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Angiotensin-Converting Enzyme-2

**Databanks**

**MEROPS name:** angiotensin-converting enzyme-2  
**MEROPS classification:** clan MA, subclan MA(E), family M2, peptidase M02.006  
**IUBMB:** EC 3.4.17.23 (BRENDA)  
**Tertiary structure:** Available  
**Species distribution:** subkingdom Metazoa  
**Reference sequence from:** Homo sapiens (UniProt: Q9BYF1)

**Name and History**

Two independent approaches converged in the discovery of ACE2 or angiotensin-converting enzyme homologue (ACEH) in 2000. ACE2 was identified from screening a human genome database for novel zinc metallopeptidases, hence recognized as a close homolog of ACE (see Chapter 98), then cloned from a human lymphoma cDNA library [1]. In parallel the same sequence was identified from a human heart failure cDNA library [2].

**Activity and Specificity**

Despite high similarity in sequence to ACE, particularly around the active site, ACE2 functions as a carboxypeptidase, rather than a peptidyl dipeptidase, cleaving the C-terminal amino acid from susceptible substrates. The distinct binding specificity of ACE2 was demonstrated by the failure of ACE inhibitors to block its activity. Given the overall similarity to ACE, the first potential substrates to be examined were angiotensin peptides and bradykinin [1]. The decapeptide angiotensin I (Ang I) is converted by ACE2 to Ang(1–9) but not further metabolized whereas the vasoconstrictor octapeptide, Ang II, is converted to the vasodilatory Ang(1–7). Subsequent detailed kinetic analysis revealed that Ang II was highly preferred as an ACE2 substrate over Ang I, being hydrolyzed 400-fold more efficiently: Ang II (Km 2 μM, kcat 3.5 s⁻¹), Ang I (Km 6.9 μM, kcat 0.0034 s⁻¹) [3]. Bradykinin is not hydrolyzed by ACE2 but des-Arg9-bradykinin, the natural ligand for the bradykinin B1 receptor, was a substrate. When screened against a panel of 126 biologically active peptides, only 11 were hydrolyzed by purified ACE2, in all cases by single carboxypeptidase action. The three most efficiently hydrolyzed substrates were angiotensin II, apelin-13 and dynorphin A-(1–13). Only for Ang II as a substrate is a physiological role for ACE2 established, its cleavage product being angiotensin-(1–7) (Ang(1–7)), which acts on the mas receptor to oppose the actions of Ang II [4]. However, there is emerging evidence that ACE2 may further control blood pressure by controlling the degradation of apelin-13, another vasoactive peptide [5, 6]. Comparison of sequences around the cleavage site of susceptible substrates suggested a consensus sequence for carboxypeptidase action of Pro-X((1–3 residues))-Pro-Hydrophobic/basic [7]. ACE2 has an optimum pH of 6.5 and, as a zinc peptidase, is inhibited by metal chelating agents [1, 8]. Several selective ACE2 inhibitors have been developed since its discovery, the first such being MLN-4760 [9], which was designed exploiting the knowledge that Ang I was an ACE2 substrate. It is a non-peptide inhibitor with which the inhibitor-bound crystal structure was subsequently solved [10]. MLN-4760 (also known as GL1001) was originally, but unsuccessfully, developed as a potential anti-obesity drug and more recently is being examined as an anti-inflammatory agent [11]. A peptide inhibitor of ACE2, DX600, was identified from phage libraries and displayed a Ki of 2.8 nM [12]. More recently a phosphinic-peptide inhibitor has been developed, which mimics the enzyme transition state. This pseudo-peptide inhibitor was designated 416F2 and has a Ki of 0.4 nM [13].

