Journal of Molecular Liquids xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

Conformations and interactions of ACE inhibitor tripeptide in aqueous and DMSO solution by all-atom MD simulations and 2D-NMR spectra

Guodong Huang, Rong Zhang *, Wei Zeng, Lin Chen, Wenjuan Wu

Laboratory of Physical Chemistry, School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, People's Republic of China

ARTICLE INFO

Article history:
Received 20 November 2014
Received in revised form 31 January 2015
Accepted 2 February 2015
Available online xxxx

Keywords:
All-atom molecular dynamic simulation
IEY
2D-NOESY
Different solutions

ABSTRACT

All-atom molecular dynamic simulations and 2D nuclear Overhauser effect spectroscopy (2D-NOESY) were used to study the conformations and hydrogen bonds of ACE inhibitor tripeptide IEY in different solutions. Intramolecular distances, root-mean-square deviation, radius of gyration, and solvent-accessible surface area were adopted to characterize the properties of IEY in the simulations. Interestingly, the IEY molecule showed different behaviors in different solutions. In aqueous solution, IEY was very flexible; it could shift between extended and folded states. However, in DMSO solution, folded conformations were not observed. IEY preferred more extended conformations in DMSO than in aqueous solution. The interesting phenomena were confirmed by 2D-NOESY.

1. Introduction

Hypertension is an important public health challenge worldwide because of its high frequency and concomitant risks of cardiovascular and kidney disease [1,2]. Hypertension has been identified as the leading risk factor for mortality, and is ranked third as a cause of disability-adjusted life years [3]. Angiotensin-converting enzyme (ACE) plays a key role in the treatment of hypertension.

ACE is a zinc metallopeptidase that is distributed in vascular endothelial, absorptive epithelial, neuroepithelial, and male germinal cells. The influences of ACE on blood pressure make it an ideal target, and various synthesized ACE inhibitors, such as captopril, enalapril, lisinopril, and ramipril, have been widely used in the clinical treatment of hypertension. Although synthesized ACE inhibitory drugs have demonstrated their usefulness, they are not entirely without side effects. Side effects include dry cough [4], fetotoxicity, intrauterine growth retardation, anuria, hypocalvaria, renal failure, and death [5]. ACE inhibitory drugs with few side effects are urgently needed, and bioactive peptides show good prospects in the treatment of hypertension.

Bioactive peptides, such as antibacterial peptide [6], immune active peptide [7], antihypertensive peptides, and antioxidant peptides [8], play an important role in maintaining life activities. Bioactive peptides have no side effects, so they are increasingly becoming important as starting points for drugs and drug-related compounds [9,10]. A considerable number of ACE inhibitory peptides were recently discovered from enzymatic hydrolysates of different food proteins; they have exhibited ACE inhibitory activity in vitro.

The quantitative structure–activity relationship (QSAR) of ACE inhibitory peptides has been the continuous focus of researchers to elucidate the structural requirements for the inhibition of ACE. Results can lead to the design of a new generation of drugs that are more potent than currently available compounds. In a previous study [11], a QSAR model of ACE inhibitory tripeptides with tyrosine as C-terminal was established. According to the model, a new ACE inhibitory active tripeptide IEY was synthesized. IEY tripeptide is made of isoleucine (I), glutamic acid (E), and tyrosine (Y). Its IC_{50} value (inhibitor concentration that reduced enzyme activity by 50%) was determined to be 0.37 μM by direct spectrophotometric measurement [12], and it is very close to the predicted value by the model.

Knowledge of the 3D conformations and dynamics of ACE inhibitory peptides is important for understanding their biochemical roles. Molecular dynamic (MD) simulations and NMR spectra are often used to investigate the conformations and properties of biochemical molecules in solutions [13–19]. Most biomolecules are active in aqueous solutions. Another solvent of interest is dimethyl sulfoxide (DMSO). DMSO is a widely used cryoprotectant of biological structures, including membranes and proteins [20,21], with two hydrophobic CH_3 groups and a highly polar S═O group. This polar group can form strong hydrogen bonds, and nonpolar sites in the molecule can initiate the hydrophobic hydration of DMSO. The biological properties of DMSO are of particular importance. In this study, we employed MD simulations and 2D nuclear Overhauser effect (2D-NOESY) spectrum to investigate the conformations of ACE inhibitory peptide IEY in aqueous and DMSO solutions.

* Corresponding author.
E-mail address: zhangr_zju@hotmail.com (R. Zhang).

http://dx.doi.org/10.1016/j.molliq.2015.02.003
0167-7322/

2. Computational and experimental methods

2.1. Molecular models

Simple rigid models were used for water, DMSO, and IEY. The non-bonded interactions are denoted by a sum of Coulomb and Lennard–Jones terms in Eq. (1).

$$E_{ab} = \sum_{i}^{ona} \sum_{j}^{onb} \left[q_i q_j e^2 / r_{ij} + 4\varepsilon_{ij} \left(\sigma_{ij}^{12} / r_{ij}^{12} - \sigma_{ij}^{6} / r_{ij}^{6} \right) \right] f_{ij} \quad (1)$$

where E_{ab} is the interaction energy between two molecules a and b; q is the partial charge on atom; ε and σ are the well depth parameter and collision diameter in Lennard–Jones functions, respectively; and r denotes the distance between atoms. Standard combination rules were used via Eqs. (2) and (3).

$$\sigma_{ij} = \left(\sigma_{ii} \sigma_{jj} \right)^{1/2} \quad (2)$$

$$\varepsilon_{ij} = \left(\varepsilon_{ii} \varepsilon_{jj} \right)^{1/2} \quad (3)$$

The same expression was used for intramolecular non-bonded interactions between all the pairs of atoms ($i<j$) separated by three or more bonds. In Eq. (1), f_{ij} equals 1.0 except for intramolecular 1,4 interactions with $f_{ij} = 0.5$.

