bis(4-nitrophenyl) phosphate, <4> pH 8.0, 30°C, Mn^{2+}-enzyme [17]) [17]
12.3 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Mn-homo-dinuclear aminopeptidase [30]) [30]
12.8 <8> (L-Phe-O-methyl ester, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]

12.8 <3> (bis(4-nitrophenyl) phosphate, <3> in presence of Mn-Ni-heterodinuclear aminopeptidase [30]) [30]
14.9 <4> (4-nitrophenyl phenylphosphonate, <4> pH 8.0, 50°C, Zn^{2+}-enzyme [17]) [17]
15.6 <8> (L-Leu-NH_2, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
15.8 <8> (L-Leu-O-methyl ester, <8> pH 8.0, 37°C, recombinant wild-type enzyme [15]) [15]
16.1 <8> (L-Leu-O-methyl ester, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
24.2 <8> (L-Phe-NH_2, <8> pH 8.0, 37°C, recombinant mutant F221I [15]) [15]
42.7 <8> (L-Leu-NH_2, <8> pH 8.0, 37°C, recombinant mutant F221A [15]) [15]
Additional information <3,8> (<3> kinetics [3,5]; <3> kinetics and thermodynamics [23]; <8> kinetics of recombinant wild-type and F221 mutant enzymes [15]; <3> kinetic analysis, effects of pH, solvent isotope effects, overview [25]; <3> kinetics,enzyme-substrate interaction, overview [26]) [3,5,15,23,25,26]

K_i-Value (mM)

1.1e-005 <3> (amastatin, <3> pH 8.0, 37°C, recombinant enzyme [16]) [16]
1.6e-005 <3> (amastatin, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
0.0005 <3> (L-leucine chloromethyl ketone, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
0.00055 <3> (amastatin, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
0.00079 <8> (amastatin, <8> pH 8.0, 37°C, recombinant enzyme [16]) [16]
0.0019 <3> (L-phenylalanine chloromethyl ketone, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
0.0026 <3> (bestatin, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
0.0039 <3> (bestatin, <3> pH 8.0, 37°C, recombinant enzyme [16]) [16]
0.0053 <3> (L-phenylalanine chloromethyl ketone, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
0.0054 <3> (bestatin, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
0.0055 <3> (L-leucine chloromethyl ketone, <3> pH 8.0, 30°C, in absence of Ca^{2+} [5]) [5]
0.065 <8> (bestatin, <8> pH 8.0, 37°C, recombinant enzyme [16]) [16]
1.1 <3> (fluoride, <3> substrate Leu-4-nitroanilide, in presence of Mn-Mn-homo-dinuclear aminopeptidase [30]) [30]
2.31 <3> (bis(*p*-nitrophenyl)phosphate, <3> pH 5.0, 30°C [25]) [25]
8.7 <3> (L-methionine, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
9.1 <3> (L-methionine, <3> pH 6.5, 30°C, in presence of Ca^{2+} [5]) [5]
10.3 <3> (leucine, <3> pH 8.0, 30°C [25]) [25]
11.4 <3> (fluoride, <3> pH 8.0 [26]) [26]
12.4 <3> (L-leucine, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
12.7 <3> (L-phenylalanine, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
12.8 <3> (L-leucine, <3> pH 6.5, 30°C, in presence of Ca^{2+} [5]) [5]

17 <3> (fluoride, <3> substrate Leu-4-nitroanilide, in presence of Mn-Co-hetero-dinuclear aminopeptidase [30]) [30]
28 <3> (fluoride, <3> substrate Leu-4-nitroanilide, in presence of Co-Co-homo-dinuclear aminopeptidase [30]) [30]
29 <3> (L-leucine, <3> above, pH 8.0, 37°C, recombinant enzyme [16]) [16]
36.6 <3> (D-phenylalanine, <3> pH 8.0, 30°C, in presence of Ca^{2+} [5]) [5]
70 <3> (fluoride, <3> substrate Leu-4-nitroanilide, in presence of Mn-Zn-hetero-dinuclear aminopeptidase [30]) [30]
75 <3> (fluoride, <3> substrate Leu-4-nitroanilide, in presence of Mn-Ni-hetero-dinuclear aminopeptidase [30]) [30]
82 <3> (fluoride, <3> substrate Leu-4-nitroanilide, in presence of Ni-Ni-homo-dinuclear aminopeptidase [30]) [30]
100 <8> (L-leucine, <8> above, pH 8.0, 37°C, recombinant enzyme [16]) [16]
108 <3> (fluoride, <3> substrate Leu-4-nitroanilide, in presence of Zn-Zn-homo-dinuclear aminopeptidase [30]) [30]
Additional information <3> (<3> inhibition kinetics [25]) [25]

pH-Optimum

6.5-8 <3> (<3> assay at [26]) [26]
7 <2,5> (<5> assay at [6]) [6,19,24]
7.8 <3> (<3> assay at [10]) [10]
8 <3,4,6,7,8> (<3,4,6,7,8> assay at [2,3,7,10,15,17,18,22,25]) [2,3,7,10,15,17,18,22,25]
8.4 <3> (<3> about [11]) [11]
8.5 <3,8> [16]
9 <3> [30]

pH-Range

4-10 <3> (<3> bell-shaped pH-profile [25]) [25]
6-8 <2> (<2> pH 6: about 55% of maximal activity, pH 11: about 35% of maximal activity [24]) [24]
6.3-9.5 <3> (<3> at 50 mM Ca^{2+} [20]) [20]
6.3-11.5 <3> (<3> at 0.05 mM Ca^{2+} [20]) [20]
Additional information <3,8> (<3,8> pH profile [16]; <3> pH-dependent activity is altered by Ca^{2+} concentration [20]; <3> a single proton transfer is involved in catalysis at pH 8.0, whereas two proton transfers are implicated at pH 6.5 [26]) [16,20,26]

pi-Value

5.2 <3> (<3> isoelectric focusing [10]; <3> recombinant enzyme, isoelectric focusing and amino acid sequence calculation [10]) [10]

Temperature optimum (°C)

22 <3> (<3> assay at [7]) [7]
24 <3> (<3> assay at [10]) [10]
25 <6> (<6> assay at [2]) [2]
30 <3,4> (<3> assay at [3,18,25,30]; <4> assay at, substrate bis(4-nitrophenyl) phosphate [17]) [3,17,18,25,30]
37 <1,3,7,8> (<1,3,7,8> assay at [10,15,21,22]) [10,15,21,22]

50 <4> (<4> assay at, substrate 4-nitrophenyl phenylphosphonate [17]) [17]
65 <3> (<3> in absence of Ca^{2+} [16]) [16]
70 <5> (<5> assay at [6]) [6]
75 <3,8> (<3> in presence of Ca^{2+} [16]) [16]

Temperature range (°C)
19-56 <3> [26]
20-60 <3> [25]
30-75 <3> (<3> in absence of Ca^{2+} [16]) [16]
30-90 <3,8> (<3> in presence of Ca^{2+} [16]) [16]

4 Enzyme Structure

Molecular weight
21000 <6> (<6> API, gel filtration [2]) [2]
22500 <6> (<6> APII, gel filtration [2]) [2]
26800 <3> (<3> recombinant enzyme, gel filtration [10]) [10]
29000-29730 <3> (<3> native PAGE and mass spectrometry [5]) [5]
29720 <6> (<6> amino acid sequence determination [4]) [4]
30000 <3> (<3> crystal structure determination [8]) [8]
170000 <5> (<5> aminopeptidase II, gel filtration [6]) [6]
290000 <2> (<2> gel filtration [24]) [19,24]
450000 <5> (<5> above, aminopeptidase I, gel filtration [6]) [6]
Additional information <3,6> (<6> enzymes are retarded on the Superose gel [2]; <3> extracellular enzymes show a low MW of 20-30 kDa [14]) [2,14]

Subunits
dimer <1> (<1> the enzyme forms an inactive dimer in the crystal with a large internal cavity with the active sites located at the opposing ends of the cavity essentially inaccessible from the outside [21]) [21]
hexamer <2> (<2> 6 * 48000, SDS-PAGE [24]; <2> 6 * 48000 [19]) [19,24]
monomer <3,6> (<3> 1 * 30000 [13,26]; <6> 1 * 29723, amino acid sequence determination [4]; <3> 1 * 29728-29731, mass spectrometry [5]; <3> 1 * 30000, crystal structure determination [8]; <3> 1 * 30800, recombinant enzyme, SDS-PAGE [10]; <6> 1 * 33000, API, SDS-PAGE, 1 * 34000, APII, SDS-PAGE [2]) [2,4,5,8,10,13,26]

5 Isolation/Preparation/Mutation/Application

Source/tissue
commercial preparation <3,6> (<3> pronase preparation, containing no prolidase acivity [7]; <3,6> protease type XIV i.e. pronase E, a protease mixture form Streptomyces griseus [2,3,5,8]) [2,3,5,7,8]
commercial product <3,6> (<3,6> protease type XIV i.e. pronase E, a protease mixture form Streptomyces griseus [4,9]) [4,9]

Additional information <3> (<3> the enzyme is contained in the pronase enzyme mixture from strain K-1 [20]) [20]

Localization

extracellular <3> (<3> excretion [8]) [1,8,10,14,20]
intracellular <3> [14]

Purification

<1> (recombinant His-tagged enzyme from Escherichia coli strain BL21(DE3) by nickel affinity chromatography, and gel filtration) [21]
<2> [19,24]
<3> (DEAE Sephacel column) [30]
<3> (from commercial product) [5,8]
<3> (from commercial product, 2 peaks API and APII, endopeptidase is eliminated) [2]
<3> (from commercial product, purification of the acetylated enzyme derivatives by DEAE cellulose chromatography) [7]
<3> (recombinant enzyme from Streptomyces lividans cells by diafiltration, hydrophobic interaction chromatography, and gel filtration to homogeneity) [10]
<3> (recombinant from Streptomyces lividans cell culture medium, high purity, 19% recovery) [10]
<3> (recombinant soluble wild-type and mutant enzymes 28fold from Escherichia coli strain BL21(DE3) by heat treatment for 20 min at 50°C and anion exchange chromatography) [18]
<3> (to homogeneity from strain K-1 pronase) [20]
<5> (partial) [6]
<8> (native enzyme by ion exchange chromatography, ammonium sulfate fractionation, and gel filtration, recombinant enzyme from Escherichia coli strain BL21(DE3) culture supernatant by ion exchange chromatography) [16]
<8> (recombinant wild-type and mutant enzymes from Escherichia coli strain Bl21(DE3)) [15]
<9> [29]

