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Molecular mechanism of interactions between inhibitory tripeptide GEF and angiotensin-converting enzyme in aqueous solutions by molecular dynamic simulations

Chunyan Qi [a,b], Rong Zhang [a,*], Fuzhi Liu [a], Tong Zheng [a], Wenjuan Wu [a]

[a] Laboratory of Physical Chemistry, School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, People's Republic of China
[b] Guangdong Provincial Institute of Food Inspection, Guangzhou, 510000, People's Republic of China

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ABSTRACT

A novel ACE-inhibitory tripeptide Gly-Glu-Phe (GEF) was used to study the molecular mechanism of interactions with ACE. Molecular docking and molecular dynamics simulations were performed to investigate the interactions between GEF and ACE of different domain (C-domain and N-domain) in the aqueous solutions. *RMSD*, *SASA*, *RMSF*, binding energy and hydrogen bonding were analyzed to reveal the molecular mechanism between ligand and acceptor in a micro level.

1. Introduction

Angiotensin I-converting enzyme (ACE) is a zinc metallopeptidase that catalyzes the transformation of angiotensin I to angiotensin II, which is a potent vasoconstrictor, and also inactivates bradykinin, which is a potent vasodilator [1,2]. ACE exists as two isoforms: testis ACE (tACE) which has a single active domain, and somatic ACE (sACE), comprised of two homologous C- and N-domain (C-ACE and N-ACE) [3,4]. As the sequence of tACE and the C-domain of sACE are identical, except for the 36 N-terminal residues, the C- and N-domain structures can be compared by using tACE [5]. The C- and N-domains of sACE were consisted of 650 and 612 amino acid residues, severally. And the sequence identity was almost 60% [6]. When N- and C-domains were superimposed (based on tACE), the most easily observable difference is the extra length of the N-domain at the N and C termini, the latter of which includes the inter-domain linker. Furthermore, only one chloride was bound to the N-domain, rather than two for tACE [5]. The functions of ACE in regulating blood pressure make it an ideal target in treatment of hypertension. Therefore, more and more researches have been focused on structure-based rational design of inhibitors that target ACE [7].

Synthetic ACE inhibitors, such as captopril, lisinopril, and enalapril, are widely used as clinical pharmaceuticals to treat hypertension and related cardiovascular diseases [8]. However, side effects of these synthetic ACE inhibitors should not be neglected, such as dry cough, angioedema, and fetotoxicity [9,10]. Therefore, inhibitory peptides derived from food proteins have become the focus [11–17], because they have no unknown side effects [18]. Peptides have abilities to bind and antagonize target protein with high affinity and outstanding specificity, when comparing with synthetic inhibitors [19].

According to previous study, tripeptides showed potent inhibitory ability for ACE [20]. GEF is a novel ACE-inhibitory tripeptide which is made of glycine (G), glutamic acid (E), and phenylalanine (F). And its IC_{50} value was 12.66 μM [21]. The molecular mechanism between GEF and ACE was not provided. As a supplement to the experiment, molecular simulations are widely used to investigate the targeted binding of ligand to acceptor at the molecular level [22,23]. Thus, molecular dynamics simulations were used to reveal the molecular mechanism between GEF and ACE in aqueous solutions.

2. Experimental and computational methods

2.1. Structure preparation

The crystal structures of ACE bound with lisinopril were obtained from Protein Data Bank (ID: 1O86 and 2C6N). 1O86 represented for C-domain (tACE, which is identical to the C-domain of sACE), and 2C6N was for N-domain of sACE. The structures were shown in Fig.1. All

* Corresponding author.
E-mail address: zhangr@gdpu.edu.cn (R. Zhang).

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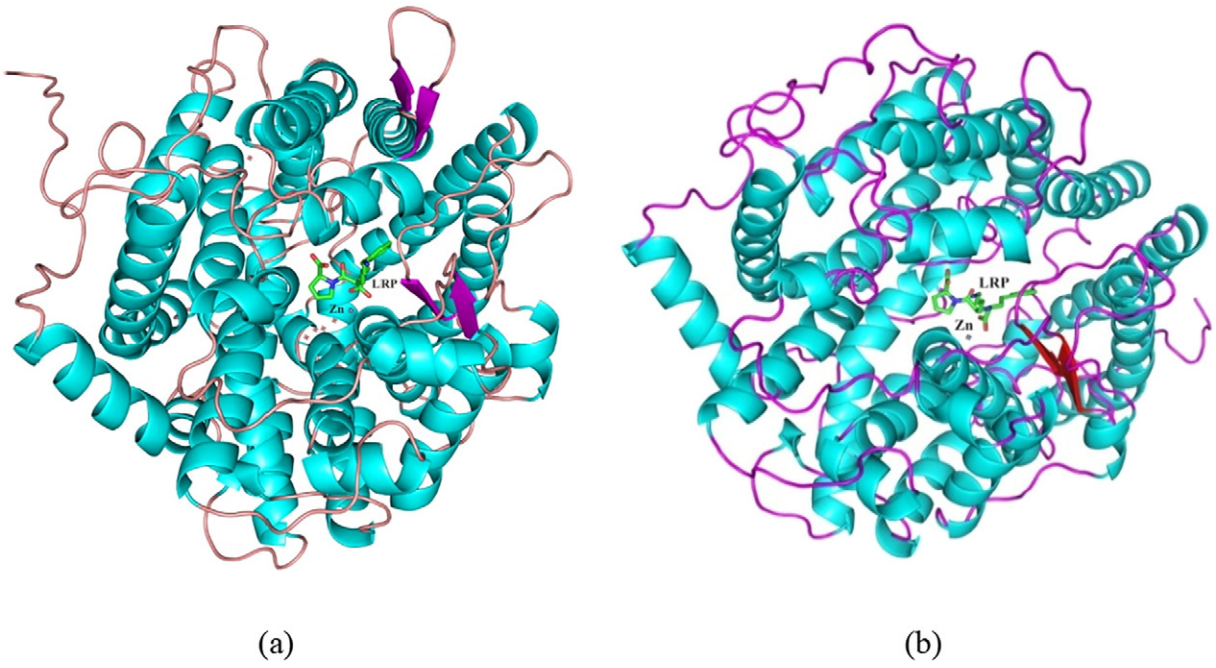

