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REVIEW PAPER

Structure and Function of Angiotensin Converting Enzyme and Its Inhibitors

Yulan Zhao, and Chuanlian Xu

College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou 310018, China

Abstract: Angiotensin converting enzyme (ACE, EC 3.4.15.1) is a membrane-bound, zinc dependent dipeptidase that catalyzes the conversion of the decapeptide angiotensin I to the potent vasopressor octapeptide angiotensin II by removing the two C-terminal amino acids. ACE is well known to be a key part of the rennin-angiotensin system that regulates blood pressure. The inhibitors of ACE have the potency of treating hypertension. This article reviews the structure-function relationship of ACE as well as its gene polymorphism and inhibitor development. In particular, it has been found that the catalytic mechanisms of the two active sites of somatic ACE in the cleavage of angiotensin I and bradykin are different. Therefore, by specifically targeting the individual active sites of somatic ACE, it will likely offer a new way to develop novel ACE inhibitors with fewer side effects.

Keywords: angiotensin converting enzyme; structure and function; gene polymorphism; inhibitor

Introduction

Angiotensin converting enzyme (ACE, EC 3.4.15.1) is a chloride and zinc dependent dipeptidyl carboxypeptidase ubiquitously existing in mammalian species. The main isoform predominantly expressed in lung is a membrane-bound singe peptide glycoprotein. As a bioactive component of renin-angiotensin system (RAS) and kallikrein-kinin system (KKS), ACE plays a significant role in blood pressure regulation, fluid and electrolyte balancing, cardiovascular system development and vascular remodeling by hydrolyzing angiotensin I into a potent vasopressor peptide angiotensin II and deactivating the vasodepressor peptide bradykinin. Its inhibitors, such as captopril, enalapril, benazepril, fosinopril *et al*, which were developed in recent years, have been tested for treating hypertension and a nice vasodepressive effect in the clinical trial was obtained. But some side effects have been reported, such as cough and sexual hypoacusis, when these synthetic drugs are used for a long time. For further screening of natural antihypertensive drugs, the properties of ACE need to be understood in depth. This article reviews the structure-function relationship of ACE as well as its gene polymorphism and inhibitor development.

1 Structure and function

1.1 Molecular structure

There are three splicing transcripts existing in humans, which are encoded by the same ACE gene. Transcript 1 is ubiquitously distributed in many tissues and termed somatic ACE (sACE), which consists of 1306 amino acids. Transcript 2 and transcript 3, referred to testicle ACE (tACE), were expressed in testis and mature sperm consisting of 732 amino acids and 739 amino acids, respectively.

The molecular weight of sACE is 149 723 Da, which contains one N-terminal signal peptide sequence (1–30aa), two large homologous catalytically active sites (referred N-domain and C-domain), one hydrophobic transmembrane domain (1257–1276aa), and one cytosolic domain. There is a metalloprotease cleavage site at the residue 1232, from

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Corresponding author: Chuanlian Xu. Tel: +86-571-86843566; Fax: +86-571-86843335; E-mail: chuanlianxu@163.com

which sACE shed from plasma membrane by truncating its C-terminus, and secreted into serum in a soluble form. So sACE can exist in two forms: tissue ACE and plasma ACE. Tissue ACE anchors to plasma membrane through the transmembrane domain, whereas plasma ACE is also named as soluble ACE. Each form contains only one catalytic domain. The transcript 2 has a molecular weight of 80073. Its N-terminal signal peptide sequence is from residue 1 to 31. Transcript 2 from residue 68 to its C-terminus is identical to the C-terminal domain of sACE except some slight differences in signal peptide and N-terminus[1,2]. Transcript 3 has a molecular weight of 83989, whose sequence is identical to transcript 2, except for some differences in residue 657 to the carboxyl end because of frameshift mutation.

1.2 Pre-mRNA alternative splicing

The molecular differences in ACE transcripts are rooted from the alternative splicing and terminal sites. The encoding gene of ACE locates on the base 17q23 in the chromosome. It is about 21 kb in length and contains 26 exons and 25 introns. The expression products sACE and tACE are derived from the same gene using alternative promoters[3], which contributes to the difference of N -terminal sequence. sACE is transcribed from exons 1–12 and 14–26, whereas tACE is transcribed from 13–26. The promoter for sACE is in the 5′-flanking region of the first exon, whereas the promoter of tACE is located within intron 12. The gene not only initiates at two alternative sites but also contains two potential polyadenylation sites downstream termination codon. They are 628 bp apart. tACE uses the proximal site whereas sACE uses the distal one [4]. Analysis of the structure of the ACE gene suggests that the both catalytically active sites of sACE are similar not only in sequence but also in exon structure (exons 4–11 and 17–24, respectively). They are both characterized by a consensus zinc-binding motif (HEGMH).This suggested that ACE gene arises from the same duplication coded by an ancestral gene segment [5,6].