Crystallization

<1> (purified recombinant enzyme, sitting drop vapour diffusion method, the reservoir solution contains 0.1 M HEPES/NaOH, pH 7.6, 2.0 M ammonium sulfate, and 2% PEG 400, 4 weeks, X-ray diffraction structure determination and analysis at 1.8 A resolution) [21]
<3> (hanging drop vapour diffusion method, 18-25 mg/ml purified enzyme in 10 mM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM $CaCl_2$, plus an equal volume of sodium acetate buffer at pH 5.0 to 6.0, 16-20% w/v polyethylene glycol 4000, suspended over 1 ml reservoir solution of sodium acetate, pH 5.0-6.0, 16-20% PEG 4000, 3-4 weeks, X-ray diffraction structure determination at 47.2 to 1.9 A resolution and analysis) [8]
<3> (protein with or without bound Zn^{2+} or replaced with Hg^{2+}, hanging drop vapour diffusion method, 20 mg/ml purified enzyme in 10 mM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM $CaCl_2$, plus an equal volume of sodium acet-

ate buffer at pH 5.0 to 6.0, 16-20% w/v polyethylene glycol 4000, suspended over 1 ml reservoir solution of sodium acetate, pH 5.0-6.0, 16-20% PEG 4000, 4-5 weeks to full size crystals, X-ray diffraction structure determination at 2.1 to 1.75 A resolution and analysis) [9]
<3> (purified enzyme complexed with L-methionine, L-phenylalanine, or L-leucine, hanging-drop vapour diffusion method, protein solution: 18 mg/ml protein, 10 mM Tris-HCl, pH 8.0, 20 mM NaCl, 6 mM $CaCl_2$, 100 mM L-methionine or 200 mM L-leucine, plus equal volume of precipitant solution: 24% w/v PEG 4000, 0.1 M ammonium sulfate, equilibrated against 1 ml of reservoir precipitant solution, 3-4 days, cyrstals of enzyme complexed with L-Phe were precipitated with 0.1 M acetate buffer, pH 5.5 instead in the same procedure within 8-10 weeks, X-ray complex structure determination at 1.6 A resolution and analysis) [11]
<3> (purified enzyme complexed with methionine, hanging-drop vapour diffusion method, protein solution: 18 mg/ml protein, 10 mM Tris-HCl, pH 8.0, 20 mM NaCl, 6 mM $CaCl_2$, 0.1 M methionine, plus equal volume of precipitant solution: 24% w/v PEG 4000, 0.1 M ammonium sulfate, equilibrated against 1 ml reservoir of the precipitant solution, 3-4 days, X-ray diffraction structure determination at 1.53 A high resolution and analysis) [1]
<3> (purified enzyme in complex with tryptophan or 4-iodo-L-phenylalanine, hanging drop vapour diffusion method, 18 mg/ml protein in 10 mM Tris-HCl, pH 8.0, 20 mM NaCl, 6 mM $CaCl_2$, and 100 mM tryptophan or 2 mM 4-iodo-L-phenylalanine, equal volumes of protein and reservoir solution are mixed, the latter containing 18% w/v PEG 4000 and 0.1 M sodium acetate, pH 5.5, equilibration against 1 ml reservoir solution for 1 day, microseeding, 3-4 days, X-ray diffraction structure determination and analysis at 1.3 A resolution) [12]
<3> (purified native enzyme in complex with product analogous weak inhibiting amino acids phenylalanine, leucine, and methionine, hanging drop vapour diffusion method, 18 mg/ml protein in 10 mM Tris-HCl, pH 8.0, 20 mM NaCl, 6 mM $CaCl_2$, and 100 mM L-methionine or 200 mM L-leucine, the precipitant solution contains 24% w/v PEG 4000 and 0.1 M ammonium sulfate, equilibration against 1 ml reservoir solution, microseeding, 3-4 days, with phenylalanine a protein solution containing 18 mg/ml protein, 10 mM Tris-HCl, pH 8.0, 20 mM NaCl, 6 mM $CaCl_2$, and 100 mM Phe is mixed with a reservoir solution containing 18% w/v PEG 4000, and 0.1 M acetate buffer, pH 5.5, microseeding, 3-4 days, X-ray diffraction structure determination and analysis at 1.8 A, 1.7 A, and 1.53 A resolution, respectively, structure modelling) [13]
<3> (structure determination and analysis) [20]

Cloning

<1> (expression of the His-tagged enzyme in Escherichia coli strain BL21(DE3)) [21]
<3> (DNA and amino acid sequence determination and analysis) [20]
<3> (DNA and amino acid sequence determination, subcloning and expression of the recombinant enzyme possessing a Asp70 and Asp184 residues in

Escherichia coli strain BL21(DE3), the soluble enzyme is inducible by growth on 1 M sorbitol, optimization of the expression method) [18]
<3> (expression in Streptomyces lividans by insertional cloning and protoplast transformation, the expression system contains the constitutive Streptomyces fradiae aph promoter) [10]
<3> (gene SGAP, standard protoplast transformation and expression in Streptomyces lividans, excretion to the medium) [10]
<3> (gene Sgap, DNA and amino acid sequence determination and analysis, overexpression as secreted enzyme in Escherichia coli) [16]
<7> (gene Sgap, screening of 21 strains with strain NBRC12875 showing the highest activity) [22]
<8> (expression of wild-type and mutant enzymes in Escherichia coli strain BL21(DE3)) [15]
<8> (gene Ssap, genomic library screening, DNA and amino acid sequence determination and analysis, overexpression as secreted enzyme in Escherichia coli strain BL21(DE3)) [16]
<9> (expression in Escherichia coli BL21) [27,29]

Engineering

D3A/D262G <7> (<7> site-directed mutagenesis, the mutant shows increased activity compared to the wild-type enzyme [22]) [22]
E131 <3> (<3> site-directed mutagenesis, the mutant enzyme shows reduced activity compared to the wild-type enzyme [18]) [18]
E131D <3> (<3> a general acid-base mutant, thermodynamic parameters for the reaction are similar to the wild-type enzyme, but the k_{cat} of the mutant is 4fold reduced, while the activation energy is elevated compared to the wild-type enzyme [26]) [26]
E196A <7> (<7> site-directed mutagenesis, the mutant shows reduced activity compared to the wild-type enzyme [22]) [22]
F221A <8> (<8> site-directed mutagenesis, the mutant shows altered substrate specificity compared to the wild-type enzyme [15]) [15]
F221G <8> (<8> site-directed mutagenesis, the mutant shows altered substrate specificity compared to the wild-type enzyme [15]) [15]
F221S <8> (<8> site-directed mutagenesis, the mutant shows altered substrate specificity compared to the wild-type enzyme [15]) [15]
S502C <9> (<9> mutant without aminopeptidase activity but with peptide synthesis activity [28]) [28]
Y246 <3> (<3> site-directed mutagenesis, the mutant enzyme shows highly reduced activity compared to the wild-type enzyme [18]) [18]
Additional information <7,8> (<8> alteration of leucine aminopeptidase to phenylalanine aminopeptidase by site-directed mutagenesis [15]; <7> construction of chimeric enzymes from the enzymes of Streptomyces griseus and Streptomyces septatus by DNA in vivo shuffling and site-directed mutagenesis for calcium activation and binding studies, overview [22]) [15,22]

Application

analysis <7> (<7> the enzyme is clinically important as a model for understanding the structure and mechanism of action of other metallopeptidases [22]) [22]
biotechnology <3> (<3> enzyme is attractive for diverse applications, e.g. the processing of recombinant DNA proteins and fusion protein production due to its heat stability, high activity, and small size [11]; <3> the enzyme is useful in many biotechnological applications e.g. in processing of recombinant DNA, proteins, and fusion protein products [13]) [11,13]

6 Stability

Temperature stability

40-60 <8> (<8> 30 min, recombinant enzyme, stable [16]) [16]
50 <3> (<3> 20 min, stable [18]) [18]
69 <3,6> (<3> recombinant enzyme, stable at for at least 1 h [10]; <6> stable during heating for 5 h at 69°C as part of the purification process [2]) [2,10]
70 <3> (<3> stable at [5]; <3> 30 min, 50% loss of activity, in absence of Ca^{2+} [16]; <3> stable at pH 6.0-10.5 [20]) [5,16,20]
75 <3> (<3> rate constant for inactivation Kin is 0.00028/s in absence of Ca^{2+}, and 0.000025/s in presence of Ca^{2+} [5]) [5]
78 <8> (<8> 30 min, recombinant enzyme, 50% loss of activity [16]) [16]
80 <3> (<3> 30 min, 50% loss of activity, in presence of Ca^{2+} [16]) [16]
90 <5> (<5> stable at for 15 min, pH 7.0 [6]) [6]
95 <9> (<9> enzyme inactivation after 30 min at 95°C [28]) [28]
Additional information <3,8> (<3> the enzyme is heat-stable [13]) [13,16]

General stability information

<3>, Ca^{2+} stabilizes [5]

Storage stability

<2>, 4°C, 50% loss of activity after 4 days, 80% loss of activity after 8 days. Remains active for 2 months, when concentrated and kept in a suspension with 4 M ammonium sulfate in 20 mM MOPS/Tris, pH 7 [24]
<3>, -20°C, liquid and freeze-dried, stable for at least 8 weeks, no loss of activity [10]

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angiotensin-converting enzyme 2 3.4.17.23

1 Nomenclature

EC number
3.4.17.23

Recommended name
angiotensin-converting enzyme 2

Synonyms
ACE <4> [12]
ACE 2 <10,12,13> [74]
ACE-2 <2,3,4,9> [38,68]
ACE-related carboxypeptidase <9> [3]
ACE2 <1,2,3,4,5,6,9,10,11,12,13> [1,2,3,5,6,9,14,15,16,17,19,20,22,25,26,30,37,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,65,66,67,68,69,70,71,72,73,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97]
ACE2 homologue <2> [41]
ACEH <9> [7]
Ang converting enzyme 2 <10,12,13> [74]
angiotensin II converting enzyme 2 <3> [81]
angiotensin converting enzyme 2 <2,3,4,6,9,10,11,12,13> (<3> functions as a carboxypeptidase [41]) [14,41,43,46,47,51,55,57,58,60,63,74,78,84]
angiotensin converting enzyme II <9> [95]
angiotensin converting enzyme-2 <2,3,4> [38]
angiotensin-converting enzyme <4,9> [22]
angiotensin-converting enzyme 2 <4,8,9,10,12,13> [10,19,67,68,70,76]
angiotensin-converting enzyme homolog <9> [7]
angiotensin-converting enzyme homologue <9> [6]
angiotensin-converting enzyme type 2 <13> [79]
angiotensin-converting enzyme-2 <3,4> [37,53,97]
angiotensin-converting enzyme-like protein <9> [7]
angiotensin-converting enzyme-related carboxypeptidase <9> [1,6]
angiotensinase <9> [4]
hACE2 <3,10,13> [36,67,74]

CAS registry number
328404-18-8

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2 Source Organism

<1> *Cricetulus griseus* [83]
<2> *Mus musculus* [26,33,34,38,41,44,45,47,50,51,56,83,86,89,93]
<3> *Homo sapiens* [15,16,17,21,24,26,28,29,30,31,32,35,36,38,40,41,42,49,52,53,58,61,81,84,85,91,92,94]
<4> *Rattus norvegicus* [5,12,13,14,19,20,22,23,25,27,37,38,39,44,46,54,55,59,62,82,87,88,90,93,96,97]
<5> *Sus scrofa* [75]
<6> *Oryctolagus cuniculus* [48,60,90]
<7> *Chlorocebus aethiops* [18]
<8> *Rhipicephalus microplus* (UNIPROT accession number: Q17248) [10]
<9> *Homo sapiens* (UNIPROT accession number: Q9BYF1) [1,2,3,4,6,7,8,9,11,22,43,63,68,69,72,77,80,95]
<10> *Mus musculus* (UNIPROT accession number: Q8R0I0) [57,63,67,74]
<11> *Felis silvestris* (UNIPROT accession number: Q56H28) [63]
<12> *Rattus norvegicus* (UNIPROT accession number: Q5EGZ1) [63,65,66,70,71,74,77]
<13> *Homo sapiens* (UNIPROT accession number: Q9FYF1) [64,72,73,74,76,78,79]

