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]

<4> (overexpression of ACE2, by usage of a recombinant adeno-associated virus 6 delivery system, in myocardium of stroke-prone spontaneously hypertensive rats, gene expression profiling, overview) [90]
<9> (ACE2 expressed in Chinese hamster ovary cells specifically binds to glutathione-S-transferase-calmodulin, but not glutathione-S-transferase alone) [68]
<9> (ACE2 expression analysis) [95]
<9> (DNA and amino acid sequence determination, gene maps to choromosomal location Xp22, expression in CHO cells of the wild-type and of the soluble truncated mutant, the latter as c-Myc- and His-tagged protein) [7]
<9> (Sf21 cells via infection with baculovirus, mIgG-tagged protein) [1]
<9> (expressed in the endothelial cell line Eahy926) [43]
<9> (expression in HEK-293 cells) [69]
<9> (expression in Spodoptera frugiperda Sf9 cells via infection with baculovirus) [8]
<9> (expression of extracellular domain and wild-type, both as FLAG-tagged proteins, in Spodotera frugiperda Sf9 cells via baculovirus infection) [9]
<9> (expression of recombinant ACE2 in P-selectin-transfected Chinese hamster ovary cells) [22]
<9> (expression of the mutant enzyme in CHO cells) [2]
<9> (gene ACE2, DNA sequence determination and analysis, expression in CHO K1 cells, secretion of the active enzyme from transfected cells by cleavage N-terminal to the transmembrane domain) [3]
<11> (expressed in Escherichia coli BL21(DE3) cells) [63]
<13> (development of a transgenic mouse model (syn-hACE2) where the full open reading frame of the human ACE2 gene is under the control of a synapsin promoter, allowing the hACE2 protein to be expressed specifically in neurons) [74]

Engineering

H345A <3> (<3> no activity with (7-methoxycoumarin-4-yl)acetyl-APK-2,4-dinitrophenyl [24]) [24]
H345L <3> (<3> no activity with (7-methoxycoumarin-4-yl)acetyl-APK-2,4-dinitrophenyl [24]) [24]
H505A <3> (<3> 1.5% of wild-type activity with (7-methoxycoumarin-4-yl)acetyl-APK-2,4-dinitrophenyl as substrate [24]) [24]
H505L <3> (<3> no activity with (7-methoxycoumarin-4-yl)acetyl-APK-2,4-dinitrophenyl [24]) [24]
K481Q <9> (<9> angiotensin I cleavage activity is 21% of wild-type activity, angiotensin II cleavage activity is 71.8% of wild-type activity [69]) [69]
L584A <3> (<3> the point mutation in the ACE2 ectodomain markedly attenuates shedding. The resultant ACE2-L584A mutant trafficks to the cell membrane and facilitates SARS-CoV entry into target cells [84]) [84]
N580A <3> (<3> the mutation in the ectodomain has no effect on sACE2 release [84]) [84]
P583A <3> (<3> the mutation in the ectodomain has no effect on sACE2 release [84]) [84]

R169Q <3,9> (<3> as active as wild-type enzyme with (7-methoxycoumarin-4-yl)acetyl-APK-2,4-dinitrophenyl as substrate [24]; <9> angiotensin I cleavage activity is 5.2% of wild-type activity, angiotensin II cleavage activity is 1.1% of wild-type activity. The mutant enzyme does not show any activity with angiotensin I in the absence of chloride ions [69]) [24,69]
R169QK481QR514Q <9> (<9> angiotensin I cleavage activity is 53.2% of wild-type activity, angiotensin II cleavage activity is 203.4% of wild-type activity [69]) [69]
R273K <3> (<3> no activity with (7-methoxycoumarin-4-yl)acetyl-APK-2,4-dinitrophenyl [24]) [24]
R273Q <3> (<3> no activity with (7-methoxycoumarin-4-yl)acetyl-APK-2,4-dinitrophenyl [24]) [24]
R514Q <3,9> (<3> about 10% of wild-type activity with (7-methoxycoumarin-4-yl)acetyl-APK-2,4-dinitrophenyl as substrate [24]; <9> angiotensin I cleavage activity is 52% of wild-type activity, angiotensin II cleavage activity is 179.3% of wild-type activity, enhancement of angiotensin II cleavage is a result of a 2.5-fold increase in V_{max} compared with the wild-type [69]) [24,69]
R582A <3> (<3> the mutation in the ectodomain has no effect on sACE2 release [84]) [84]
V581A <3> (<3> the mutation in the ectodomain has no effect on sACE2 release [84]) [84]
V604A <3> (<3> the mutation in the ectodomain has no effect on sACE2 release [84]) [84]
W271A <9> (<9> angiotensin I cleavage activity is 5.3% of wild-type activity, angiotensin II cleavage activity is 0.9% of wild-type activity. Lacks any significant chloride sensitivity with the substrate angiotensin I [69]) [69]
Additional information <1,2,3,4,6,9> (<9> construction of a soluble truncated mutant enzyme lacking the transmembrane and cytosolic domains [2,7]; <6> ACE2 overexpression leads to markedly increased myocyte volume, assessed in primary rabbit myocytes [90]; <3> construction of cytoplasmic tail deletion mutants by introduction of a stop codon at position amino acid 763. Construction of chimeric proteins containing portions of human ACE2 and portions of human CD4 or human β-defensin-2, both showing loss of domain shedding [84]; <3> construction of several transgenic linages with differential virological and immunological outcome of severe acute respiratory syndrome coronavirus infection in susceptible and resistant transgenic mice expressing human ACE2, overview. Transgenic lineages AC70 and AC22, representing those susceptible and resistant to the lethal SARS-CoV infection, respectively, are both permissive to SARS-CoV infection, causing elevated secretion of many inflammatory mediators within the lungs and brains, viral infection appears to be more intense in AC70 than in AC_{22} mice, especially in the brain, differential SARS-CoV-induced morbidity and mortality between AC70 and AC22 mice, overview [92]; <2> generation of triple-transgenic-model mice with brain ACE2 overexpression on a chronically hypertensive, AngII-increased background. The transgenic mice show dramatically decreased baseline spontaneous baroreflex sensitivity and brain ACE2 activity compared with nontransgenic mice, whereas peripheral

