Structural basis of the lisinopril-binding specificity in N- and C-domains of human somatic ACE

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Abstract

Angiotensin I-converting enzyme (ACE, EC 3.4.15.1) plays a key role in the cardiovascular homeostasis and regulation of blood pressure, by generating the potent vasoconstrictor angiotensin II (A-II) after cleavage of the C-terminal dipeptide from angiotensin I (A-I) [1,2]. In addition, the ACE inactivates the bradykinin, blocking its hypotensive effects [3,4]. The discovery of the natural inhibitors of ACE isolated from Brazilian snake venoms [5-7] allowed the development of several highly potent and specific ACE inhibitors widely employed as orally active drugs for the treatment of hypertension, cardiac failure, and diabetic nephropathies [8,9].

ACE is mainly expressed as a somatic isoform that is an ectoenzyme found on the surface of vascular endothelial, epithelial, and neuroepithelial cells [8], and as a germinal form found in male developing spermatidis and in mature sperm, the testis-specific ACE (tACE) [10-13]. The somatic ACE (sACE) displays two highly homologous active sites, one at the C-domain (sACEc) and another at the N-domain (sACEn) of the protein, each of which is catalytically active and functionally independent [14]. Although the two domains are highly efficient to convert A-I into A-II and to inactivate Bk [15], the N-site is several times more efficient in hydrolyzing the peptides angiotensin 1–7 [16] and the AcSDKP, a negative regulatory factor of the hematopoietic stem cell differentiation and proliferation [17]. More recently, we have shown that some natural inhibitors of ACE were also able to selectively block the N- and C-terminal active sites of the sACE [18,19]. Both the human sACE and tACE are encoded by the same gene [4]. Indeed, the tACE corresponds to the C-domain of the sACE with an additional N-terminal sequence [12]. In human tACE, amino acid residues 68–732 are identical to the residues 642–1306 of the human sACE. In spite of a very similar active site sequence (including a highly conserved motif HEXXH), distinct peptide hormone

Keywords: Angiotensin I-converting enzyme; Somatic ACE; BPPs; Lisinopril; Homology modeling
substrates have been found for each active site of the sACE and tACE. Other common substrates are cleaved at different rates [15,17].

Each sACE active site seems to be relevant for distinct physiological function [14–17], and the development of selective inhibitors has been employed in order to specifically probe each function [20]. However, the recent description of the crystal structure of the *Drosophila* AnCE [21] and human tACE [22] provided a new possibility to access this question. In this work, we describe the molecular model of the N-domain active site of human sACE (sACEn) and the analysis of lisinopril and sACEn complex obtained by homology modeling. We used Modeller [23] for simultaneous modeling of the sACEn–lisinopril complex with zinc (Zn), chloride cofactors (Cl\textsuperscript{−}), and important structural waters in the active site. The Java Protein Dossier (SMS 3.0) [24] was used for the initial analysis of hydrogen bonds including electrostatic interactions. The refinement of the obtained model using energy minimization and restrained molecular dynamics in Gromacs 3.0 [25] was the second step of our work, and the obtained sACEn–lisinopril interactions were compared to the already described ones in the initial model. The data presented here may aid enzymological studies by rationalizing the design of specific inhibitors for each active site, as well as provide an important tool to study the physiological function of each domain in the regulation of blood pressure and other events involving activity of ACE isoforms.

**Materials and methods**

**Alignments.** Sequences of the N- and C-domains of the human somatic angioten- sin 1-converting enzyme (sACE, P12621 [26]) and human testicular ACE (tACE [22]) were aligned using ClustalW [27] software. The alignment was further refined by removing the first 42 N-terminal and the last 76 C-terminal amino acids of the sACE sequence and used in the modeling procedure.

