Biophysical Chemistry 168–169 (2012) 60–66

Contents lists available at SciVerse ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Molecular mechanism of the interactions between inhibitory tripeptides and angiotensin-converting enzyme

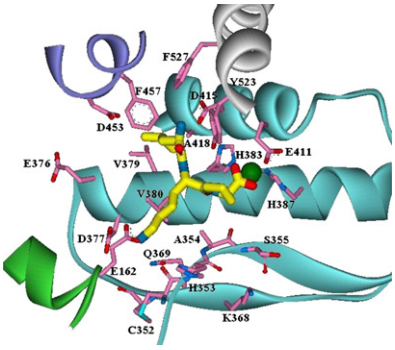
Min Zhou [a], Kun Du [a], Peijun Ji [b], Wei Feng [a,*]

[a] Department of Biochemical Engineering, Beijing University of Chemical Technology, Beijing, China
[b] Department of Chemical Engineering, Beijing University of Chemical Technology, Beijing, China

HIGHLIGHTS

► MD simulations have identified a hydrophobic subsite in the active site of cACE.
► The interactions of the side chains of the tripeptides with the hydrophobic residues determine the binding positions of the tripeptides.
► This work presents the molecular mechanism of the interactions between the inhibitory tripeptides and ACE.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 April 2012
Received in revised form 13 May 2012
Accepted 19 May 2012
Available online 23 May 2012

Keywords:
Angiotensin-converting enzyme
Tripeptide
Molecular dynamics simulation

ABSTRACT

Angiotensin I-converting enzyme (ACE) is a key therapeutic target for combating hypertension and related cardiovascular diseases. ACE inhibitory peptides offer the prospect of enhanced potency, high specificity, and no or low side effect. The ACE inhibitory tripeptides LKP and IKP differ from each other by one amino acid but their inhibitory potencies for ACE differ significantly. To uncover the molecular mechanism underlying this phenomenon, we have investigated the tripeptide/ACE complexes through molecular dynamics simulations coupled with quantum mechanical simulations. Comparative structural analysis has identified a hydrophobic subsite in the active site of cACE comprising hydrophobic residues Val379, Val380, Phe457, Phe527, and Ala418. The interactions of the side chains of Leu and Ile with the hydrophobic residues determine the binding positions of N-terminal residues of the tripeptides, that influence the interaction of the residues of tripeptides with the active site of cACE. This work presents the molecular mechanism of the interactions between the inhibitory tripeptides and ACE, and deciphers the structural basis for the high affinity LKP inhibition of ACE.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Angiotensin I-converting enzyme (ACE) is a zinc metallopeptidase that can catalyze the proteolysis angiotensin I to the vasopressor angiotensin II, which is a potent vasoconstrictor, and inactivate bradykinin, which is a peptide that causes blood vessels to enlarge [1–3]. ACE exists as two isoforms: somatic ACE (sACE), comprised of two homologous N- and C-domains (cACE and nACE), and testis ACE (tACE) which has a single active domain [4,5]. ACE is a key therapeutic target for combating hypertension and related cardiovascular diseases [6]. Toward this end, much of the efforts have been focused on library search for and structure-based rational design of inhibitors that target ACE [7,8].

Synthetic ACE inhibitors, such as captopril, lisinopril, enalapril and fosinopril, are used as pharmaceuticals to treat hypertension, congestive

* Corresponding author at: Department of Biochemical Engineering, Beijing University of Chemical Technology, Beijing, China. Tel.: +86 10 64446249; fax: +86 10 64446249.
E-mail address: fengwei@mail.buct.edu.cn (W. Feng).

0301-4622/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2012.05.002

heart failure, and myocardial infarction [9]. However, these synthetic ACE inhibitors are known to have strong side effects, such as cough, skin rashes, and angioedema [10,11]. In recent years, inhibitory active peptides derived from the food protein have gained a lot of interest [11–16], because they have not shown these side effects yet [13]. ACE inhibitory peptides have been discovered in various food sources such as milk [11,13], pea [12], potato [14], and chicken meat [16]. Compared with synthetic inhibitors, peptides are capable of binding and antagonizing target proteins, often with high affinity and unsurpassed specificity [17].

Tripeptides have been shown to have inhibitory potency for ACE [18]. Some tripeptide exhibits much higher inhibitory potency than others. LKP, for example, has an IC_{50} of 0.32 μM while the IC_{50} value of IKP is 1.60 μM [18]. The inhibitory potency of LKP is 5-fold that of IKP. The superior inhibitory potency of LKP over IKP was surprising, partly because the tripeptide IKP differs from LKP by one amino acid, and Leu and Ile are isomeric amino acid residues. Unfortunately, the mechanism behind the experimental result was not provided. More insight into the inhibitory mechanisms may hold the key to further improvements in the design of ACE inhibitors.

As a complement to experiment in the study of ACE inhibitors, molecular simulation offers unique possibilities for investigating molecular-level phenomena that are difficult to probe experimentally [19–21]. These studies include the interactions of ACE with gonadotropin-releasing hormone by using molecular simulations [19]; molecular docking study for dual ACE/neutral endopeptidase inhibitors to investigate the molecular environment of the catalytic sites and the specific interactions between the inhibitors and amino acid residues [20]; the binding patterns of inhibitors of ACE [21].