**Structural Chemistry**

The ACE2 gene is located on chromosome Xp22 and contains 18 exons, many of which resemble the corresponding exons in the ACE gene. The complete cDNA sequence of ACE2 encodes a 805 amino acid protein, which resembles a chimera composed of a single ACE-like catalytic ectodomain fused to the transmembrane protein, collectrin, or TMEM27 [14], which plays a role in regulation of renal amino acid transport and has been implicated in insulin exocytosis and β-cell proliferation.
The homology of ACE and ACE2 is particularly striking around the HEXXH zinc-binding motif which is identical (HEMGH) in the two proteins. The crystal structure of ACE2 was solved within 4 years of its discovery [10]. This established the structural basis for the unique catalytic activity of ACE2 compared to ACE, which is the result of subtle differences in their active sites. For example, the presence of an arginine at position 273 in ACE2 compared with glutamine in ACE removes the S2 pocket present in the active site of ACE, thereby eliminating the peptidyl dipeptidase activity [10]. Upon substrate or inhibitor binding the two catalytic subdomains of ACE2 undergo a hinge-bending movement of 16 Å towards each other, which closes the active site cleft to initiate substrate hydrolysis [10].

Key active site residues of ACE2 (Figure 100.1) were identified by site-directed mutagenesis based on the structure of the single domain testicular form of ACE [15]. The fidelity of this model was verified by the inhibitor-bound crystal structure. The formation of a salt bridge by Arg273 was found to be critical for substrate recognition since its mutation abolished enzymatic activity. Like ACE, a histidine in ACE2 (His345) stabilizes the tetrahedral peptide intermediate by acting as the hydrogen bond donor/acceptor. Mutation of His505 to alanine reduced ACE2 activity by 300-fold and therefore also established His505 as a critical active site residue.

The activities of both ACE and ACE2 are chloride-dependent, which is due to the presence of CL1 and CL2 sites in ACE and a comparable CL1 site in ACE2. This chloride regulation is, however, substrate-dependent [7,16]. The chloride dependency of the ACE2 CL1 site is mediated by several critical residues: Arg169, Trp271 and Lys481, which correspond to Arg186, Trp279 and Arg489 in testicular ACE. Additionally, Arg514 in ACE2 was identified by mutagenesis as a residue important for substrate selectivity. Effective inhibition of ACE2 is also dependent on the presence of chloride, the IC50 for MLN-4760 being 10-fold lower in the presence of 500 mM NaCl compared to its absence [16].

**Preparation**

ACE2 protein has been expressed in functional form in mammalian cells (Chinese hamster ovary) [1] and in a baculovirus-infected insect cell (Sf9) expression system [8]. Characterization of ACE2 has involved the use of a truncated, secreted form of the protein which lacks the transmembrane and cytosolic domains [1,2]. The baculovirus-expressed protein was purified to homogeneity by sequential chromatography on a QAE anion exchanger, hydrophobic interaction chromatography, Mono-Q ion exchange and gel filtration. This form was subsequently used for the crystallization of the protein [10].

**Biological Aspects**

The main tissue sites of expression of ACE2 were originally identified as testis, heart and kidney [1], where it was shown to be localized on the apical membrane of polarized cells [17]. However, it has now been identified more widely, for example in liver, intestine and lung [18,19]. More recently ACE2 has been localized in the brain [20], where it appears to act as a central regulator of cardiovascular function [21–24]. The major identified physiological function of ACE2 is the conversion of the vasoconstrictor Ang II to Ang(1–7), a vasodilator, antifibrotic and anti-inflammatory peptide [25,26]. Deletion of the ACE2 gene in mice was shown to produce a major defect in cardiac contractility and an upregulation of hypoxia-inducible genes in the heart [27]. Deletion of both ACE and ACE2 genes led to a rescue of the observed cardiac phenotype [27]. However, subsequent gene deletion studies of ACE2 have produced mixed results with much less effect on baseline cardiac function [28]. Overall, it appears that the actions of ACE2 may primarily be relevant in pathological states such as hypertension and cardiac hypertrophy. For example, levels of ACE2 have been shown to increase in the failing heart [29] and post-myocardial infarction (MI) in rat models [30,31], as well as in failing human heart tissue. Viral over-expression of ACE2 can attenuate heart hypertrophy and preserve cardiac function post-MI [32]. Overall, therefore, ACE2 does appear to play a role in cardiac homeostasis and these observations have led to the
consensus view that ACE2 plays a cardioprotective role acting as a counterbalance to the actions of ACE [33,34]. Additionally, a decrease in ACE2 expression is associated with diabetic nephropathy [35] and ACE2 plays a major anti-fibrotic role in the liver [36].