**Modeling.** The structure of the human tACE complexed with lisinopril (pdb 1o86) [22] has been used to predict the three-dimensional (3D) structure of the C- and N-terminal domains of human sACE. We have taken advantage of the 100% sequence identity between tACE and sACEc to directly map the sACEc sequence in the 3D structure described for tACE in the 1o86 structure. The atomic coordinates of the 1o86 pdb structure, solved at 2.0Å resolution, were used as a template in comparative modeling by satisfaction of spatial restraints [23] implemented in the program Modeller 6v2 [28]. The program uses methods of conjugated gradients and CHARMM [29] energy terms for molecular dynamics with simulated annealing [30]. In the sACEn modeling procedure, we generated 25 different models of the protein structure. The quality of the predicted fold was evaluated in Modeller using the score of the variable target function method [28]. The stereochemical quality of the five best scoring models was assessed by Procheck program [31] at the same resolution as the 1o86 structure. The final model was selected based on the overall stereochemical quality. Using the same modeling procedure, the sACEn structure was modeled in complex with Zn, Cl\textsuperscript{−} ions, structural waters, and lisinopril inhibitor. The obtained model was subjected to energy minimization using Gromacs [25].

**Energy minimization of the complex using Gromacs.** To refine the molecular model of the sACEn–lisinopril complex, additional energy minimization and equilibrating molecular dynamics simulations were carried out with the program Gromacs 3.0 [25] on a dual-CPU Intel PIII Linux workstation. Initial sACEn protein model was submitted to a steepest-descent (SD) energy minimization (1000 steeps) to remove bad van der Waals contacts. In parallel, the Gromacs topology of the lisinopril ligand was obtained in the PRODRG server [32]. To direct the docking of the lisinopril to the minimized sACEn, the already modeled structure of the complex was used (Fig. 3A). The obtained structure of the complex was used in a second SD energy minimization (3000 steeps) and for further relaxation, a restrained molecular dynamic was performed for 50 ps with Berendsen-type temperature (300 K) and pressure (1 atm) coupling in a 40 × 40 × 40 simulation cell [25,33,34], implementing the PME method [35]. An unrestrained multiple step conjugate-gradient (CG) minimization process was used (0.1 kJ mol \textsuperscript{−1} nm \textsuperscript{−1}) to obtain the minimized structure of the complex.

**Evaluation of interactions in sACE–lisinopril complex.** In modeling procedures we have created conditions for the comparison of binding pocket with those of sACEn and sACEc–lisinopril. For evaluation of the sACE–lisinopril and sACE–Cl\textsuperscript{−} interactions, the Java Protein Dossier (JPD) program of the Sting Millennium Suite (SMS 3.0) [24] was used. The cutoff distances for hydrogen bonds, salt bridges, and aromatic interactions were 2–3.2, 2–6, and 4.0 Å, respectively. For the superimposition of N- and C-terminal sACE modeled domain backbones, combinatorial extension method (CE) [36] and PsISM program [37] were used.

**Results and discussion**

**Sequence alignment**

The aligned sequences of N- and C-terminal domains of sACE are represented in Fig. 1. There were 2 gaps with a total of 5 insertions included and 265 differences between the two domains over the 592 aligned residues, representing 55% of sequence identity. The secondary structure elements described for tACE [22] identical to sACEc were mapped in the alignment. A lower conservation of amino acids was mainly found at the $\alpha_1$-helix (a$\alpha_1$) to 3–10-helix $\alpha_2$ (H$\alpha_2$) and at a$\alpha_8$ to a$\alpha_2$ secondary structure elements (Fig. 1). The central sequence of the domains (at a$\alpha_8$ to H$\alpha_7$ elements), containing and surrounding the active site (HEXXH motif), is more conserved. The observed overall identity and relative residue conservation in the secondary structure elements predicted for sACEn, if compared to that of the tACE structure (sACEc in our alignment), makes the comparative modeling of sACEn domain feasible.