3 Reaction and Specificity

Catalyzed reaction

angiotensin II + H_2O = angiotensin-(1-7) + L-phenylalanine (<3> a transmembrane glycoprotein with an extracellular catalytic domain. ACE2 functions as a carboxypeptidase, cleaving a single C-terminal residue from a distinct range of substrates [41]; <9> ACE2 catalytic efficiency is 400-fold higher with angiotensin II (1-8) as a substrate than with angiotensin I (1-10). ACE2 also efficiently hydrolyzes des-Arg9-bradykinin, but it does not hydrolyze bradykinin [8])

Reaction type

hydrolysis of peptide bond

Natural substrates and products

S angiotensin I + H_2O <9> (Reversibility: ?) [95]
P angiotensin(1-9) + L-Phe
S angiotensin I + H_2O <4,8,9> (<4> ACE2 contributes to the production of angiotensin(1-7) from angiotensin I in proximal straight tubule [14]) (Reversibility: ?) [1,2,3,5,6,7,8,9,10,11,14]
P angiotensin-(1-9) + Leu
S angiotensin II + H_2O <2,3,4,6,9> (<3> ACE2, a homologue of ACE, EC 3.4.15.1, converts angiotensin II into Ang(1-7). Ang(1-7) shows vasoprotective effects, serum autoantibodies to ACE2 predispose patients with connective tissue diseases to constrictive vasculopathy, pulmonary arter-

ial hypertension, or persistent digital ischemia [85]; <2,4> angiotensin II has many adverse cardiovascular effects when acting through the AT1 receptor [93]; <4> high levels of angiotensin II induces pulmonary arterial hypertension [97]) (Reversibility: ?) [85,86,87,88,89,90,91,93,95,96,97]

P angiotensin(1-7) + L-Phe (<2> Ang(1-7) is a vasodilator peptide [89]; <9> Ang-(1-7) is a potential endogenous inhibitor of the classical renin-angiotensin system cascade [95])

S angiotensin II + H_2O <3> (<3> the enzyme is involved in the renin angiotensin system [81]) (Reversibility: ?) [81]

P angiotensin-(1-7) + L-Phe

S angiotensin II + H_2O <3,4,12,13> (<4> ACE2 is highly regulated at transcription. ACE2 plays a critical role in regulating the balance between vasoconstrictor and vasodilator effects within the RAS cascade. Angiotensin II may be a stimulus determining cardiac ACE2 gene expression, because either reduction in its levels or prevention of angiotensin II binding to the AT1 receptor increases ACE2 mRNA. ACE2 serves as the cellular entry point for severe acute respiratory syndrome (SARS) virus [27]; <3> the uteroplacental location of angiotensin (1-7) and ACE2 in pregnancy suggests an autocrine function of angiotensin(1-7) in the vasoactive regulation that characterizes placentation and establishes pregnancy [35]; <12> hepatic production of Ang-(1-7) is catalysed by ACE2 [65]; <13> the major role of ACE2 in Ang peptides metabolism is the production of Ang-(1-7). ACE2 also participates in the metabolism of other peptides non related to the renin-angiotensin system: apelin-13, neurotensin, kinetensin, dynorphin, [des-Arg9]-bradykinin, and [Lys-des-Arg9]-bradykinin [74]) (Reversibility: ?) [27,35,65,74]

P angiotensin-(1-7) + Phe

S Additional information <2,3,4,7,9,10,12,13> (<2> ACE2 is a crucial SARS-CoV receptor. SARS-CoV infections and the Spike protein of the SARS-CoV reduce ACE2 expression. Injection of SARS-CoV Spike into mice worsens acute lung failure in vivo that can be attenuated by blocking the renin-angiotensin pathway [33]; <7> angiotensin-converting enzyme 2: a functional receptor for SARS coronavirus [18]; <3> presence of ACE2 alone is not sufficient for maintaining viral infection. Other virus receptors or coreceptors may be required in different tissues [32]; <3> the enzyme has a function in blood pressure regulation, blood flow and fluid regulation. Loss of ACE2 impairs heart function [17]; <3> the enzyme is involved in diesease condition including hypertension, diabetes and cardiac function. ACE2 is the SARS virus receptor [16]; <9> ACE2 ectodomain shedding and/or sheddase(s) activation regulated by calmodulin is independent from the phorbol ester-induced shedding [68]; <13> ACE2 is down-regulated and ACE is up-regulated in hypertensive nephropathy. Ang II, once released, can act to up-regulate ACE but down-regulate ACE2 via the AT1 receptor-mediated mechanism. Activation of the ERK1/2 and p38 MAP kinase pathway may represent a key mechanism by which Ang II down-regulates ACE2 [64]; <9> ACE2 is involved in the regulation of heart function, ACE 2 is a functional receptor for the coro-

navirus that causes the severe acute respiratory syndrome [72]; <12> ACE2 plays a crucial role in liver fibrogenesis [71]; <13> ACE2 plays a key role in pulmonary, cardiovascular and hypertensive and diabetic kidney diseases. ACE2 plays a pivotal role in maintaining a balanced status of the RAS synergistically with ACE by exerting counter-regulatory effects [78]; <10> ACE2 plays a pivotal role in the central regulation of blood pressure and volume homeostasis [67]; <13> ACE2 plays a protective role in organs directly related to hypertension and associated diseases [73]; <13> the affinity for Ang-I is poor in comparison with ACE, therefore the conversion of Ang-I to Ang-(1-9) is not of physiological importance, except maybe under conditions in which ACE activity is inhibited [74]; <2,4> ACE2 activation promotes antithrombotic activity. ACE2 is an ACE, EC 3.4.15.1, homologue [93]; <3> ACE2 is a terminal carboxypeptidase and the receptor for the SARS and NL63 coronaviruses. Soluble sACE2 acts as receptor binding SARS-CoV glycoprotein S pseudo-typed FIV virus and blocks virus infection of target cells [84]) (Reversibility: ?) [16,17,18,32,33,64,67,68,71,72,73,74,78,84,93]

P ?

Substrates and products

S (7-methoxycoumarin-4-yl)-YVADAPK-(2,4-dinitrophenyl)-OH + H_2O <4> (Reversibility: ?) [38]

P (7-methoxycoumarin-4-yl)-YVADAP + N^6-(2,4-dinitrophenyl)-L-lysine

S (7-methoxycoumarin-4-yl)-acetyl-APK(2,4-dinitrophenyl) + H_2O <3> (Reversibility: ?) [49,53]

P (7-methoxycoumarin-4-yl)-acetyl-AP + N^6-(2,4-dinitrophenyl)-L-Lys

S (7-methoxycoumarin-4-yl)-acetyl-APK(2,4-dinitrophenyl)-OH + H_2O <4> (Reversibility: ?) [39]

P (7-methoxycoumarin-4-yl)-acetyl-AP + N^6-(2,4-dinitrophenyl)-L-Lys

S (7-methoxycoumarin-4-yl)-acetyl-Ala-Pro-Lys(2,4-dinitrophenyl) + H_2O <3> (Reversibility: ?) [52]

P (7-methoxycoumarin-4-yl)-acetyl-Ala-Pro + N^6-(2,4-dinitrophenyl)-L-Lys

S (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH + H_2O <3> (Reversibility: ?) [42]

P (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro + N^6-(2,4-dinitrophenyl)-L-Lys

S (7-methoxycoumarin-4-yl)-acetyl-YVADAPK-(2,4-dinitrophenyl)-OH + H_2O <2,3> (Reversibility: ?) [38]

P (7-methoxycoumarin-4-yl)-acetyl-YVADAP + N^6-(2,4-dinitrophenyl)-L-Lys

S (7-methoxycoumarin-4-yl)acetyl-APK(2,4-dinitrophenyl) + H_2O <3,4,9> (Reversibility: ?) [22,58]

P (7-methoxycoumarin-4-yl)acetyl-AP + N^6-(2,4-dinitrophenyl)-L-lysine

S (7-methoxycoumarin-4-yl)acetyl-APK(2,4-dinitrophenyl)-OH + H_2O <9> (<9> synthetic fluorogenic substrate [2,8]) (Reversibility: ?) [2,8]

P (7-methoxycoumarin-4-yl)acetyl-AP + N^6-(2,4-dinitrophenyl)-L-lysine

S (7-methoxycoumarin-4-yl)acetyl-APK-(2,4-dinitrophenyl)-OH + H_2O <3> (Reversibility: ?) [85]
P ?
S (7-methoxycoumarin-4-yl)acetyl-APK-2,4-dinitrophenyl + H_2O <3> (Reversibility: ?) [24]
P (7-methoxycoumarin-4-yl)acetyl-AP + N^6-(2,4-dinitrophenyl)-L-lysine
S (7-methoxycoumarin-4-yl)acetyl-Ala-Pro-Lys(2,4-dinitrophenyl) + H_2O <3> (Reversibility: ?) [30,36]
P (7-methoxycoumarin-4-yl)acetyl-Ala-Pro + N^6-(2,4-dinitrophenyl)-L-lysine
S (7-methoxycoumarin-4-yl)acetyl-YVADAPK(2,4-dinitrophenyl)-OH + H_2O <9> (<9> synthetic fluorogenic caspase-1 substrate [8,9]) (Reversibility: ?) [8,9]
P (7-methoxycoumarin-4-yl)acetyl-YVADAP + N^6-(2,4-dinitrophenyl)-L-lysine
S (des-Arg9)-bradykinin + H_2O <3> (Reversibility: ?) [41]
P ?
S 7-methoxycoumarin-4-acetyl-Ala-Pro-Lys-(2,4-dinitrophenyl)-OH + H_2O <4> (Reversibility: ?) [82]
P ?
S 7-methoxycoumarin-4-acetyl-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys-(2,4-dinitrophenyl)-OH + H_2O <2,4> (Reversibility: ?) [93]
P ?
S 7-methoxycoumarin-4-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys-(2,4-dinitrophenyl)-OH + H_2O <2,4> (Reversibility: ?) [93]
P ?
S KRPPGSPF + H_2O <9> (<9> i.e. Lys-des-Arg-bradykinin [8]) (Reversibility: ir) [8]
P KRPPGSP + Phe
S Lys-des-Arg9 bradykinin + H_2O <3> (Reversibility: ?) [16,17]
P KRPPGFSP + Phe
S Lys-des-Arg9-bradykinin + H_2O <2,4> (Reversibility: ?) [44]
P ?
S RPPGSPF + H_2O <9> (<9> i.e. des-Arg-bradykinin [1,8]) (Reversibility: ir) [1,8]
P RPPGSP + Phe (<9> i.e. des-Arg-bradykinin-(1-7) [1,8])
S SARS-coronavirus S_1 protein + H_2O <9,10,11,12> (Reversibility: ?) [63]
P ?
S TBC5046 + H_2O <9> (<9> synthetic fluorogenic peptide, i.e. des-Arg-bradykinin with N-terminal *o*-aminobenzoic acid and a 3-nitrophenylalanine instead of Phe at the C-terminus [1]) (Reversibility: ir) [1]
P *o*-aminobenzoic acid-des-Arg-bradykinin-(1-7) + 3-nitrophenylalanine
S YPVEPFI + H_2O <9> (<9> i.e. β-casomorphin [8]) (Reversibility: ir) [8]
P YPVEPF + Ile
S angiotensin I + H_2O <9> (Reversibility: ?) [95]
P angiotensin(1-9) + L-Phe