Fig. 1. The crystal structure of C-domain (a) and N-domain (b). Lisinopril was depicted as sticks in green, and Zn^{2+} is shown as sphere.

heteroatoms were removed except for zinc and chloride ions. Chimera 1.9 was used to add the missing structure and atoms [24–27].

2.2. Molecular docking

The AutoDock 4.2 package was used for docking simulation [28], and the AutoDock-Tools was applied to the visual inspection of the docking results. Formal charges and van der Waals parameters were assigned to the zinc and chloride ions based on GROMOS 96 force field. For all protein atoms, Kollman partial charges were assigned. The maps for C-domain and N-domain of ACE centered at the zinc-binding site were calculated using AutoGrid with $40 \times 40 \times 40$ grid points of 0.375 Å spacing. For docking, the Lamarckian genetic algorithm was used, and the AutoDock 4.2 parameters were selected as Zhou et al. [29]. Docking conformations were clustered using a tolerance of 2.0 Å root mean square deviations. The predicted binding energy from the docking provided a ranking of the docked conformations.

2.3. Molecular dynamic simulation

Molecular dynamic simulations were performed by using GROMACS 5.1.2 software package [30,31], and GROMACS 96 force field [30,32–34] was used. The complex of protein and inhibitor was solvated with the explicit SPC water [35,36]. Then, a box was generated by using the genbox program embedded in Gromacs. The type of the box is truncated cubic. The dimensions of the box were based on setting the box edge approx 1.0 nm from the surface of the protein. Sodium ions were added to neutralize the system by replacing water molecule randomly. Temperature and pressure were stayed at 300 K and 101.3 kPa, respectively. The particle mesh Ewald algorithm [37] was applied to calculate the electrostatic interactions, with a grid spacing of 0.16 nm and an interpolation order of 4. The cutoff for van der Waals interactions were set to 1.0 nm. Both equilibration and production runs were used an integration time step of 2 fs. Followed by energy minimization, each system was simulated under NVT conditions for 100 ps and under NPT conditions for 100 ps. Molecular simulations were then carried out to simulate the dynamic binding of GEF to ACE.

3. Results and discussions

3.1. Overall conformation of ACE bound with tripeptides

The conformation of ACE was checked by analyzing the backbone root mean square deviations (*RMSD*) from the starting crystal structure over the course of the trajectory. *RMSD* for the complexes of ACE with GEF showed that the structures of the two systems equilibrated well after 5 ns of MD simulations (Fig.2). The radius of gyration (*Rg*) is an indicator for structural change in a protein [29]. It was calculated to analyze structural changes of ACE, when inhibitors were bound. The average *Rg* values throughout the simulation time are 2.440 nm for C-ACE-GEF and 2.470 nm for N-ACE-GEF (Fig.3). While for the ACE not bound to the inhibitor, the average *Rg* values are 2.442 nm for C-ACE and 2.475 nm for N-ACE. As seen, upon the binding of the inhibitor,

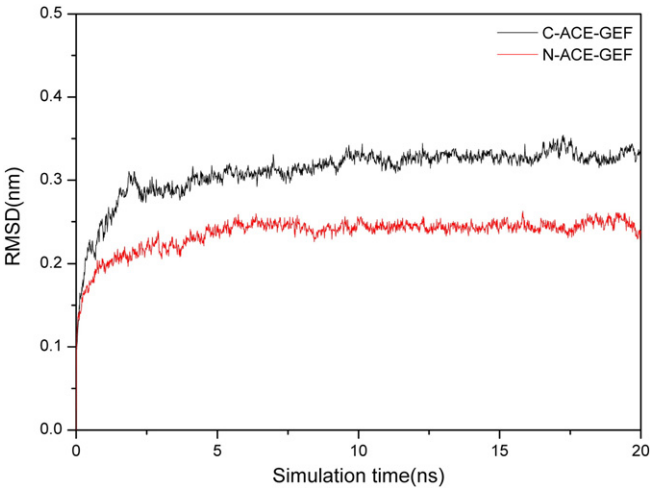

Fig. 2. The *RMSD*s of complexes of ACE with inhibitor dependent on time over 20 ns MD simulations.