Potent peptide inhibitors against ACE are needed as useful templates for structure-based rational design of different classes of ACE inhibitors for potential therapeutic use. Here, tripeptide/ACE systems are investigated. Based on quantum mechanics (QM) geometry optimization, a parameterization for the zinc ion and coordinating atoms is provided. Molecular dynamic simulations are performed to study the molecular mechanism of the interactions of inhibitory tripeptides LKP and IKP with ACE. The simulations interpret that, compared to LKP, IKP is much less inhibitory toward ACE.

2. Materials and methods

2.1. Structure preparation

The crystal structure of ACE bound with lisinopril was taken from Protein Data Bank at Brookhaven. The entry codes are 1O86 and 2C6N for the C-domain (tACE, which is identical to the C domain of sACE) and N-domain of sACE, respectively. Fig. S1 shows the inhibitor lisinopril in the active site. Except zinc and chloride ions, all heteroatoms were removed. Swiss-PdbViewer [22] was used to add the missing atoms and hydrogen atoms. The program H++ was used to predict the protonation state of ionizable groups at pH 7.0 by utilizing a continuum electrostatic model with the Poisson–Boltzmann method [23]. The added atoms were subjected to 500 rounds of energy minimization with steepest descent gradients using the GROMACS software package (version 4.05) [24] using the GROMOS-96 force field [25–28], while all other atoms were kept fixed [19]. The size of the system is listed in Table 1.

2.2. Docking of the inhibitors

The AutoDock 4.2 package was used for docking simulation [29], the visual inspection of the docking results was done using AutoDock-Tools. For the zinc and chloride ions, formal charges and van der Waals parameters from GROMOS 96 force field were assigned, and Kollman partial charges were assigned to all protein atoms. The maps for each domain of ACE centered at the zinc-binding site were calculated using AutoGrid with $40\times40\times40$ grid points of 0.375 Å spacing. The Lamarckian genetic algorithm was used, and the following AutoDock 4.2 parameters are selected: a population size of 150 individuals; a maximum number of 2.5×10^6 energy evaluations; a maximum number of 27,000 generations; an elitism value of 1; a mutation rate of 0.02; and a crossover rate of 0.80 [19]. For all the calculations, 250 docking rounds were performed with step sizes of 0.2 Å for translations and 5° for orientations and torsions. Docked conformations were clustered using a tolerance of 1.5 Å root mean square deviation. The predicted binding energy from the dockings provided a ranking of the docked conformations. The zinc coordination sphere in native ACE comprises His383/361, His387/365, and Glu411/389 (C-/N-domain numbering) [19]. Many inhibitors have been synthesized, and they differ in the nature of their zinc-binding ligands and other interaction groups. Captopril, for example, coordinates strongly the zinc atom in the active site of ACE by its sulfhydryl group [30], while enalaprilat and lisinopril contain a carboxylate group for coordinating the zinc atom [31]. In this work, the results of top-ranked binding modes indicate that the inhibitors LKP and IKP coordinate the zinc in the active site of ACE by their carboxylate groups. The docking results are used for the quantum mechanics (QM) and molecular dynamics (MD) simulations in the following sections.

Table 1
The size of the system.

System	No. of residues	Water	Total atoms
C-domain/LKP	574	20,221	66,717
C-domain/IKP	574	20,228	66,738
N-domain/LKP	612	21,668	71,481
N-domain/IKP	612	21,671	71,490

2.3. QM geometry optimization and zinc force field parameter

Due to the promiscuous ability of zinc to assume a variety of coordination states, zinc catalytic centers have long been a challenge for molecular modeling. Both nonbonded models [32–34] and bonded models [19,35–38] are suggested for the parameterization of zinc. In nonbonded approach [32–34], optimized electrostatics and van der Waals terms are used to enforce the correct coordination geometry. The bonded models utilize the bond terms including bond stretching, angle bending, and torsional terms [19,36–38]. These approaches require "freezing" a specific zinc coordination and a predefined valence of the coordinating metal.

Here, the bonded approach is adopted with explicit bonds between the zinc and its coordinating atoms. A model of the active site (Fig. 1) was extracted from the high-resolution crystal structure of tACE. The QM method was used to handle the zinc–ligand charge transfer, inter atom distances, and angles upon binding to the zinc. To reduce the convergence time for geometry optimization, the complex structure predicted by the best docking score was subjected to energy minimization using the steepest descent method. The model of the active site structure shown in Fig. 1 is used for the geometric optimization and the calculation of the zinc–inhibitor charge transfer. In this model, the zinc-binding motif was retained, except that Met was truncated into Gly and Glu411 into butyrate [19].

The docking of the two tripeptides with N- and C-domains was done independently. And then the geometric optimization for the ACE catalytic site was performed separately. The geometric optimization for the ACE catalytic site (Fig. 1) was performed with the Gaussian 03 package using the B3LYP method and 6-31G* basis set [39]. When performing MD simulations, the distances of Zn–Pro O1 were set to the values determined by the QM geometric optimization.

The calculated electrostatic potential was fitted with the RESP (restrained electrostatic potential) program [40] to handle the zinc–inhibitor charge transfer between the zinc and its coordinating atoms. For these atoms, the charge difference between the Amber charge and the RESP charge was calculated. The charge from GROMOS-96 force

Download English Version:

https://daneshyari.com/en/article/5371206

Download Persian Version:

https://daneshyari.com/article/5371206

Daneshyari.com