S angiotensin I + H_2O <2,3,4,8,9,10,13> (<9> C-terminal bond between His-Leu is cleaved [6]; <9> no angiotensin-converting activity, i.e. no conversion of the decapeptide angiotensin I to the octapeptide angiotensin II [3]; <9> wild-type and truncated mutant [7]; <4> ACE2 contributes to the production of angiotensin(1-7) from angiotensin I in proximal straight tubule [14]; <3> poor affinity [41]; <13> the affinity for Ang-I is poor in comparison with ACE, therefore the conversion of Ang-I to Ang-(1-9) is not of physiological importance, except maybe under conditions in which ACE activity is inhibited [74]) (Reversibility: ?) [1,2,3,4,5,6,7,8,9,10,11,14,15,16,17,41,43,44,45,46,50,53,57,69,74,78]

P angiotensin-(1-9) + Leu

S angiotensin II + H_2O <2,3,4,6,9> (<2,4> i.e. Asp-Arg-Val-Tyr-Ile-His-Pro-Phe [87,93]; <3> ACE2, a homologue of ACE, EC 3.4.15.1, converts angiotensin II into Ang(1-7). Ang(1-7) shows vasoprotective effects, serum autoantibodies to ACE2 predispose patients with connective tissue diseases to constrictive vasculopathy, pulmonary arterial hypertension, or persistent digital ischemia [85]; <2,4> angiotensin II has many adverse cardiovascular effects when acting through the AT1 receptor [93]; <4> high levels of angiotensin II induces pulmonary arterial hypertension [97]; <4> i.e. Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, detection of myocardial ACE2 activity by surface enhanced laser desorption lionization time of flight mass spectroscopy, SELDI-TOF-MS [88]) (Reversibility: ?) [85,86,87,88,89,90,91,93,95,96,97]

P angiotensin(1-7) + L-Phe (<2> Ang(1-7) is a vasodilator peptide [89]; <9> Ang-(1-7) is a potential endogenous inhibitor of the classical renin-angiotensin system cascade [95]; <2,4> i.e. Asp-Arg-Val-Tyr-Ile-His-Pro [87,88,93])

S angiotensin II + H_2O <3> (<3> the enzyme is involved in the renin angiotensin system [81]) (Reversibility: ?) [81]

P angiotensin-(1-7) + L-Phe

S angiotensin II + H_2O <2,3,4,9,10,12,13> (<9> preferred substrate [4]; <3> efficient cleavage [41]; <9> 400fold higher activity than with angiotensin I [8]; <9> wild-type and truncated mutant [7]; <4> ACE2 is highly regulated at transcription. ACE2 plays a critical role in regulating the balance between vasoconstrictor and vasodilator effects within the RAS cascade. Angiotensin II may be a stimulus determining cardiac ACE2 gene expression, because either reduction in its levels or prevention of angiotensin II binding to the AT1 receptor increases ACE2 mRNA. ACE2 serves as the cellular entry point for severe acute respiratory syndrome (SARS) virus [27]; <3> the uteroplacental location of angiotensin (1-7) and ACE2 in pregnancy suggests an autocrine function of angiotensin(1-7) in the vasoactive regulation that characterizes placentation and establishes pregnancy [35]; <10> primary substrate [57]; <12> hepatic production of Ang-(1-7) is catalysed by ACE2 [65]; <13> the major role of ACE2 in Ang peptides metabolism is the production of Ang-(1-7). ACE2 also participates in the metabolism of other peptides non related to the renin-angiotensin system: apelin-13, neurotensin, kinetensin, dynorphin, [des-

Arg9]-bradykinin, and [Lys-des-Arg9]-bradykinin [74]; <13> ACE2 has approximately a 400fold greater affinity for Ang-II than Ang-I [74]) (Reversibility: ?) [2,4,7,8,15,16,17,22,26,27,35,37,38,39,40,41,42,43,44,45,46,47,49,50,51,52,53,54,55,56,57,58,59,61,62,65,69,74,78]

P angiotensin-(1-7) + Phe
S angiotensin IV + H_2O <3> (Reversibility: ?) [16]
P VYIHP + Phe
S angiotensin-(3-8) + H_2O <9> (Reversibility: ir) [2]
P angiotensin-(3-7) + Phe
S angiotensin-(4-8) + H_2O <9> (Reversibility: ir) [2]
P angiotensin-(4-7) + Phe
S angiotensin-(5-8) + H_2O <9> (Reversibility: ir) [2]
P angiotensin-(5-7) + Phe
S apelin-13 + H_2O <3,4> (Reversibility: ?) [16,17,46]
P QRPRLSHKGPMP + Phe
S apelin-13 + H_2O <9> (Reversibility: ?) [8]
P apelin-12 + Phe
S apelin-13 + H_2O <2,3,4> (<2,4> high catalytic efficiency [44]) (Reversibility: ?) [41,44]
P ?
S apelin-36 + H_2O <4,9> (Reversibility: ?) [8,46]
P apelin-35 + Phe
S apelin-36 + H_2O <2,3,4> (<2,4> high catalytic efficiency [44]) (Reversibility: ?) [16,17,44]
P ?
S β-casomorphin + H_2O <3> (Reversibility: ?) [16,17]
P YPFVEP + Ile
S β-casomorphin + H_2O <2,4> (Reversibility: ?) [44]
P ?
S casomorphin + H_2O <4> (Reversibility: ?) [46]
P ?
S des-Arg10-Lys-bradykinin + H_2O <4> (Reversibility: ?) [46]
P KRPPGFSP + Phe
S des-Arg9-bradykinin + H_2O <3> (Reversibility: ?) [16,17]
P RPPGFSP + Phe
S des-Arg9-bradykinin + H_2O <2,4> (Reversibility: ?) [44]
P ?
S des-Arg9-bradykinin + H_2O <4> (Reversibility: ?) [46]
P bradykinin (1-7) + Phe
S dynorphin A + H_2O <2,4> (Reversibility: ?) [44,46]
P ?
S dynorphin A 1-13 + H_2O <9> (Reversibility: ir) [8]
P dynorphin A 1-12 + Lys
S dynorphin A(1-13) + H_2O <3> (Reversibility: ?) [16,17]
P YGGFLRRIRPKL + Lys
S ghrelin + H_2O <3> (Reversibility: ?) [16]
P ?

S ghrelin + H_2O <9> (Reversibility: ir) [8]
P ghrelin minus C-terminal amino acid + arginine
S kinetensin + H_2O <4> (Reversibility: ?) [46]
P ?
S neocasomorphin + H_2O <9> (Reversibility: ir) [8]
P neocasomorphin minus C-terminal amino acid + isoleucine
S neurotensin + H_2O <2,4> (Reversibility: ?) [44]
P ?
S neurotensin 1-13 + H_2O <4> (Reversibility: ?) [46]
P ?
S neurotensin(1-11) + H_2O <3> (Reversibility: ?) [16]
P pELYENKPRRP + Tyr
S neurotensin(1-8) + H_2O <3> (Reversibility: ?) [16]
P pELYENKP + Arg
S neurotensin-(1-8) + H_2O <9> (Reversibility: ir) [8]
P neurotensin-(1-7) + arginine
S Additional information <2,3,4,7,9,10,12,13> (<2> ACE2 is a crucial SARS-CoV receptor. SARS-CoV infections and the Spike protein of the SARS-CoV reduce ACE2 expression. Injection of SARS-CoV Spike into mice worsens acute lung failure in vivo that can be attenuated by blocking the renin-angiotensin pathway [33]; <7> angiotensin-converting enzyme 2: a functional receptor for SARS coronavirus [18]; <3> presence of ACE2 alone is not sufficient for maintaining viral infection. Other virus receptors or coreceptors may be required in different tissues [32]; <3> the enzyme has a function in blood pressure regulation, blood flow and fluid regulation. Loss of ACE2 impairs heart function [17]; <3> the enzyme is involved in diesease condition including hypertension, diabetes and cardiac function. ACE2 is the SARS virus receptor [16]; <3> angiotensin I is not a good substrate for recombinant human ACE2 [26]; <3> no activity with angiotensin (1-9) and angiotensin(1-7) [15]; <3> no hydrolysis of angiotensin (1-9), angiotensin (1-7), bradikinin, bradykinin(1-7), neurotensin(1-13) [16]; <2,4> ACE2 functions as a carboxymonopeptidase with a preference for C-terminal Leu or Phe, ACE2 counterbalances the enzymatic actions of ACE, ACE2 does not metabolize bradykinin [44]; <3> the ACE2 ectodomain can be cleaved from the cell membrane and released into the extracellular milieu by stimulation of phorbol esters and ADAM17, calmodulin inhibits shedding of the ACE2 ectodomain from the membrane [53]; <9> ACE2 ectodomain shedding and/or sheddase(s) activation regulated by calmodulin is independent from the phorbol ester-induced shedding [68]; <13> ACE2 is down-regulated and ACE is up-regulated in hypertensive nephropathy. Ang II, once released, can act to up-regulate ACE but down-regulate ACE2 via the AT1 receptor-mediated mechanism. Activation of the ERK1/2 and p38 MAP kinase pathway may represent a key mechanism by which Ang II down-regulates ACE2 [64]; <9> ACE2 is involved in the regulation of heart function, ACE 2 is a functional receptor for the coronavirus that causes the severe acute respiratory syndrome [72]; <12> ACE2 plays a crucial role in liver fibrogenesis [71];

<13> ACE2 plays a key role in pulmonary, cardiovascular and hypertensive and diabetic kidney diseases. ACE2 plays a pivotal role in maintaining a balanced status of the RAS synergistically with ACE by exerting counter-regulatory effects [78]; <10> ACE2 plays a pivotal role in the central regulation of blood pressure and volume homeostasis [67]; <13> ACE2 plays a protective role in organs directly related to hypertension and associated diseases [73]; <13> the affinity for Ang-I is poor in comparison with ACE, therefore the conversion of Ang-I to Ang-(1-9) is not of physiological importance, except maybe under conditions in which ACE activity is inhibited [74]; <13> ACE2 functions predominantly as a carboxymonopeptidase with a substrate preference for hydrolysis between proline and a hydrophobic or basic C-terminal residue [78]; <13> hydrolyses its substrates by removing a single amino acid from their respective C-terminal [74]; <2,4> ACE2 activation promotes antithrombotic activity. ACE2 is an ACE, EC 3.4.15.1, homologue [93]; <3> ACE2 is a terminal carboxypeptidase and the receptor for the SARS and NL63 coronaviruses. Soluble sACE2 acts as receptor binding SARS-CoV glycoprotein S pseudotyped FIV virus and blocks virus infection of target cells [84]) (Reversibility: ?) [15,16,17,18,26,32,33,44,53,64,67,68,71,72,73,74,78,84,93]

P ?