**Overall description of the sACEn initial model**

The SMS Ramachandran plot [24] for the modeled complex sACEn–lisinopril (Fig. 2) shows 93.9% of the residues in the most favorable regions, 5.0% in additional allowed regions, and 1.2% in generously allowed regions. No residues lie in the disallowed regions and stereochemical parameters checked in Procheck [31] were inside or better than expected at 95% confidence level.
Fig. 1. Sequence alignment of C (sACEc) and N-domains (sACEn) of human sACE. Clustal W [28] alignment was used as input in the modeling procedure. Identical residues (55%) are represented in a black background. Secondary structure elements are represented on the top of the alignment (α, α-helices, β, β-strands, H, and 3–10-helices) according to the resolved tACE crystal structure [23]. On the bottom of the alignment are marked the important residues that are involved in the lisinopril (*), Zn (+), and Cl⁻ (-) binding.

* Residues involved in lisinopril binding.
+ Residues involved in Zn binding
. Residues involved in Cl⁻ binding.

Fig. 2. SMS Ramachandran plot [25] for the sACEn model. The residues displayed on the right are located in the generously allowed regions. No residue is located in the disallowed regions. Glycine residues are displayed as triangles.
Predicted sACEn fold maintains the overall structure of ellipsoid shape with a central groove already described for human tACE [23] and Drosophila AnCE [21]. The secondary structure of modeled sACEn was calculated using the DSSP program [38] and it is composed of 21 α-helices, six 3–10-helices, and six antiparallel β-strands. The most important differences between the tACE and Drosophila AnCE secondary structures (in Fig. 1) were the lack of the 3–10-helix structure at the H1 position, the lateral addition of the Met349 to the β-barrel formed by β4- and β5-strands, and the insertion of a small α-helix at His522–Asn525 positions. The backbone superimposition of sACEn initial model and tACE structures using CE program [36] and PrISM [37] showed backbone rmsd equal to 0.3 and 0.4 Å, respectively, indicating high conservation in the initial model domain structure. The zinc ion, critical for the catalysis, is coordinated by the conserved His392, His396, Glu420, and carboxylic group of the lisinopril inhibitor (Fig. 3A) in a penta-coordinated geometry. The additional Glu393 from the HEXXH zinc-binding motif, conserved in the sequence of the gluzincin family of metalloproteases, interacts directly with the carboxylic moiety of lisinopril and coordinates the position of the important catalytic

![Fig. 3. Structural model of the active site of human sACEn and residues responsible for lisinopril binding. (A) Stereo representation of the sACEn active site and important residues contacting lisinopril inhibitor (LPR), Zn cofactor (Zn), and Cl− ions (Cl-1 and Cl-2). Important structural waters (w) are represented as small dark spheres. (B) Superimposed structures of the binding pockets of human sACEn–lisinopril minimized complex (thick line, upper labeled residues) and C-domain (thin dark line, lower labeled residues).](image-url)
water molecule (W_{385}) placed near the enzyme active center (Fig. 3A). A similar zinc cofactor–inhibitor interaction in penta-coordinated geometry was described in tACE–lisinopril complex (pdb 1o86) [22] and AnACE–lisinopril complex [21].

**Lisinopril–sACEc interactions in the initial model**

Lisinopril is a carboxylic inhibitor of ACE that binds to human sACE with $K_i = 0.39$ nM [39] and appears to mimic peptide substrates. The main interactions between sACEc and lisinopril inhibitor are ionic and hydrogen bonds and aromatic stacking (Table 1 and Fig. 3A). The active sites of the enzyme are placed in the $\alpha_13$-helix (HEXXH motif) and $\alpha_14$-helix (Glu_{429} residue in Fig. 3A) that coordinated zinc ion and catalytic water molecule. However, the lisinopril-binding pocket is completed by the $\alpha_{17}$- and H_{6}-helices and the 360–363 loop residues (Fig. 3A). The Java Protein Dossier program [24] was used to identify the lisinopril-binding pocket. The obtained interactions and distances are shown in Table 1.