1.3 Tissue distribution

By quantitative RT-PCR, Harmer *et al* (2002) found ACE is expressed in all 72 tissues examined. The expression was particularly high in ileum, jejunum, duodenum, testis, lung, pulmonary blood vessels, and prostate. sACE is expressed in most human tissues, whereas tACE is only found in testis at adolescence with regulation by hormone. sACE and tACE are the products of tissue-specific and stage-specific expression. It implied that they exerted specific biological function[7].

1.4 Biological function

In the presence of Zn^{2+}, ACE hydrolyzes the peptide bond of Phe^{8}-His^{9} of decapeptide angiotensin I (Ang I) to release octapeptide angiotensin II (Ang II) and C-terminal dipeptide His-Leu. Ang II is one of the most potent vasoconstrictor. Upon binding with its type 1 receptor, Ang II contracts vascular smooth muscle, stimulates the secretion of aldosterone and Na^{+}, K^{+}-reabsorption in kidney, and induces water-sodium retention and blood volume increase. These subsequently result in blood pressure increase and the positive inotropic effect and chronotropic effect in heart. ACE inactivates the vasodilator by the sequential cleavage of two C-terminal dipeptides. It was named as kininase 2, and besides the substrates of angiotensin I and bradykinin, ACE can also hydrolyze a wide range of vasoactive peptide, such as enkephalin, substance P, and neurotensin. It is demonstrated that ACE can degrade amyloid β-peptide overexpression in Alzheimer's disease *in vitro*[8].

In testicular germ cells, Kondoh *et al* (2005) identified ACE as the glycosylphosphatidylinositol (GPI)-anchored protein-releasing (GPIase) factor. In addition to the peptidase activity, ACE also plays the role of GPIase releasing GPI-anchored proteins from sperm membrane. Because of the ACE-knockout, sperm lost the egg-binding ability[9].

2 Gene polymorphism and disease

There is significant relationship between ACE *ID/DD* genotypes and the development of cardiovascular diseases. A polymorphism of ACE involves the insertion or deletion(*I/D*) of a 287-base pair Alu repeat sequence near the 3′end of intron 16.This is partially associated with the concentration of ACE in the circulation. As the serum ACE activity of the *DD* genotype is twice as high as that of the *II* genotype, ACE polymorphism is reported to associate with hypertension, cardiovascular diseases, left ventricle hypertrophy, myocardial infarction, and diabetes. The *DD* genotype is shown as a risk factor for cardiovascular diseases [10]. Significant association of cardiovascular diseases, as hypertension, with D allele of the ACE gene had been documented in African-American, Chinese, Japanese, Bangladeshi, and Turkish population. But some other influences from population backgrounds and environmental heterogeneity are also found. On the study associating ACE *I/D* gene polymorphism with hypertension in Bangladeshi population, Morshed *et al* (2002) found the following: among the three ACE *I/D* variants, the *DD* genotype was associated with the highest value of the mean systolic blood pressure and mean diastolic blood pressure, and *II* genotype was associated with least value. However, the association of *I/D* polymorphism with hypertension was tested as sex-specific. The relationship between *DD* genotype and hypertension is much closer in men than in women. One reason was that estrogen in women might play a protective effect against hypertension. This sex-difference was more remarkable in younger subjects than in elderly subjects [10, 11]. Bradykinin which stimulates the release of tissue plasminogen activator (tPA) in endothelial cells can be