Inhibitors

(2S)-3-(biphenyl-4-yl)-2-((3S)-2-mercapto-3-methylpentanamido)propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[(2-methyl-2-sulfanylpropanoyl)amino]propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[(2-sulfanylpropanoyl)amino]propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[(sulfanylacetyl)amino]propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[[(2R)-2-sulfanylbutanoyl]amino]propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[[(2R)-3-methyl-2-sulfanylbutanoyl]amino]propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[[(2R)-3-phenyl-2-sulfanylpropanoyl]amino]propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[[(2S)-2-phenyl-2-sulfanylacetyl]amino]propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[[(2S)-2-sulfanylhexanoyl]amino]propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[[(2S)-3-phenyl-2-sulfanylpropanoyl]amino]propanoic acid <3> [42]
(2S)-3-biphenyl-4-yl-2-[[cyclobutyl(sulfanyl)acetyl]amino]propanoic acid <3> [42]
(S)-3-(biphenyl-4-yl)-2-((2R,3R)-2-mercapto-3-methylpentanamido)propanoic acid <3> [42]
(S)-3-(biphenyl-4-yl)-2-((R)-2-cyclohexyl-2-mercaptoacetamido)propanoic acid <3> [42]

(S)-3-(biphenyl-4-yl)-2-((R)-2-cyclopentyl-2-mercaptoacetamido)propanoic acid <3> [42]
(S)-3-(biphenyl-4-yl)-2-((R)-2-mercapto-3-(naphthalen-2-yl)propanamido)propanoic acid <3> [42]
(S)-3-(biphenyl-4-yl)-2-((R)-2-mercapto-4,4-dimethylpentanamido)propanoic acid <3> [42]
(S)-3-(biphenyl-4-yl)-2-((R)-2-mercapto-4-methylpentanamido)propanoic acid <3> [42]
(S)-3-(biphenyl-4-yl)-2-((R)-2-mercapto-4-phenylbutanamido)propanoic acid <3> [42]
(S)-3-(biphenyl-4-yl)-2-((R)-3-cyclohexyl-2-mercaptopropanamido)propanoic acid <3> [42]
(S,S)-2-[1-carboxy-2-[3-(3,5-dichlorobenzyl)-3H-imidazol-4-yl]-ethylamino]-4-methylpentanoic acid <3> (<3> MLN-4760 [41]) [41]
(S,S)-2-{1-carboxy-2-[3-(3,5-dichlorobenzyl)-3H-imidazol-4-yl]-ethylamino}-4-methylpentanoic acid <13> (<13> i.e MLN-4760 [72]) [72]
1,3,8-trihydroxy-6-methylanthraquinone <3> (<3> 1,3,8-trihydroxy-6-methylanthraquinone (emodin) blocks interaction between the SARS corona virus spike protein and its receptor angiotensin-converting enzyme 2, 94.12% inhibition at 0.05 mM [40]) [40]
1,4-bis-(1-anthraquinonylamino)-anthraquinone <3> (<3> slight inhibition [40]) [40]
1,8,dihydroxy-3-carboxyl-9,10-anthraquinone <3> (<3> 1,8,dihydroxy-3-carboxyl-9,10-anthraquinone (rhein) exhibits slight inhibition [40]) [40]
1N-08795 <3> (<3> 90% inhibition at 0.2 mM [58]) [58]
1N-26923 <3> (<3> 93% inhibition at 0.2 mM [58]) [58]
1N-27714 <3> (<3> 89% inhibition at 0.2 mM [58]) [58]
1N-28616 <3> (<3> 93% inhibition at 0.2 mM [58]) [58]
1S-90995 <3> (<3> 11% inhibition at 0.2 mM [58]) [58]
1S-91206 <3> (<3> 75% inhibition at 0.2 mM [58]) [58]
2-[(2-carboxy-3-phenyl-propyl)-hydroxy-phosphinoyl]-pyrrolidine-1-carboxylic acid benzyl ester <3> [61]
2-[(2-carboxy-4-methyl-pentyl)-hydroxy-phosphinoyl]-pyrrolidine-1-carboxylic acid benzyl ester <3> [61]
2-[(2-carboxy-propyl)-hydroxy-phosphinoyl]-pyrrolidine-1-carboxylic acid benzyl ester <3> [61]
2-benzyl-3-(hydroxy-pyrrolidin-2-yl-phosphinoyl)-propionic acid <3> [61]
2-benzyl-3-[(1-benzyloxycarbonylamino-2-phenyl-ethyl)-hydroxy-phosphinoyl]-propionic acid <3> [61]
2-benzyl-3-[(1-benzyloxycarbonylamino-3-methyl-butyl)-hydroxy-phosphinoyl]-propionic acid <3> [61]
2-benzyl-3-[(1-benzyloxycarbonylamino-ethyl)-hydroxy-phosphinoyl]-propionic acid <3> [61]
2-methylphenyl-benzylsuccinic acid <9> [6]
3,4-dimethylphenyl-benzylsuccinic acid <9> [6]
3,5-dichloro-benzylsuccinate <9> [6]
3,5-dimethylphenyl-benzylsuccinic acid <9> [6]

3-([1-[2-acetylamino-3-(1H-imidazol-4-yl)-propionyl]-pyrrolidin-2-yl]-hydroxy-phosphinoyl)-2-(3-phenyl-isoxazol-5-ylmethyl)-propionic acid <3> [61]
3-([1-[2-acetylamino-3-(1H-imidazol-4-yl)-propionyl]-pyrrolidin-2-yl]-hydroxy-phosphinoyl)-2-benzyl-propionic acid <3> [61]
3-([1-[2-acetylamino-3-(1H-imidazol-4-yl)-propionylamino]-3-methyl-butyl]-hydroxy-phosphinoyl)-2-(3-phenyl-isoxazol-5-ylmethyl)-propionic acid <3> [61]
3-([1-[2-acetylamino-3-(1H-imidazol-4-yl)-propionylamino]-3-methyl-butyl]-hydroxy-phosphinoyl)-2-benzyl-propionic acid <3> [61]
3-([1-[2-acetylamino-3-(4-hydroxy-phenyl)-propionyl]-pyrrolidin-2-yl]-hydroxy-phosphinoyl)-2-benzyl-propionic acid <3> [61]
3-[(1-amino-2-phenyl-ethyl)-hydroxy-phosphinoyl]-2-benzylpropionic acid <3> [61]
3-[(1-amino-3-methyl-butyl)-hydroxy-phosphinoyl]-2-benzylpropionic acid <3> [61]
3-[(1-amino-ethyl)-hydroxy-phosphinoyl]-2-benzyl-propionic acid <3> [61]
3-[[1-(2-acetylamino-3-methyl-butyryl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid <3> [61]
3-[[1-(2-acetylamino-3-phenyl-propionyl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid <3> [61]
3-[[1-(2-acetylamino-4-methyl-pentanoyl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-(3-phenyl-isoxazol-5-ylmethyl)-propionic acid <3> [61]
3-[[1-(2-acetylamino-4-methyl-pentanoyl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid <3> [61]
3-[[1-(2-acetylamino-4-methyl-pentanoylamino)-2-phenylethyl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid <3> [61]
3-[[1-(2-acetylamino-6-amino-hexanoyl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid <3> [61]
3-[[1-(2-acetylamino-propionyl)-pyrrolidin-2-yl]-hydroxyphosphinoyl]-2-benzyl-propionic acid <3> [61]
3-methylphenyl-benzylsuccinic acid <9> [6]
3S-95223 <3> (<3> 40% inhibition at 0.2 mM [58]) [58]
4-acetylamino-5-[2-[(2-carboxy-3-phenyl-propyl)-hydroxyphosphinoyl]-pyrrolidin-1-yl]-5-oxo-pentanoic acid <3> [61]
4-methylphenyl-benzylsuccinic acid <9> [6]
4-nitrophenyl-benzylsuccinic acid <9> [6]
4S-14713 <3> (<3> 70% inhibition at 0.2 mM [58]) [58]
4S-16659 <3> (<3> 76% inhibition at 0.2 mM [58]) [58]
5,7-dihydroxyflavone <3> (<3> 5,7-dihydroxyflavone (chrysin) is a weak inhibitor [40]) [40]
5115980 <3> (<3> 1% inhibition at 0.2 mM [58]) [58]
7490938 <3> (<3> 20% inhibition at 0.2 mM [58]) [58]
7850455 <3> (<3> 20% inhibition at 0.2 mM [58]) [58]
7857351 <3> (<3> 27% inhibition at 0.2 mM [58]) [58]
7870029 <3> (<3> 11% inhibition at 0.2 mM [58]) [58]
Ac-GDYSHCSPLRYYPWWKCTYPDPEGGG-NH_2 <9> (<9> strong inhibition, most potent inhibitory peptide, i.e. DX600 [9]) [9]

Ac-GDYSHCSPLRYYPWWPDPEGGG-NH_2 <3> (<3> i.e. DX600 [91]) [91]
Cl^- <9> (<9> inhibition is substrate dependent, inhibitory with substrate angiotensin II [2]; <9> ACE2 activity is regulated by chloride ions. The presence of chloride increases the hydrolysis of angiotensin I by ACE2, but inhibits cleavage of the vasoconstrictor angiotensin II [69]) [2,69]
Cu^{2+} <3> (<3> 69% inhibition at 0.01 mM [52]) [52]
DX600 <2,3,4> (<2> 0.01 mM, 99% inhibition [51]; <3> IC50: 10 nM [16]; <2,3,4> competitive inhibitor, 0.1 mM [38]; <2> a decrease in thrombus ACE2 activity is associated with increased thrombus formation in nude mice [93]; <4> a decrease in thrombus ACE2 activity is associated with increased thrombus formation in spontaneously hypertensive rats [93]) [16,38,41,44,51,93,97]
EDTA <2,9> (<2> complete inhibition at 10 mM [51]; <9> no inhibition by benzylsuccinate, no inhibition by lisinopril, no inhibition by captopril, no inhibition by enalaprilat [7]) [7,51]
Ile-Pro-Pro <5> (<5> inhibits EC 3.4.15.1 at one-thousandth of the concentration needed to inhibit ACE2 [75]) [75]
Leu-Pro-Pro <5> (<5> inhibits EC 3.4.15.1 at one-thousandth of the concentration needed to inhibit ACE2 [75]) [75]
MLN 4760 <2,3> (<2,3> IC50: 3 nM [26]) [26]
MLN-4760 <2,3,4,9> (<3> 0.01 mM [49]; <4> 0.001 mM [37]; <3> 0.0001 mM [52]; <3> i.e. (SS) 2-[(1)-carboxy-2-[3-(3,5-dichlorobenzyl)-3H-imidazol-4-yl]ethylamino]-4-methyl-pentanoic acid, IC50: 0.44 nM [16]; <4> specific inhibitor, 1 mM [39]; <2> total inhibition at 0.01 mM [50]; <9> ACE2-specific inhibitor. Inhibition of wild-type ACE2 was sensitive to chloride concentration [69]; <9> i.e. ((S,S)-2-[1-carboxy-2-[3-(3,5-dichlorobenzyl)-3H-imidazol4-yl]-ethylamino]-4-methylpentanoic acid) [72]) [16,37,39,49,50,52,56,59,69,72,82]
MLN4760 <3> [30]
N-[(1S)-1-carboxy-3-methylbutyl]-3-(3,5-dichlorobenzyl)-L-histidine <9> (<9> enzyme-specific inhibitor [4]) [4]
N-[(1S)-1-carboxy-3-methylbutyl]-3-(3,5-dichlorophenyl)-L-histidine <1> (<1> i.e. C_{16}, a ACE2 specific inhibitor [83]) [83]
Pro-Phe <3> (<3> IC50: 0.15 mM [16]) [16,41]
T0507-4963 <3> (<3> 41% inhibition at 0.2 mM [58]) [58]
T0513-5544 <3> (<3> 4% inhibition at 0.2 mM [58]) [58]
T0515-3007 <3> (<3> 13% inhibition at 0.2 mM [58]) [58]
Val-Pro-Pro <5> (<5> inhibits EC 3.4.15.1 at one-thousandth of the concentration needed to inhibit ACE2 [75]) [75]
angiotensin I <3,9> [6,16]
angiotensin II C-terminal analogs <3> (<3> screening of a library of angiotensin II C-terminal analogs identifies a number of tetrapeptides with increased ACE2 inhibition, and identifies residues critical to the binding of angiotensin II to the active site of ACE2 [81]) [81]
anthraquinone <3> (<3> slight inhibition [40]) [40]
benzylsuccinate <2> (<2> essentially abolishes the formation of Ang(1-9) by ACE2 [50]) [50]
benzylsuccinic acid <9> [6]