The accommodation of the lisinopril carboxy-terminal proline moiety is mediated by the hydrophobic interaction with the aromatic ring of the Tyr_{532} and by the lateral hydrophobic pocket formed by aromatic rings of Tyr_{529}, Phe_{466}, and Phe_{521}. The lisinopril lysine moiety is contacted by the Gln_{386} and Asp_{385} residues through the water-mediated hydrogen bonds besides charge attractive contact with Asp_{385}. The lisinopril amino-terminal phenyl moiety is accommodated by the aromatic stacking with the Phe_{521} residue. The His_{361} and Ala_{362}, contacting the amino and carboxyl groups of the S2=–S1′ peptide bond, and the Glu_{393} contacting the carboxylic lisinopril moiety, are positioned in a ring form, comprising the $\alpha_{13}$, $\alpha_{17}$, H_{6}, and the 360–365 loop positions (Fig. 3A).

The residues coordinating lisinopril lysine moiety in our sACEc model are different from those described in the tACE–lisinopril complex [22] (see sACEc in Table 1), although the position of the inhibitor is mainly unchanged in the superposition of the sACEc and sACEc–lisinopril complexes (Fig. 3B).

**Chloride-binding site of human sACEc**

The location of the chloride ions in the modeled human sACEc and the enzyme interacting residues was determined using the Ligand Pocket function in the Java Protein Dossier (SMS 3.0) [24] and the obtained results are given in Table 2.

Recently, it has been proposed that the chloride ions might interact with the substrate [40]. Although Cl$^-$ dependence of hydrolysis is substrate specific, the chloride ion location at 20 Å for Cl-1 and 10 Å for Cl-2 (see Fig. 3A for Cl$^-$ nomenclature) from the active site makes a direct interaction with short peptides unlikely [22]. Moreover, Cl-2 is located in the N-cavity and may directly help in the accommodation of the substrate in the active site. Similarly as described for the tACE [22], and also mapped in the sACEc, the primary ligand for Cl-2 in modeled sACEc is Arg_{531}, located in the

### Table 1

Contacts between lisinopril and sACEc-N- and C-domain residues and mapped distances in the Java Protein Dossier program [25]