The simple point charge (SPC) model [22] and optimized potentials for the liquid simulation-all atom model [23–25] were used for water and IEY molecules, respectively. The improved OPLS-AA is a suit force field for peptides in MD simulations used in many researches and also in our previous work [26–29]. The IEY molecule is ionized in aqueous solution and maintains the charged state in the neutral solution. The structure of IEY is illustrated in Fig. 1.

2.2. Simulation details

MD calculations were performed using a modified TINKER 5.1 molecular modeling package [30]. The simulations were carried out in the NPT ensemble at T = 298 K and P = 101 kPa. The IEY molecule was placed in the center of a cubic box and solvated by 512 SPC water molecules or 256 DMSO molecules to study the conformations in the dilute solutions. Periodic boundary conditions were adopted with a spheric cutoff. The particle-mesh Ewald method was used for long-range electrostatics. Energies of the initial configurations were minimized by the MINIMIZE program in the TINKER 5.1 package. Simulations of 5 ns were used for equilibrium, and simulations of 30 ns were used for analysis. Configurations were saved every 1 ps. The initial conformation was obtained from the beginning conformation of the molecule in MD simulations.

2.3. Definitions

The radius of gyration (Rg) is defined as follows [31]:

$$Rg = \sqrt{\sum_{i=1}^{N} \left(r_i - r_g \right)^2 / N} \quad (4)$$

where Rg represents the position of the molecular center, ri represents the position of the i atom, and N is the number of atoms. Solvent-accessible surface areas (SASAs) of IEY were calculated using the Connolly algorithm [32]. The root-mean-square deviation (RMSD) of each atom in IEY from the initial conformation was also calculated. The distance is defined between terminal Ile-C_δ and Tyr-O_{OH}.

2.4. NMR experiments

^{1}H NMR, ^{1}H–^{1}H COSY, and 2D-NOESY spectra were measured using a Bruker DMX 500 spectrometer operating at 500 MHz with an accuracy of ±0.1 K at 298 K. The mixing time was 80 ns, and the number of scans was set to 16. IEY was dissolved in DMSO (with TMS) solution and aqueous solution (90% H_2O and 10% D_2O), which is used in biological NMR [33–36]. The chemical shift reference of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used in the aqueous solutions.

3. Results and discussions

3.1. Conformational analysis

The distance (Dis), Rg, RMSD, and SASA are four of the most important factors to be considered when analyzing the flexibility and conformation of biomolecules in solution. Interestingly, these factors show different values in different solutions.

The average values of distances between Ile-C_δ and Tyr-O_{OH} in DMSO were larger than those in water, as shown in Fig. 2. The shorter the intramolecular distance, the more folded the conformations. The distances in aqueous and DMSO solutions were in the regions of 3–16 and 7–15 Å, respectively. The large distance region indicated that IEY molecules in aqueous solution were more flexible than those in DMSO, and they could rapidly transform from extended to folded conformations. Folded conformations of IEY molecules could be observed in water but not in DMSO.

RMSD and SASA variation ranges were larger in aqueous solution than those in DMSO solution (Figs. 3 and 4). These findings indicated

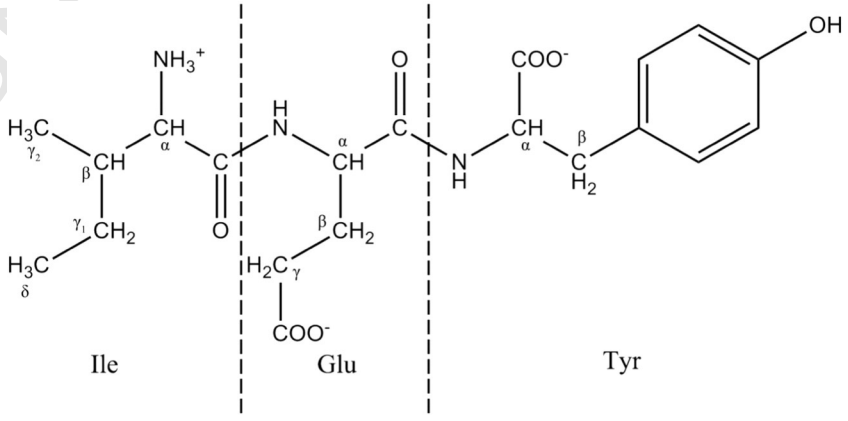

Fig. 1. Structure of IEY molecule.