ACE2 activity/expression remains unaffected [89]; <1> M2-mutant CHO cells, mutated in tumor necrosis factor α-converting enzyme, TACE, show reduced shedding of the ectodomain of ACE2 and increased release of the larger soluble enzyme form, compared to the smaller one, overview. Tandem mutation in the juxtamembrane region also causes a decreaee in the small soluble enzyme form [83]; <3> overexpression of ACE 2 might have a protective effect by inhibiting cell growth and vascular endothelial growth factor a production in vitro [94]; <4> overexpression of ACE2 favorably affects the pathological process of left ventricular remodeling after myocardial infarction by inhibiting ACE activity, reducing AngII levels and upregulating Ang(1-7) expression [88]; <4> overexpression of ACE2, by usage of a recombinant adeno-associated virus 6 delivery system, in myocardium of stroke-prone spontaneously hypertensive rats mediates onset of experimental severe cardiac fibrosis [90]) [2,7,83,84,88,89,90,92,94]

Application

analysis <2,3> (<2,3> mass spectrometric assay for angiotensin-converting enzyme 2 using angiotensin II as substrate will have applications in drug screening, antagonist development, and clinical investigations [26]) [26]

medicine <2,3,4,7,9,10,12,13> (<9> potential important target in cardio-renal disease [2]; <3> ACE2 protects against acute lung injury in several animal models of acute respiratory distress syndrome. Increasing ACE2 activity might be a novel approach for the treatment of acute lung failure in several diseases [21]; <4> angiotensin-converting enzyme 2 is a target for gene therapy for hypertension disorders [23]; <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. These effects are associated with significant decreases in aortic medial thickness and may represent an important protective mechanism in the prevention of cardiovascular events in hypertensive subjects [12]; <7> identification of ACE2 as a receptor for SARS-CoV will contribute to the development of antivirals and vaccines [18]; <2> recombinant ACE2 can protect mice from severe acute lung injury [34]; <2,3,4> ACE-2 protects against lung fibrogenesis by limiting the local accumulation of the profibrotic peptide angiotensin II [38]; <3> ACE2 is a functional receptor for the causative agent of severe acute repiratory syndrome, the SARS coronavirus, ACE2 also plays a role in the development of liver fibrosis and subsequent cirrhosis [41]; <2> ACE2 is a key factor for protection from ARDS/acute lung injury and it functions as a critical SARS receptor in vivo, recombinant ACE2 protein might not only be a treatment to block spreading of SARS but also to protect SARS patients from developing lung failure [45]; <2> ACE2 may be a target for therapeutic interventions that aim to reduce albuminuria and glomerular injury [56]; <2> ACE2 protects murine lungs from acute respiratory distress syndrome [47]; <13> ACE2 activators are a reliable approach which could lead to the development of a novel class of antihypertensive and cardioprotective drugs [73]; <10> ACE2 offers a new target for the treatment of hypertension and other cardiovascular diseases [67]; <12> administration of ACE2

activators may be a valid strategy for antihypertensive therapy [70]; <13> differential regulation of ACE2 activity during the progression of atherosclerosis suggest that this novel molecule of the renin-angiotensin system may play a role in the pathogenesis of atherosclerosis [76]; <13> enhancing ACE2 action may serve to provide additional therapeutic benefits patients with cardiovascular and diabetic kidney disease. Increased ACE2 activity by the use of human recombinant ACE2 and/or a small molecule activator(-xanthenone) of ACE2 may represent potential new therapies for lung, cardiovascular and kidney diseases by providing dual beneficial effects by antagonizing angiotensin II action while generating angiotensin-(1-7) [78]; <9> reduction of ACE2 expression by RNA interference promotes the proliferation of cultured pancreatic cancer cells. ACE2 may have clinical potential as a novel molecular target for the treatment of pancreatic ductal adenocarcinoma [80]) [2,12,18,21,23,34,38,41,45,47,56,67,70,73,76,78,80]
pharmacology <3,4,9> (<9> design and synthesis of first potent and selective enzyme inhibitors may be useful as pharmacological tools to help understanding the biological relevance and potantial role of the enzyme in human disease [6]; <4> ACE2 is a potential therapeutic target in the treatment of heart failure [88]; <3> ACE2 might be a target for treatment of non-small cell lung cancer [94]) [6,88,94]

6 Stability

Temperature stability

22 <9> (<9> recombinant enzyme, at room temperature, stable for 6 h [8]) [8]

General stability information

<9>, Zn^{2+} stabilizes [8]

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