<table>
<thead>
<tr>
<th>Position</th>
<th>Lisinopril</th>
<th>sACEc</th>
<th>Distance (Å)</th>
<th>sACEc</th>
<th>Distance (Å)</th>
<th>sACEc-N minimized</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Aromatic ring</td>
<td>Ring-Phe_{1117}</td>
<td>3.7</td>
<td>Ring-Phe_{521}</td>
<td>3.8</td>
<td>Ring-Phe_{521}</td>
<td>3.6</td>
</tr>
<tr>
<td>0</td>
<td>COOH-(C=O)</td>
<td>W_{47}</td>
<td>2.8$^a$</td>
<td>W_{632}</td>
<td>2.8</td>
<td>W_{632}</td>
<td>2.8</td>
</tr>
<tr>
<td>0</td>
<td>COOH-(C=O)</td>
<td>OE2-Glu_{469}</td>
<td>2.7</td>
<td>OE2-Glu_{393}</td>
<td>2.7</td>
<td>OE2-Glu_{393}</td>
<td>3.6</td>
</tr>
<tr>
<td>0</td>
<td>COOH-(C=O)</td>
<td>W_{47}–OE2-Glu_{469}</td>
<td>2.8–3.2</td>
<td>W_{385}–OE2-Glu_{393}</td>
<td>2.8–3.2</td>
<td>W_{385}–OE2-Glu_{393}</td>
<td>3.6</td>
</tr>
<tr>
<td>0</td>
<td>COOH-(O')</td>
<td>OH-Tyr_{1128}</td>
<td>2.8</td>
<td>OH-Tyr_{332}</td>
<td>2.7</td>
<td>OH-Tyr_{332}</td>
<td>2.9</td>
</tr>
<tr>
<td>0</td>
<td>COOH-(O')</td>
<td>Zn</td>
<td>2.1</td>
<td>Zn</td>
<td>2.3</td>
<td>Zn</td>
<td>2.0</td>
</tr>
<tr>
<td>S1$^a$</td>
<td>Lys-(NH$_2$)</td>
<td>W_{671}–OD1–Asp$_{385}$</td>
<td>3.0–2.7</td>
<td>W_{718}–OD1–Asp$_{385}$</td>
<td>3.2–2.7</td>
<td>W_{718}–OD1–Asp$_{385}$</td>
<td>3.7</td>
</tr>
<tr>
<td>S1$^a$</td>
<td>Lys-(NH$_2$)</td>
<td>OE2-Glu_{386}</td>
<td>3.4</td>
<td>OE2-Asp$_{71}$</td>
<td>5.0</td>
<td>OE2-Asp$_{71}$</td>
<td>4.3</td>
</tr>
<tr>
<td>13-Helix</td>
<td>Lys-(NH$_2$)</td>
<td>W$_{103}$</td>
<td>3.5</td>
<td>W$_{632}$</td>
<td>3.1</td>
<td>W$_{632}$</td>
<td>3.1</td>
</tr>
<tr>
<td>S1$^a$</td>
<td>Cys-(NH)</td>
<td>O-Ala$_{363}$</td>
<td>2.9</td>
<td>O-Ala$_{363}$</td>
<td>2.9</td>
<td>O-Ala$_{363}$</td>
<td>2.3</td>
</tr>
<tr>
<td>S1$^a$</td>
<td>Cys-(NH)</td>
<td>OE-Glu$_{393}$</td>
<td>3.4</td>
<td>OE-Glu$_{393}$</td>
<td>3.4</td>
<td>OE-Glu$_{393}$</td>
<td>2.3</td>
</tr>
<tr>
<td>S1$^a$</td>
<td>Cys-(C=O)</td>
<td>NE2-His$_{393}$</td>
<td>2.8</td>
<td>NE2-His$_{393}$</td>
<td>2.7</td>
<td>NE2-His$_{393}$</td>
<td>2.1</td>
</tr>
<tr>
<td>S1$^a$</td>
<td>Cys-(C=O)</td>
<td>NE2-His$_{393}$</td>
<td>3.1</td>
<td>NE2-His$_{393}$</td>
<td>3.1</td>
<td>NE2-His$_{393}$</td>
<td>2.5</td>
</tr>
<tr>
<td>S2$^a$</td>
<td>Pro-(C=O)</td>
<td>W$_{749}$</td>
<td>2.8</td>
<td>W$<em>{644}$–NZ-Lys$</em>{520}$</td>
<td>2.7–3.0</td>
<td>W$<em>{644}$–NZ-Lys$</em>{520}$</td>
<td>3.1</td>
</tr>
<tr>
<td>S2$^a$</td>
<td>Pro-(C=O)</td>
<td>W$<em>{360}$–NZ-Lys$</em>{520}$</td>
<td>2.7</td>
<td>NZ-Lys$_{520}$</td>
<td>2.9</td>
<td>NZ-Lys$_{520}$</td>
<td>2.0</td>
</tr>
<tr>
<td>S2$^a$</td>
<td>Pro-(C=O)</td>
<td>NZ-Lys$_{520}$</td>
<td>2.7</td>
<td>NZ-Lys$_{520}$</td>
<td>2.7</td>
<td>NZ-Lys$_{520}$</td>
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<td>Pro-(C=O)</td>
<td>NZ-Lys$_{520}$</td>
<td>2.7</td>
<td>NZ-Lys$_{520}$</td>
<td>3.6</td>
<td>NZ-Lys$_{520}$</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*In the comparison are included mapped distances in the initial model, including structural waters (sACEc-N) and final model (sACEc-N minimized). Principal differences between N- and C-domains are shaded in gray.

*According to [23].
between lisinopril lysine moiety and Glu385, and carboxylic mapped were the stabilization of the salt bridges be-
pocket residues, the only differences with the already
shown in Table 2.