cyclohexyl-benzylsuccinic acid <9> [6]
dicyclohexyl-benzylsuccinic acid <9> [6]
phenylbenzylsuccinic acid <9> [6]
telmisartan <2> (<2> specific angiotensin II type 1 receptor blocker [56]) [56]
Additional information <2,3,4,9> (<9> no inhibition by captopril [3]; <9> construction of 6 constrained peptide libraries, selected from peptide libraries displayed on phage, peptides, 21-27 amino acids, with inhibitory effects on the enzyme, specificity and stability, selection of inhibitory sequence motifs, best CXPXRXXPWXXC, overview [9]; <9> no inhibition by enalaprilat [4]; <9> no inhibition by lisinopril [2]; <9> no inhibition by lisinopril, no inhibition by captopril, no inhibition by enalaprilat [6]; <4> rampiril does not influence the mRNA content in renal tubules [5]; <3> carboxylalkyl compounds cilazaprilat, indolaprilat, perindoprilat, quinaprilat and spiraprilat, the thiol compounds rentiapril and zofenapril, and the phosphoryl compounds ceranopril and fosinoprilat fail to inhibit the hydrolysis of either angiotensin I or angiotensin II by ACE2 at concentrations that abolished activity of EC 3.4.15.1 [15]; <2,3,4> ACE-2 mRNA and activity are severely downregulated in lung fibrosis [38]; <3> GM6001 does not have any effect on the activity of ACE2 and little effect on basal shedding of ACE2 [53]; <3> not inhibited by Ca^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} [52]; <3> not inhibited by captopri and lisinopril [41]; <2> not inhibited by captopril and benzyloxycarbonyl-Pro-Pro [51]; <3> not inhibited by rentiapril, ceranopril, indolaprilat, zofenoprilat, spiraprilat, quinaprilat, perindoprilat, fosinoprilat, cilazaprilat, captopril, lisinopril, and enalaprilat [58]; <2> the Spike protein of the SARS-coronavirus reduces ACE2 expression [47]; <4> ACE2 is insensitive to ACE inhibitors [87]; <2> central angiotensin II type 1 receptors reduce ACE2 expression/activity in hypertensive mice [89]; <4> chronic cigarette smoke administration causes an reduction in ACE2 activity and increases angiotensin II levels in the lung [97]) [2,3,4,5,6,9,15,38,41,47,51,52,53,58,87,89,97]

Activating compounds

8-[[2-(dimethylamino)ethyl]amino]-5-(hydroxymethyl <12> (<12> enhances ACE2 activity in a dose-dependent manner and causes considerable reductions in blood pressure and a striking reversal of cardiac and renal fibrosis in the spontaneously hypertensive rat model of hypertension [70]) [70]
XNT <2,4> (<2> activates ACE2, reduces platelet attachment to injured vessels, reduces thrombus size, and prolonges the time for complete vessel occlusion in mice. Thrombus area is reduced by 60%, whereas time for thrombus formation is prolonged by 45% in XNT-treated mice [93]; <4> treatment at 10 mg/kg resultes in a 30% attenuation of thrombus formation in the SHR [93]) [93]
all-trans retinoic acid <6> [48]
losartan <2,4> (<4> a specific angiotensin II receptor antagonist, is a well-known antihypertensive drug with a potential role in positively regulating ACE2 in the lung [97]; <2> an angiotensin II type 1 receptor blocker, in-

creases central ACE2 activity. Losartan also restores brain ACE2 activity in transgenic RA mice, overview [89]) [89,97]
resorcinolnaphthalein <12> (<12> enhances ACE2 activity in a dose-dependent manner [70]) [70]
Additional information <2> (<2> no activation by PD123319, an angiotensin II type 2 antagonist [89]) [89]

Metals, ions

Cl^- <9> (<9> binding ligands are Tyr207 and Arg514, possible model for chloride activation, effect is substrate dependent: activation with angiotensin I and (7-methoxycoumarin-4-yl)acetyl-APK(2,4-dinitrophenyl)-OH, inhibition with angiotensin II [2]; <9> enhances activity by about 10fold [8]; <9> required, highest activity at 1.5 M NaCl [1]; <9> ACE2 activity is regulated by chloride ions. The presence of chloride increases the hydrolysis of angiotensin I by ACE2, but inhibits cleavage of the vasoconstrictor angiotensin II [69]) [1,2,8,69]
F^- <9> (<9> enhances activity by about 10fold [8]) [8]
Zinc <3> (<3> zinc carboxypeptidase [81]) [81]
Zn^{2+} <2,3,4,9,10,12,13> (<10,12,13> metallopeptidase [74]; <9,12> zinc metalloprotease [77]; <3> dependent [41]; <9> contains zinc-binding consensus sequence HEXXH, amino acids 374-378, zinc-binding protease [6]; <9> zinc-binding motif HEXXH and third zinc ligand glutamate402, contains zinc-binding consensus sequence HEXXH, amino acids 374-378, zinc-binding protease [7]; <9> zinc-binding motif HEXXH and third zinc ligand glutamate402, zinc-binding protease [2]) [2,6,7,41,74,77,82,93]
Additional information <9> (<9> metalloprotease [3]; <9> no effect of Br^- [8]) [3,8]

Turnover number (s^{-1})

2 <9> (angiotensin I, <9> pH 6.5, room temperature [6,8]) [6,8]
2.9 <3> (angiotensin I, <3> 37°C, pH 7.4 [15]) [15]
12.8 <3> (angiotensin II, <3> 37°C, pH 7.4 [15]) [15]
84 <9> (angiotensin 4-8, <9> pH 7.4, 37°C [2]) [2]
162 <9> (angiotensin 3-8, <9> pH 7.4, 37°C [2]) [2]
1110 <9> (angiotensin II, <9> pH 7.4, 37°C [2]) [2]
1518 <9> (angiotensin 5-8, <9> pH 7.4, 37°C [2]) [2]
6840 <9> ((7-methoxycoumarin-4-yl)acetyl-APK(2,4-dinitrophenyl)-OH, <9> pH 6.5, room temperature [8]) [8]

K_m-Value (mM)

0.005 <9> (angiotensin II, <9> pH 7.4, 37°C [2]) [2]
0.0057 <3> (angiotensin II, <3> 37°C, pH 7.4 [15]) [15]
0.0069 <9> (angiotensin I) [6]
0.0091 <9> (angiotensin 3-8, <9> pH 7.4, 37°C [2]) [2]
0.0126 <9> (angiotensin 4-8, <9> pH 7.4, 37°C [2]) [2]
0.0245 <9> (angiotensin 5-8, <9> pH 7.4, 37°C [2]) [2]
0.053 <9> (angiotensin II, <9> pH 7.4, 37°C, mutant enzyme R514Q [69]) [69]

0.0586 <9> (angiotensin II, <9> pH 7.4, 37°C, wild-type enzyme [69]) [69]
0.0868 <3> (angiotensin I, <3> 37°C, pH 7.4 [15]) [15]
0.147 <9> ((7-methoxycoumarin-4-yl)acetyl-APK(2,4-dinitrophenyl)-OH, <9> pH 6.5, room temperature [8]) [8]

K_i-Value (mM)

0.00000035 <3> (3-[[1-(2-acetylamino-3-methyl-butyryl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid) [61]
0.0000004 <3> (3-([1-[2-acetylamino-3-(1H-imidazol-4-yl)-propionyl]-pyrrolidin-2-yl]-hydroxy-phosphinoyl)-2-(3-phenyl-isoxazol-5-ylmethyl)-propionic acid) [61]
0.00000125 <3> (3-[[1-(2-acetylamino-4-methyl-pentanoyl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-(3-phenyl-isoxazol-5-ylmethyl)-propionic acid) [61]
0.0000014 <3> ((2S)-3-biphenyl-4-yl-2-[[(2R)-2-sulfanylbutanoyl]amino]propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0000014 <3> ((S)-3-(biphenyl-4-yl)-2-((R)-2-mercapto-4-methylpentanamido)propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0000015 <3> ((2S)-3-(biphenyl-4-yl)-2-((3S)-2-mercapto-3-methylpentanamido)propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0000015 <3> ((2S)-3-biphenyl-4-yl-2-[[(2R)-3-methyl-2-sulfanylbutanoyl]amino]propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0000016 <3> ((S)-3-(biphenyl-4-yl)-2-((2R,3R)-2-mercapto-3-methylpentanamido)propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0000018 <3> ((2S)-3-biphenyl-4-yl-2-[[(2S)-2-sulfanylhexanoyl]amino]propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0000018 <3> ((S)-3-(biphenyl-4-yl)-2-((R)-2-cyclopentyl-2-mercaptoacetamido)propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in

0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0000021 <3> (3-([1-[2-acetylamino-3-(1H-imidazol-4-yl)-propionyl]-pyrrolidin-2-yl]-hydroxy-phosphinoyl)-2-benzyl-propionic acid) [61]
0.0000024 <3> ((2S)-3-biphenyl-4-yl-2-[[cyclobutyl(sulfanyl)acetyl]amino]-propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0000028 <3> (DX600) [16]
0.0000052 <3> (3-([1-[2-acetylamino-3-(4-hydroxy-phenyl)-propionyl]-pyrrolidin-2-yl]-hydroxy-phosphinoyl)-2-benzyl-propionic acid) [61]
0.0000052 <3> (3-[[1-(2-acetylamino-3-phenyl-propionyl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid) [61]
0.0000065 <3> (3-[[1-(2-acetylamino-6-amino-hexanoyl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid) [61]
0.0000066 <3> (3-[[1-(2-acetylamino-4-methyl-pentanoyl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid) [61]
0.0000069 <3> ((2S)-3-biphenyl-4-yl-2-[(2-sulfanylpropanoyl)amino]propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.000007 <3> (4-acetylamino-5-[2-[(2-carboxy-3-phenyl-propyl)-hydroxy-phosphinoyl]-pyrrolidin-1-yl]-5-oxo-pentanoic acid) [61]
0.0000071 <3> ((S)-3-(biphenyl-4-yl)-2-((R)-2-mercapto-4,4-dimethylpentanamido)propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0000075 <3> (3-[[1-(2-acetylamino-propionyl)-pyrrolidin-2-yl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid) [61]
0.000044 <3> ((S,S)-2-[1-carboxy-2-[3-(3,5-dichlorobenzyl)-^{3}H-inidazol-4-yl]-ethylamino]-4-methylpentanoic acid) [41]
0.000065 <3> ((S)-3-(biphenyl-4-yl)-2-((R)-2-cyclohexyl-2-mercaptoacetamido)propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.000084 <3> ((2S)-3-biphenyl-4-yl-2-[[(2S)-2-phenyl-2-sulfanylacetyl]amino]propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.000086 <3> ((2S)-3-biphenyl-4-yl-2-[[(2R)-3-phenyl-2-sulfanylpropanoyl]amino]propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in