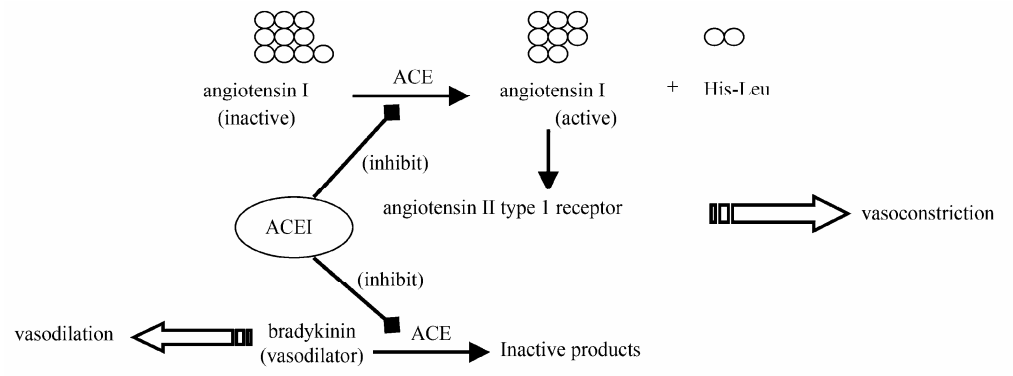

Fig. 1 Role of ACE and its inhibitor in renin-angiotensin system

ACE: angiotensin converting enzyme; ACEI: angiotensin converting enzyme inhibitor

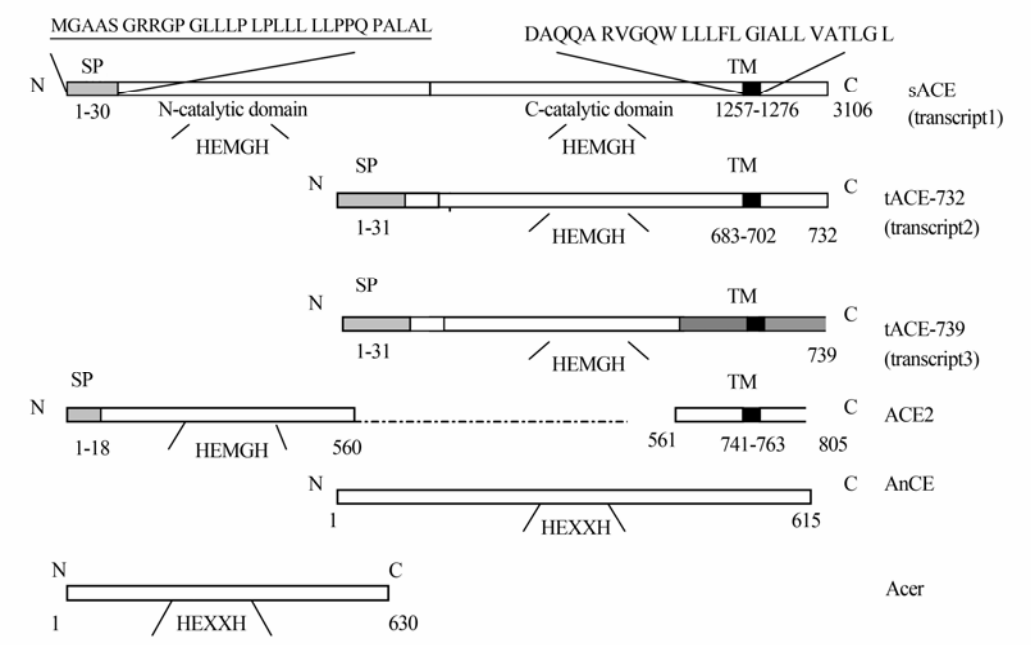

Fig. 2 Schematic drawing of human somatic ACE(sACE), testicle ACE(tACE), ACE2, *Drosophila* AnCE, and *Drosophila* Acer

The sequence of tACE is identical to that of the C-domain of sACE, except tACE first 36 residues. Human sACE and tACE-732 have the same carboxy-terminal transmembrane and cytosolic sequences, while tACE-732 and tACE-739 have the same amine-terminal and the distinct transmembrane and cytosolic sequences. None of the *Drosophila* AnCE and Acer has a membrane-anchoring sequence. The carboxyl end of ACE2 is homologous to collectin, a nonenzymatic protein associated with renal injury. N: amine-terminus; C: carboxy-terminus; SP: signal peptide; TM: transmembrane domain; HEMGH: the locations of active-site zinc-binding motif; HEXXH: locations of the active-site zinc-binding motif

degraded and inactivated by ACE. Thus, ACE inhibitor has been shown to reduce the incidence of myocardial infarction by retaining tPA. On the study of relationship between *I/D* polymorphism and coronary thrombogenesis, the *DD* genotype is associated with higher ACE activity contributed to coronary thrombogenesis and myocardial infarction by inhibiting bradykinin[12,13]. The sequence of tACE is identical to that of the C-domain of sACE, except for its first 36 residues. Human sACE and tACE-732 have the same carboxy-terminal transmembrane and cytosolic sequences. tACE-732 and tACE-739 have the same amine terminal, and a distinct transmembrane and cytosolic sequences. Neither the *Drosophila* AnCE nor the Acer has a membrane-anchoring sequence. The carboxyl end of ACE2 is homologous to collectin, a nonenzymatic protein associated with renal injury.