Unexpected roles for the ACE2 protein independent of its catalytic activity have emerged. Firstly, the cellular receptor for the severe acute respiratory syndrome (SARS) coronavirus, which causes severe acute lung failure, was identified as ACE2 [37]. It also serves as a receptor for another coronavirus, NL63 [38]. Binding of either virus causes downregulation of surface expression of ACE2. The loss of surface ACE2 on SARS infection results in a diminished ability of the lung to respond to inflammatory lung cell damage due to an increased ratio in the levels of Ang II:Ang(1–7). Administration of recombinant human ACE2 in vivo can protect from severe acute lung injury [39].

ACE2 also functions as a chaperone for the small neutral amino acid transporter (B0AT1) in the intestine, transporting it from intracellular sites of synthesis to the cell surface. This chaperone role is mediated by the collectrin-like domain of ACE2, with collectrin serving as the main transporter of B0AT1 in the kidney. In Hartnup disorder, an inherited disease leading to severe aminoaciduria, a mutation in B0AT1 reduces its affinity for ACE2 and collectrin leading to its intracellular sequestration [40].

Like ACE, ACE2 is found as a soluble, circulating form in plasma and is also detectable in urine as a result of renal excretion. The soluble form represents the ectodomain, which is shed from the membrane-bound form following proteolytic cleavage by ADAM17 [41]. At present it is unclear if the circulating form is physiologically active since its activity in plasma seems to be masked by an endogenous inhibitor. The retention of ACE2 on the cell membrane is regulated by calmodulin binding and calmodulin inhibitors increase the cellular release of ACE2 [42].

Given the generally protective role of ACE2 in cardiovascular, hepatic and lung pathologies, its upregulation would seem to have therapeutic potential. Several strategies have been investigated to date to achieve these ends, including viral delivery and the administration of recombinant soluble ACE2, in a range of in vivo disease models [43–45]. ACE2 activators have also been developed using a rational drug design approach [46]. A molecular docking model based on the open (substrate-free) and closed conformations of ACE2 led to the identification of xanthenone as a compound that binds at the hinge site on ACE2 between subdomains 1 and 2. This compound increased ACE2 activity by approximately 2-fold in vitro and was shown to reduce the blood pressure of spontaneously hypertensive rats [46] and attenuate thrombus formation in the same model of hypertension [47].

Distinguishing Features

ACE2 is a multifaceted enzyme with a broad range of biological functions. As discussed above, ACE2 resembles ACE in many structural aspects but differs from it in specificity since it acts as a carboxypeptidase. The cytoplasmic domains of ACE and ACE2 show no homology and have distinct functions. ACE2 does not hydrolyze bradykinin or the synthetic ACE substrate Hip-His-Leu. ACE2 is not inhibited by inhibitors of ACE or CP-A and specifically designed ACE2 inhibitors such as MLN-4760 do not inhibit ACE.

Related Peptidases

Other metallocarboxypeptidases known to contain an HEXXH zinc binding motif are bacterial in origin and include carboxypeptidase Taq [48,49] (Chapter 279) and the cobalt-activated carboxypeptidase from the hyperthermophilic archaeon Pyrococcus furiosus [50,51] (Chapter 286). ACE2 shares some similarities in specificity with these carboxypeptidases but their characterization remains somewhat limited.

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Further Reading

For reviews, see Hamming et al. [52], Lambert et al. [53] and Kuba et al. [54].

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