0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.00022 <3> (3-([1-[2-acetylamino-3-(1H-imidazol-4-yl)-propionylamino]-3-methyl-butyl]-hydroxy-phosphinoyl)-2-(3-phenyl-isoxazol-5-ylmethyl)-propionic acid) [61]
0.0003 <3> (2-[(2-carboxy-3-phenyl-propyl)-hydroxy-phosphinoyl]-pyrrolidine-1-carboxylic acid benzyl ester) [61]
0.00032 <3> ((2S)-3-biphenyl-4-yl-2-[(sulfanylacetyl)amino]propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.00042 <3> ((S)-3-(biphenyl-4-yl)-2-((R)-3-cyclohexyl-2-mercaptopropanamido)propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.00055 <3> ((S)-3-(biphenyl-4-yl)-2-((R)-2-mercapto-3-(naphthalen-2-yl)-propanamido)propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0008 <3> (3-([1-[2-acetylamino-3-(1H-imidazol-4-yl)-propionylamino]-3-methyl-butyl]-hydroxy-phosphinoyl)-2-benzyl-propionic acid) [61]
0.00086 <3> ((S)-3-(biphenyl-4-yl)-2-((R)-2-mercapto-4-phenylbutanamido)-propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.00092 <3> (3-[[1-(2-acetylamino-4-methyl-pentanoylamino)-2-phenylethyl]-hydroxy-phosphinoyl]-2-benzyl-propionic acid) [61]
0.0014 <3> ((2S)-3-biphenyl-4-yl-2-[[(2S)-3-phenyl-2-sulfanylpropanoyl]amino]propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0022 <3,9> (angiotensin I) [6,16]
0.0023 <3> ((2S)-3-biphenyl-4-yl-2-[(2-methyl-2-sulfanylpropanoyl)amino]-propanoic acid, <3> apparent value, in (7-methoxycoumarin-4-yl)-acetyl-Tyr-Val-Ala-Asp-Ala-Pro-Lys(2,4-dinitrophenyl)-OH as substrate in 0.001 mM $Zn(OAc)_2$, 0.1 mM TCEP, 50 mM HEPES, 0.3 mM CHAPS, and 300 mM NaCl, at pH 7.5 [42]) [42]
0.0028 <9> (Ac-GDYSHCSPLRYYPWWKCTYPDPEGGG-NH_2, <9> pH 8.0, room temperature with substrate angiotensin I, pH 7.4, room temperature with substrate (7-methoxycoumarin-4-yl)acetyl-YVADAPK(2,4-dinitrophenyl)-OH [9]) [9]

0.003 <3> (2-[(2-carboxy-4-methyl-pentyl)-hydroxy-phosphinoyl]-pyrrolidine-1-carboxylic acid benzyl ester) [61]
0.003 <3> (2-[(2-carboxy-propyl)-hydroxy-phosphinoyl]-pyrrolidine-1-carboxylic acid benzyl ester) [61]
0.008 <3> (2-benzyl-3-[(1-benzyloxycarbonylamino-3-methyl-butyl)-hydroxy-phosphinoyl]-propionic acid) [61]
0.01 <3> (2-benzyl-3-(hydroxy-pyrrolidin-2-yl-phosphinoyl)-propionic acid, <3> K_i above 0.01 mM [61]) [61]
0.01 <3> (2-benzyl-3-[(1-benzyloxycarbonylamino-2-phenyl-ethyl)-hydroxy-phosphinoyl]-propionic acid, <3> Ki above 0.01 mM [61]) [61]
0.01 <3> (2-benzyl-3-[(1-benzyloxycarbonylamino-ethyl)-hydroxy-phosphinoyl]-propionic acid, <3> K_i above 0.01 mM [61]) [61]
0.01 <3> (3-[(1-amino-2-phenyl-ethyl)-hydroxy-phosphinoyl]-2-benzylpropionic acid, <3> K_i above 0.01 mM [61]) [61]
0.01 <3> (3-[(1-amino-3-methyl-butyl)-hydroxy-phosphinoyl]-2-benzylpropionic acid, <3> K_i above 0.01 mM [61]) [61]
0.01 <3> (3-[(1-amino-ethyl)-hydroxy-phosphinoyl]-2-benzyl-propionic acid, <3> K_i above 0.01 mM [61]) [61]
Additional information <9> (<9> K_i values of peptides from constrained peptide libraries [9]) [9]

pH-Optimum

6.5 <9> [8]
7 <4,9> (<4> assay at [82]) [1,82]
7.4 <1,9> (<1,9> assay at [7,83]) [7,83]
7.5 <2,4> (<2,4> assay at [93]) [93]
8 <9> (<9> assay at [9]) [9]

pH-Range

4.5-8 <9> (<9> activity drops sharply at pH 8.0, substantial activity at pH 4.5-6.5, inactive at pH 9.0 [1]) [1]

Temperature optimum (°C)

22 <9> (<9> room temperature, assay at [8]) [8]
37 <1,9> (<1,9> assay at [1,2,7,83]) [1,2,7,83]
42 <4> (<4> assay at [82]) [82]

4 Enzyme Structure

Molecular weight

27000 <2> (<2> SDS-PAGE [41]) [41]
42000 <11> (<11> His-tagged ACE219-367, SDS-PAGE [63]) [63]
80000 <4> (<4> SDS-PAGE [37]) [37]
89600 <9> (<9> recombinant enzyme, MALDI-TOF mass spectrometry [8]) [8]
90000 <9> (<9> recombinant His-tagged enzyme, SDS-PAGE [43]) [43]
92000 <4> (<4> SDS-PAGE [54]) [54]
92460 <9> (<9> DNA sequence determination [7]) [7]

Subunits

Additional information <1,2,3> (<2> ACE2 is a type I membrane-anchored protein with a catalytically active ectodomain, that undergoes shedding involving tumor necrosis factor α-converting enzyme, TACE [83]; <1> ACE2 is a type I membrane-anchored protein with a catalytically active ectodomain, that undergoes shedding resulting in the smaller soluble enzyme form and involving tumor necrosis factor α-converting enzyme, TACE, mechanism, overview [83]; <3> the membraneous enzyme contains an ectodomain which is cleaved in the shedding process resulting in the stille active soluble enzyme form, regulation, overview [84]) [83,84]

Posttranslational modification

glycoprotein <1,3,4,9> (<9> 7 potential N-glycosylation sites [7]; <1> the lager membraneous and smaller soluble enzyme forms are glycosylated [83]) [7,30,46,83]

proteolytic modification <3> (<3> ACE2 is is shed from human airway epithelia, constitutive generation of soluble ACE2 is inhibited by ADAM17 inhibitor DPC 333, i.e. (2R)-2-[(3R)-3-amino-3(4-[2-methyl-(4-quinolinyl) methoxy] phenyl)-2-oxopyrrolidinyl]-N-hydroxy-4-methylpentanamide, but not by while ADAM10 inhibitor GI254023, while phorbol ester, ionomycin, endotoxin, and IL-1β and TNFα acutely induce ACE2 release, thus, the regulation of ACE2 cleavage involves a disintegrin and metalloprotease 17, ADAM17, and ADAM10, overview. The ACE2 ectodomain regulates its release and residue L584 might be part of a putative sheddase recognition motif [84]) [84]

5 Isolation/Preparation/Mutation/Application

Source/tissue

A-549 cell <3> [94]
Calu-3 cell <3> [84]
HEK-293 cell <3> [24]
HK-2 cell <13> [64]
HT-1080 cell <3> [84]
Leydig cell <4,9> (<4,9> ACE2 may participate in the control of the testicular function [22]) [22]
Sertoli cell <9> [22]
adipose tissue <4,12> (<4> epididymal adipose tissue [96]) [77,96]
adrenal gland <4> (<4> low ACE mRNA expression [25]) [25]
alveolar cell <3> [32]
aorta thoracica <4> (<4> chronic treatment with the AT1R antagonist almesartan induces a fivefold increase in ACE2 mRNA in the aorta which leads to a significant increase in aortic angiotensin(1-7) protein expression [12]) [12]
artery <13> (<13> non-diseased mammary arteries and atherosclerotic carotid arteries. Total vessel wall expression of ACE and ACE2 is similar during all stages of atherosclerosis. The observed ACE2 protein is enzymatically ac-

tive and activity is lower in the stable advanced atherosclerotic lesions, compared to early and ruptured atherosclerotic lesions [76]) [76]
astrocyte <4> (<4> transcriptional regulation of ACE2 mRNA in astrocytes is dependent on the relative concentrations of both angiotensin II and angiotensin(1-7) as well as on interaction with their respective receptors [13]) [13]
atherosclerotic plaque <6> (<6> cells in atherosclerotic plaques co-express ACE2, Oct-4, and CD34 [48]) [48,60]
bile duct <12> [65]
blood <3> (<3> coronary sinus blood, evidence against a major role for angiotensin converting enzyme-related carboxypeptidase in angiotensin peptide metabolism in the human coronary circulation [31]) [31]
blood plasma <2,3,4> (<3> no or very low ACE2 in healthy individuals. ACE2 may be upregulated in subjects with cardiovascular disease [28]; <3> ACE2 circulates in human plasma, but its activity is suppressed by the presence of an endogenous inhibitor [52]) [28,51,52,59]
blood vessel <2,3,4> (<3> cardiac blood vessel [17]) [17,88,93]
brain <2,3,4,10,12,13> (<4> fetal, low ACE mRNA expression [25]; <4> transcriptional regulation of ACE2 mRNA in astrocytes is dependent on the relative concentrations of both angiotensin II and angiotensin(1-7) as well as on interaction with their respective receptors [13]; <13> ACE2 is widespread throughout the brain, present in nuclei involved in the central regulation of cardiovascular function like the cardio-respiratory neurons of the brainstem, as well as in non-cardiovascular areas such as the motor cortex and raphe [74]; <10> overexpression to the forebrain, essentially the subfornical organ, inhibits both pressor and drinking responses resulting from intracerebroventricular administration of Ang-II [67]; <10> predominantly in neurons [74]) [13,25,51,67,74,77,89,92]
brain stem <2,4> (<4> about 20% of the ACE2 gene expression in kidney cortex [27]) [27,51]
bronchoalveolar lavage fluid <3> [84]
cardiofibroblast <9> [95]
cardiomyocyte <3,4> [49,82]
cardiovascular regulatory neuron <4> [54]
carotid atherosclerotic plaque <13> (<13> ACE2 mRNA is expressed in early and advanced human carotid atherosclerotic lesions [76]) [76]
cell culture <4,6> [90,97]
cerebellum <4> (<4> low ACE mRNA expression [25]) [25]
cerebral cortex <2,4> (<4> about 10% of the ACE2 gene expression in kidney cortex [27]) [27,51]
ciliary body <5> [75]
colon <3,9> (<9> only moderate levels [7]) [6,7,41,77]
connective tissue <3,4> [85,87]
coronary artery <4> (<4> vascular walls and endothelium [88]) [88]
endothelial cell <3> (<3> expressed ACE2 to a high level, has not been shown to be infected by SARS-CoV. Presence of ACE2 alone is not sufficient for maintaining viral infection. Other virus receptors or coreceptors may be required in different tissues [32]) [32]