3 M2 family

The high homology of ACE catalytical active domains indicates that they are from the same ancestral gene. ACE-like proteinases have also been found in other animal

species including chimpanzee, cow, rabbit, mouse, chicken, goldfish, electric eel, house fly, mosquito, torn fly, silk worm, *Drosophila melanogaster* (*D. melanogaster*), the bacteria *Xanthomonas* sp., and *Shewanella oneidensis*. ACE and its homologs consist of the M2 gluzincin family. AnCE consisting of 615 amino acids is one form of ACE-like protein in *D. melanogaster*. Its catalytical activity resembles that of the C-domain of human sACE. Acer is the second ACE-related protein identified in *D. melanogaster*, and its catalytical activity is more similar to the N-domain of sACE. AnCE and Acer are both single-domain enzyme and a secretive ectoenzyme without C-terminal membrane -anchoring hydrophobic sequence. They are expressed successively during the pupal development of *D. melanogaster* and performed different physiological functions. Four additional ACE-like genes have been found in the *Adh* region of *D. melanogaster* chromosome 2. This reflected that an ancestral gene structure is presented in both protostome and deuterostome lineages. It further suggested that the duplication within the ACE gene in vertebrate genomes predates the divergence of these lineages [7,14,15].

The first homologue of ACE known by human is termed ACEH or ACE2. ACE2 is a zinc dependent carboxy -peptidase which contains 805 amino acids, including one N-terminal hydrophobic signal peptide sequence (1–18aa), one catalytically active site (147–555aa), and one hydrophobic transmembrane domain (740–763aa). ACE2 can also be shedd from the cell surface through proteolytic cleavage at the juxtamembrane region. ACE2 has 42% sequence identity and 61% sequence similarity with the two active sites of ACE. It is 33% identical overall to tACE. ACE2 is expressed in specific tissues such as human heart, kidney, and testis. It distributes in coronary endothelial cells, vascular smooth muscle cells, and renal tubular epithelial cells. ACE2 converts Ang I to form Ang1-9 by cleaving single C-terminal Leu, and Ang II to form Ang1-7 by removing the C-terminal Phe. The product Ang1-9 can be hydrolyzed as a C-terminal dipeptide by ACE, generating a vasodilator Ang1-7[16]. Therefore, Ang1-9 acts as a competitive inhibitor of ACE, counterbalancing its vasoconstriction. With the exception of Ang I and Ang II, the substrates of ACE2 also include neurotensin, kinetensin, and des-Arg bradykinin. However, ACE2 fails to cleave bradykinin and Hip-His-Leu, which shows substrate specificity of ACE2 and ACE. The activity of ACE2 cannot be inhibited by ACEI captopril, lisinopril, or enalaprilat. They perform different physiological functions in the sophisticated regulation in vivo. ACE2 gene orients on the human X chromosome with sex-specificity, which implies that the sex-specificity appeared in RAS and cardiovascular physiology with possible relation to ACE2[17].

It is regarded that ACE2 play a crucial role in cardiovascular physiological regulation and is closely identified as membrane receptor for SARS coronavirus. SARS-CoV infects cells by the attachment of S-protein to the cell surface. The infectivity of SARS-CoV can be inhibited by blocking S-protein bound to its functional receptor ACE2[18]. ACE2 anchoring to plasma membrane is the functional receptor for SARS-CoV, which mediates the fusion of viral and host membranes. Antibodies or compounds, with affinity to ACE2 as well as soluble ACE2, are all able to prevent virus entry. Lang *et al* (2006) found that interferon-γ and interleukin-4 downregulated expression of ACE2 in Vero E6 cells. By screening 312 controlled Chinese medicinal herbs, Ho *et al* (2006) identified that emodin inhibited the interaction of S-protein and ACE2 in Vero E6 cells[19,20].