Model refinement and complex interaction analysis

To avoid the natural consequence of the homol ogy
modeling procedure (the possible targeting of the initial
3D structure to the obtained model) we performed an
additional energy minimization and restrained MD in
the initial model of the sACEn–lisinopril complex in
GROMACS [25]. The obtained minimized complex
maintains the overall enzyme structure (sACEn
rmsd = 1.5 Å), all the described sACE–lisinopril contacts,
and the overall position of the LPR inhibitor
(LPR rmsd = 0.3 Å). The obtained structure of the li-
gand pocket residues is sowed in Fig. 3B. The Java
Protein Dossier program [24] was used to identify the
lisinopril-binding pocket in minimized complex. In the
MD process we lost the information about the forming
H-bond water molecules, and for that, all water mole-
cules were removed for interaction analysis in the final
model. The obtained interactions and distances are
shown in Table 2.

In newly analyzed contacts of the minimized ligand
pocket residues, the only differences with the already
mapped were the stabilization of the salt bridges be-
tween lisinopril lysine moiety and Glu385 and carboxylic
group of the lisinopril proline moiety and Gln 290. The
Phe466 position in the enzyme hydrophobic pocket
(Fig. 3B) is also noticeable. All these contacts (Table 2)
are maintained in 500 ps restrained and 100 ps unre-
stained MD processes (not described in this paper).

Substrate specificity of sACE-N- and C-domains

The N- and C-domains of sACE are a good model
system for investigating the basis of the substrate spec-
ificity in the gluzincin family of metalloproteases. Dis-
tinct binding affinities for each domain were previously
reported for natural substrates such as A-I and Bk, and
also for inhibitors such as BPPs [14,18,19].

The sACE active sites are located in the center of the
large internal channel, as already described for AnCE
[21] and tACE [22]. The described ligand-binding resi-
dues and catalytic Zn ion divided the channel in two
different clefts. The carboxy-terminal side of the sub-
strate-inhibitor is accommodated in the small C-cham-
ber and the amino-terminal side in the large N-chamber.
Analysis of the substrate-binding channel indicated that
the lengths of the N- and C-site chambers are approxi-
mately 17 and 8 Å, respectively (data not shown). It is
possible that the channel length is the limiting factor for
the binding and hydrolysis of longer peptides. The
length restriction might be primarily on the substrate
sequence N-terminal to the cleavage site, due to the
natural dicarboxydipeptidase activity of the sACE.

As shown in Fig. 3A, the Tyr532 presents a hydro-
phobic stacking with the lisinopril S2' proline while the
Pro ring is nearly perpendicular to the Tyr532 aromatic
ring at 3.6 Å. This stacking is strongly maintained in the
sACEc–lisinopril model (Fig. 3B and Table 1) and in
tACE–lisinopril structure [22], and reminds of an off-
centered parallel orientation of side chain aromatic
groups in π-stacking interactions [41]. Proline-aromatic
rings hydrophobic stacking were described in the bra-
dykinin–polyphenol complexes studied by NMR [42]
and are the predominant mode of complexation of
tannins by the salivary proline-rich proteins (PRPs)
which are secreted into the oral cavity [43].

The hydrophobic patch provided by the aromatic
rings of Tyr532 in sACEn and Tyr1128 in sACEc
(Tyr532,1128), Phe1123,1127, Phe466,1062, and Tyr527,1123
interacting with the S2' inhibitor position may explain why
sACE generally favors a hydrophobic residue at the
carboxy-terminal position of the substrate-inhibitors
[14,19]. At the same time, tyrosine residues could inter-
act with different types of substrate side-chain groups
due to the aromatic–hydrophilic duality of their side

### Table 2
Contacts between sACE-N- and C-domain residues and Cl\(^{-}\) ions mapped in Java Protein Dossier program [25]