endothelium <4,6> (<6> the enzyme is present in endothelia overlying neointima formation and atherosclerotic plaques, but not in endothelial layer overlying normal vessel wall [60]; <4> and vascular walls of coronary arteries [88]) [60,88]
enterocyte <3> (<3> surface enterocytes of the small intestine [32]) [32]
epithelium <3,9> (<9> of coronary and intrarenal vessels and renal tubuls [3]; <3> from airway, apical surface [84]) [3,84,92]
eye <5> (<5> vitreous body, retina and ciliary body. Counterbalancing interaction of ACE1 (EC 3.4.15.1) and ACE2 in physiological regulation of ocular circulation and pressure and possible protective role in certain ophthalmic disorders such as glaucoma and diabetic retinopathy [75]) [75]
glomerulus <2,4> [46,56]
heart <2,3,4,6,9,13> (<4> 12-day administration of agents that either inhibit the synthesis of circulating angiotensin II or block the activity of angiotensin II at the AT1 receptor induce an increase in cardiac ACE2 mRNA, accompanied by increases in cardiac membrane ACE2 activity in rats medicated with either losartan or both losartan and lisinopril [19]; <4> about 35% of the ACE2 gene expression in kidney cortex [27]; <13> the endothelium-bound carboxypeptidase is expressed in the heart and kidney [78]; <3> the enzyme is upregulated in cardiovascular disease [81]) [7,17,19,23,25,27,30,37,41,44,49,50,54,55,77,78,81,82,86,88,90,95]
heart ventricle <9> [3,6]
hepatic stellate cell <4> [87]
hippocampus <2> [51]
hypothalamus <2,4> (<4> about 15% of the ACE2 gene expression in kidney cortex [27]; <4> low ACE mRNA expression [25]; <2> brain ACE2 activity is highest in hypothalamus [51]) [25,27,51]
intestine <4> (<4> highest ACE2 mRNA expression in intestine epithelium [25]) [25,59]
kidney <2,3,4,9,10,12,13> (<4> diabetic rats, 50% reduced enzyme content in renal tubules [5]; <4> ACE2 mRNA is widely expressed, with relatively high levels in proximal straight tubule. ACE2 protein is present in tubular segments, glomeruli and endothelial cells. No activity in medullary thick ascending limb of henles loop [14]; <4> cortex and medulla, about 50% of the ACE2 gene expression in kidney cortex [27]; <3> tubular epithelium [17]; <2> ACE and ACE2 co-localized strongly in the apical brush border of the proximal tubule [56]; <4> predominantly expressed in the proximal tubule [46]; <12> in salt-sensitive Sabra hypertensive (SBH/y) rats, ACE2 mRNA and protein expression are lower than that in salt-resistant Sabra normotensive (SBN/y) rats [74]; <9> localization of ACE2 in the podocytes early in the development of diabetes indicates that it may protect against podocyte loss, thus preventing the worsening glomerular injury [77]; <13> the endothelium-bound carboxypeptidases is expressed in the heart and kidney. ACE2 is expressed in renal tubular epithelium, vascular smooth muscle cells of the intrarenal arteries and in the glomeruli [78]) [5,6,7,14,17,25,26,27,30,39,41,44,46,51,56,57,59,66,74,77,78,89,96]

liver <3,4,12> (<12> ACE2 plays a crucial role in liver fibrogenesis [71]; <3> the enzyme is upregulated in fibrotic liver [81]) [59,71,77,81,87]
lung <2,3,4,11,12> (<2> ACE2 and the AT2 receptor protect against lung injury. Exogenous recombinant human ACE2 attenuates acute lung failure in Ace knockout as well as in wild-type mice. Acute lung injury results in a marked downregulation of ACE2. Loss of ACE2 expression in acute lung injury leads to leaky pulmonary blood vessels through AT1 receptor stimulation [34]; <4> ECE2 and ACE activities are increased in the same portions in the lungs of FR30 rats (adult 4-months-old offspring from 70% food-restricted dams throughout gestation) [25]) [25,34,41,45,47,59,63,77,92,97]
lung epithelium <10> [57]
macrophage <4,6> [48,60,88]
myocardium <4> [88,90]
myocyte <4,6> [88,90]
non-small cell lung cancer cell <3> (<3> decreased ACE2 expression, expression profile in relation to clinicopathological factors, e.g. smoking, overview [94]) [94]
ovary <9> (<9> only moderate levels [7]) [7,77]
pancreas <4,9,12> (<4> low ACE mRNA expression [25]; <12> ACE-mediated inhibition of TGF-β expression may prevent islet fibrosis and loss of islet function [77]; <9> non-malignant tissues surrounding invasive pancreatic ductal adenocarcinoma [80]) [25,77,80]
pancreatic invasive ductal adenocarcinoma cell <9> [80]
pituitary gland <2,4> (<4> low ACE mRNA expression [25]) [25,51]
placenta <3,4,9,12> (<3> expression of ACE2 is similar in samples obtained from normal term or preeclamptic pregnancies, except for increased expression of ACE2 in umbilical arterial endothelium in preeclampsia. The uteroplacental location of angiotensin (1-7) and ACE2 in pregnancy suggests an autocrine function of angiotensin(1-7) in the vasoactive regulation that characterizes placentation and establishes pregnancy [35]; <12> during pregnancy, the placenta and the uterus, constitute important sources of ACE2, in addition to its normal production in the kidney, leading to an estimated twofold increase in total ACE2 activity [66]) [25,35,66,77]
podocyte <2> [56]
pulmonary artery smooth muscle cell <4> (<4> primary cell culture [97]) [97]
renal cortex <4> [39]
renal medulla <4> [39]
renal tubule <4> (<4> predominantly in proximal tubules, diabetic rats, 30% reduced enzyme content [5]) [5]
retina <4,5,9,12> (<4> ACE2 is localized predominantly to the inner nuclear layer but also to photoreceptors, in the diabetic retina ACE2 is increased, ramipril treatment has no influence [20]) [20,75,77]
rostral ventrolateral medulla <4> [54]
serum <3> [85]
skin <2,3,4> [91,93]

small intestine <3,9> (<9> only moderate levels [7]; <3> surface enterocytes [32]) [6,7,32,77]
smooth muscle cell <6> [48,60]
stomach <4> (<4> low ACE mRNA expression [25]) [25]
testis <3,4,9> (<4,9> ACE2 may participate in the control of the testicular function [22]) [3,6,7,22,41,77]
urine <3> [29,30]
uterine endometrium <13> [79]
uterus <12> (<12> during pregnancy the placenta and the uterus, constitute important sources of ACE2, in addition to its normal production in the kidney, leading to an estimated twofold increase in total ACE2 activity [66]) [66]
vein <13> (<13> total vessel wall expression of ACE and ACE2 is similar during all stages of atherosclerosis. The observed ACE2 protein is enzymatically active and activity is lower in the stable advanced atherosclerotic lesions, compared to early and ruptured atherosclerotic lesions [76]) [76]
vena cava <2,4> (<2> induced thrombus [93]; <4> induced thrombus. No differences between spontaneously hypertensive rats and Wistar Kyoto rats in ACE2 protein and ACE activity in the thrombi [93]) [93]
Additional information <1,2,3,4> (<2> no activity in plasma [26]; <4> weak or no ACE2 mRNA expression in: hippocampus, skeletal muscle, liver, spleen, testis, uterus and mammary gland [25]; <3> no detectable enzyme levels in vascular smooth muscle cell or vascular endothelium [49]; <1> no activity in CHO cell [83]; <2> no activity in EC cells [83]) [25,26,49,83]

Localization

cell membrane <4> [88]
cell surface <3> [84]
cytoplasm <3,9> (<9> ACE2 exists as bothmembrane bound and soluble forms, the latter being generated by proteolytic cleavage of the ectodomain by the tumor necrosis factor convertase ADAM17 [77]) [77,94]
membrane <1,2,3,4,9> (<9> integral membrane protein [72]; <2,3> transmembrane enzyme [41]; <9> enzyme possesses a transmembrane domain, posttranslational cleavage for secretion of the protein in vivo and in cell culture [3]; <3> ACE2 also undergoes phorbol-12-myristate-13-acetate-inducible ectodomain shedding from the membrane [49]; <9> ACE2 exists as bothmembrane bound and soluble forms, the latter being generated by proteolytic cleavage of the ectodomain by the tumor necrosis factor convertase ADAM17 [77]; <3> ACE 2 is shedded [84]; <2> ACE2 is a type I membrane-anchored protein with a catalytically active ectodomain, that undergoes shedding [83]; <1> the larger form of ACE2 is a type I membrane-anchored protein with a catalytically active ectodomain, that undergoes shedding resulting in the smaller soluble enzyme form [83]) [3,4,7,41,46,49,50,52,56,72,77,83,84,94]
plasma membrane <3> (<3> evenly distributed to detergent-soluble regions of the plasma membrane in non-polarized CHO cells, in polarized Madin-Darby canine kidney epithelial cells ACE is localized predominantly to the apical surface (92%) where it is proteolytically cleaved within the ectodomain to release a soluble form, recombinantly expressed enzymes [30]; <3> the

ACE2 ectodomain can be cleaved from the cell membrane and released into the extracellular milieu [53]) [30,53]
soluble <1,3> (<3> ACE 2 is shedded [84]; <1> smaller enzyme form without ectodomain [83]) [83,84]
Additional information <9> (<9> transmembrane domain [6]) [6]

Purification

<3> (Ni^{3+}-charged nitrilotriacetic acid-linked resin chromatography and anti-Flag column chromatography) [52]
<9> (recombinant from CHO K1 cells) [3]
<9> (recombinant from Sf21 cells as mIgG-tagged protein) [1]
<9> (recombinant from Sf9 cells, to near homogeneity) [8]
<9> (recombinant truncated extracellular form of human ACE2 (residues 1-740)) [72]
<9> (recombinant wild-type and extracellular domain as FLAG-tagged proteins from Sf9 cells) [9]
<11> (nickel-nitrilotriacetic acid agarose affinity chromatography) [63]
<13> (recombinant) [72]

Crystallization

<9> (hanging drop vapor diffusion at 16-18°C, crystal structures of the native and inhibitor(MLN-4760)-bound forms of the ACE2 extracellular domains are solved to 2.2 and 3.0 A resolution, respectively) [72]
<13> (hanging drop vapor diffusion at 16-18°C, crystal structures of the native and inhibitor-bound forms of the ACE2 extracellular domains are solved to 2.2 A and 3.0 A resolution) [72]

Cloning

<1> (expression in CHO cells) [83]
<2> (expression in E4 cells) [83]
<3> (cloning and expression of a constitutively secreted form of ACE2, WKY rats are transduced with lentiviral vector containing shACE2. The plasma ACE2 levels could be increased by lentivector-mediated shACE2 gene transfer. This provides a tool to investigate the role of this enzyme in the development of the cardiovascular disease both through the role of hyperactivity of the RAS and through infectious agents) [36]
<3> (expressed in HEK 293-T cells) [52]
<3> (expressed in HEK-ACE2 cells) [53]
<3> (expression in CHO cells and polarized Madin-Darby canine kidney epithelial cells) [30]
<3> (expression of wild-type and utant L584A ACE2 in HEK-293 cells) [84]
<4> (ACE2 expression analysis by RT-PCR) [87]
<4> (cloning of the enzyme utilizing the murine cytωlovirus immediate early gene promoter, MCMV Pr, in an adenoviral vector for ACE2 overexpression in rats as a gene therapy model. overview) [88]
<4> (expressed in CHO cells) [62]