4 ACE inhibitor (ACEI)

ACE has been a drug target for screening anti-hypertensive agents. Some snake venoms exert favorable inhibitory effects on ACE. A nonapeptide compound, termed SQ20881, has obviously a hypotensive activity. But it only works by being injected and does not work when taken orally, which restrict its medicine value. As ACE shares similar advanced structure with carboxypeptidase A, and that L-Benzylsuccinic acid is a strong inhibitor of carboxypeptidase A, succinyl amino acid was designed to be a target compound of ACEI. Because of the natural peptide-inhibitors of ACE such as snake venom with C-terminal of proline, succinylproline was synthesized as the first target compound and its physiological activity is systematically studied. Later by modifying the structure of the compounds, an oral nonpeptide compound, Captopril, was found as a new ideal hypotensive. On basis of this structure, two new oral hypotensive drugs, Enalapril and Lisinopril are further developed[21,22].

ACEI inhibits the activity of ACE in myocardium, kidney, vessel wall via decreasing blood pressure and inhibiting myocardial and vascular hypertrophy. It also can improve the autonomic nervous activity of patients with chronic heart failure. As a vasodilator initially, ACEI maybe associated with blockade of Endocrine, cardiac tissue, paracrine, and autocrine. It is tested that myocardium hypertrophy and myocardial fibrosis were reduced and ventricular remodeling was improved. Thus, ACEI is not only used as a hypotensive drug but also is widely used for the treatment of cardiovascular system,urinary system, and endocrine system diseases. It is demonstrated that this class of drugs distinctly improve heart function, decrease urinary protein in Nephropathies, and delay the progression of renal failure after long term usage. ACEI also can improve patients living quality and prolong their life span [23–25].

ACEI not only reduces the vasoconstriction induced by Ang II but also blocks the degradation of bradykinin as well

as other substrates. The increased bradykinin concentration brings some side effects such as cough[26,27]. Development of single domain specific inhibitor is expected to solve this problem. RXP407 is reported as an N-domain-specific inhibitor of sACE[29]. It increases the concentration of Ac-S-D- K-P in serum. It is a hematoregulator. This suggests that Ac-S-D-K-P is specifically cleaved by the N-domain and some differences occur between the two active sites of sACE[28]. RXPA380 was designed as a highly specific inhibitor of the C-domain of sACE in the study of Georgiadis *et al* (2003), and they focused on the roles of the two active sites in the cleavage of Ang I and bradykinin. When the two active sites of ACE in tissue or plasma were inhibited by RXP407 and RXPA380 together, the largest bradykinin protection was observed. Whereas one active site was inhibited by either RXP407 or RXPA380, and only half activity was protected. It leads to a conclusion that both active sites of sACE equally participate in the degradation of bradykinin. A full inhibition of Ang I cleavage required a blockade of both the two active sites of ACE of plasma in circulatory system. ACE N-domain in tissues was specifically inhibited without any differences on Ang I conversion. Whereas selectively inhibited its C-domain, Ang I conversion was completely blocked. This shows that both active domains of plasma sACE contribute to the hydrolyzation of Ang I. The Ang I-Ang II conversion depends exclusively on the ACE C-domain. Thus, ACE C-domain-specific inhibitor is regarded sufficiently to regulate hypertension. Between the two forms of sACE (plasma ACE or tissue ACE), the two catalytically active sites appear with different catalytical capability and have their specific substrates, which shows that membrane-bound ACE and soluble ACE appear to obey different mechanisms[26]. In the study of Van Esch *et al* (2005), it was demonstrated that selective ACE C-domain inhibitor was sufficient to prevent Ang II inducing vasoconstriction. This supplies a new path to screen safer hypotensive agents of ACEI[27]. ACE C-domain specific inhibitor is regarded as a treatment for cardiovascular diseases with fewer side effects and higher safety because it sufficiently blocks the Ang II generation with incomplete inhibition of bradykinin degradation, which maintains the bradykinin concentration. It offers a novel path for a new generation of drug structure designing.

5 Prospect

Important roles of RAS in cardiovascular system draw more and more attentions recently. ACE inhibitor has always been used as the first line treatment of hypertension and heart failure. As new members of ACEI are identified continually, research on molecular mechanism is carried out more deeply. It ranges widely from pioneer peptide-inhibitors, synthetic small molecules to domain-specific inhibitors and natural drugs. We hope screening domain-specific inhibitory leading compounds from natural products as well as traditional Chinese medicine, which selectively block the C-domain of ACE with less toxicity and side effects.

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