<table>
<thead>
<tr>
<th>sACE-C</th>
<th>Distance (Å)</th>
<th>sACE-N</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl-1</td>
<td>W(<em>{755}) (C=O)Co-Asp(</em>{111})</td>
<td>3.2–2.8</td>
<td>W(<em>{691}) (C=O)Glu-Asp(</em>{518})</td>
</tr>
<tr>
<td>Cl-1</td>
<td>NE-Arg(_{91})</td>
<td>3.2</td>
<td>NE-His(_{595})</td>
</tr>
<tr>
<td>Cl-1</td>
<td>NH-Arg(_{591})</td>
<td>3.4</td>
<td>—</td>
</tr>
<tr>
<td>Cl-1</td>
<td>NH-Arg(_{4094})</td>
<td>3.2</td>
<td>NE-Arg(_{488})</td>
</tr>
<tr>
<td>Cl-1</td>
<td>NE-Trp(_{23})</td>
<td>3.3</td>
<td>NE-Trp(_{94})</td>
</tr>
<tr>
<td>Cl-1</td>
<td>Ring-Trp(_{94})</td>
<td>3.6</td>
<td>Ring-Trp(_{94})</td>
</tr>
<tr>
<td>Cl-2</td>
<td>W(<em>{911}) (C=O)Co-Trp(</em>{22})</td>
<td>3.1–2.9</td>
<td>W(<em>{691}) (C=O)Co-Asp(</em>{22})</td>
</tr>
<tr>
<td>Cl-2</td>
<td>OH-Tyr(_{23})</td>
<td>3.0</td>
<td>OH-Tyr(_{23})</td>
</tr>
<tr>
<td>Cl-2</td>
<td>NE-Arg(_{117})</td>
<td>3.0</td>
<td>NE-Arg(_{117})</td>
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<td>Cl-2</td>
<td>NH-Arg(_{127})</td>
<td>3.5</td>
<td>NH-Arg(_{531})</td>
</tr>
<tr>
<td>Cl-2</td>
<td>(NH)C=C-Arg(_{127})</td>
<td>3.6</td>
<td>(NH)C=C-Arg(_{531})</td>
</tr>
<tr>
<td>Cl-2</td>
<td>Ring-Pro(_{106})</td>
<td>3.6</td>
<td>Ring-Pro(_{106})</td>
</tr>
<tr>
<td>Cl-2</td>
<td>Ring-Pro(_{134})</td>
<td>3.6</td>
<td>Ring-Pro(_{134})</td>
</tr>
</tbody>
</table>

Principal differences are shaded in gray.

\(\alpha_{17}\)-helix, in the vicinity of the residues Tyr\(_{532}\) and Tyr\(_{529}\),
which also directly interact with lisinopril (Fig. 3A).
chain groups, indicating inherent interaction flexibility in α17-helix (Fig. 3A).

Some of the sACE-C-domain residues altered in sACE-N-domain-binding pocket appear well positioned to interact with the lysine moiety of lisinopril inhibitor. The residue Gln386–Asp981 variation could create an electrostatically less favorable binding surface for the negatively charged residues at the inhibitor S1 contacting position, but would not change the accommodation of the lysine hydrogen bond through the structural important waters and the formed salt bridge with Glu385 (sACE-N) or Glu767 (sACEc) (Figs. 3A and B). The lisinopril access to the active site across the channel may be different for C- and N-terminal domains. Although lisinopril-binding pocket is very similar in both sACE-N and sACEc models, the already described Kₜ differences for each active site [14] may be explained by the electrostatic or hydrophobic properties that distinct residues are mapping to the channel surface. In addition, the N- and C-domain positions in sACE model suggest that the interdomain interactions may also play an important role in the substrate access to the active site (data not shown).

Our study and comparison of the overall topology of the human tACE and Drosophila AnCE crystal structure, and models for human sACE-N- and C-domains, bring new insights into the ACE active sites. This in turn can be used for the rational structure-based development of highly selective new drugs. These results will help to design more specific sACE inhibitors each targeting one of the sACE active sites.

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References


