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Binding Difference of Inhibitors ACD and TDZ to A-FABP Revealed by Molecular Dynamics Simulations

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Abstract. Adipocyte fatty-acid binding protein (A-FABP) is abundantly expressed in macrophage and adipocyte, and it is a potential target for the treatment of atherosclerosis and metabolic disease. In this work, binding differences of two inhibitors ACD and TDZ to A-FABP were studied by using principal component (PC) analysis, molecular mechanics generalized Born surface area (MM-GBSA) and solvated interaction energy (SIE) methods. The results show that the binding of inhibitor TDZ to A-FABP is stronger than that of ACD to A-FABP. The calculation of residue-based free energy decomposition and dynamics analysis of hydrogen bonds suggest that hydrophobic interactions and hydrogen bonding interactions play important roles in the structural stability of A-FABP. The information obtained from this work will provide a useful clue for design of effective drugs targeting A-FABP.

1. Introduction

Fatty acid binding proteins (FABPs) can reversibly bind to hydrophobic ligands including long chain fatty acids and these kinds of proteins have relatively low molecular weight of (14~15 kDa) [1]. Up to now, at least nine family members of FABPs have been discovered, and adipocyte fatty acid binding protein (A-FABP) is one of the most widely studied members among FABPs [2,3]. A-FABP is the fourth fatty acid binding protein to be discovered, so it is also called fatty acid binding protein 4 (namely FABP4). Structurally, A-FABP consists of two α-helices, ten β-sheets, and the helix-loop-helix domains covering the top of the structures to form a binding pocket for inhibitors (Figure 1.1(A)). This protein, mainly existing in adipose tissue and macrophages [4,5], plays a key role in the regulation of metabolism, inflammation and immune response [6,7]. The bindings of inhibitors to A-FABP can effectively inhibit the development of atherosclerosis, therefore, A-FABP has been a potential target for the treatment of inflammation, atherosclerosis and metabolic disease.

Over the past few years, a series of inhibitors of A-FABP have been reported [8,9] and a number of crystal structures of A-FABP associated with various ligands have been determined [10-12], which provides structural basis for further investigating binding modes of inhibitors to A-FABP. In this study, two inhibitors ACD and TDZ are selected to investigate their binding difference to A-FABP. The structures of ACD and TDZ are shown in Figure 1.1(B-C) [13,14]. The structural difference of two inhibitors results in different binding abilities of ACD and TDZ to A-FABP. Therefore, it is of importance for design of potent inhibitors targeting A-FABP to probe the underlying binding mechanisms of inhibitors ACD and TDZ to A-FABP at atomic levels.

The previous studies demonstrated that molecular dynamics (MD) simulations and binding free energy calculations have been universal tools for investigating structure and dynamics of A-FABP as well as ligands-protein binding mechanisms [15-24]. In current work, the conformational change of A-FABP induced by inhibitor bindings was probed by applying principal component (PC) analysis [25-30]. At the same time, molecular mechanics generalized Born surface area (MM-GBSA) [31-39] and solvated interaction energy (SIE) methods [40,41] were employed to comparatively study binding difference of ACD and TDZ to A-FABP. We expect that this work is able to provide a theoretical guidance for design of effective drugs to treat metabolic disease related with A-FABP.

2. Theoretical methods

2.1 System preparations

The crystal structures of A-FABP associated with two inhibitors ACD and TDZ were taken from Protein Data Bank (PDB): 3RZY for the *apo* A-FABP [42], 1ADL for the ACD-A-

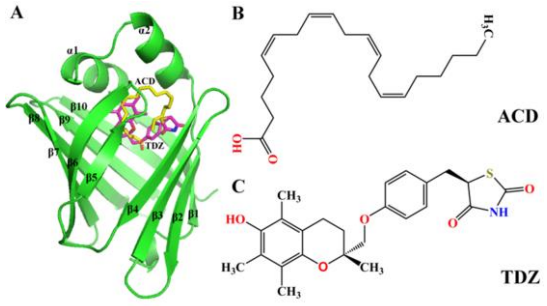

Figure 1.1: Structures of molecules: (A) structure of A-FABP in a cartoon diagram and the structures of inhibitors are shown in line modes, (B) inhibitor ACD and (C) inhibitor TDZ.

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FABP system [13] and 2QM9 for the TDZ-A-FABP compound [14]. All the crystal water molecules were remained in the starting structures. All missing hydrogen atoms were added to the corresponding heavy atoms by using the Leap module in Amber 16 [43]. The FF99SB force field was used to describe the protein and water molecules [44]. The general Amber force field (GAFF) was applied to optimize the structures of two inhibitors ACD and TDZ at a semiempirical standard [45,46] and the antechamber module was used to assign AM1-BCC charges to ACD and TDZ [47]. Then, three systems were solved in a truncated octahedral box composing of TIP3P water molecules, keeping a 12.0 Å buffer along each dimension and a certain number of counterions were added to neutralize these systems [48].

2.2 MD simulations

Before the starting of MD simulations, it is important to perform energy minimizations on three systems to remove bad contacts between the complex and solvent molecules. The energy optimization of each system was conducted in two steps. Firstly, the harmonic constant of 100 *kcal/mol·Å*$^{-2}$ was used to restrict the complex so as to better optimize the water molecules and counterions. Secondly, all atoms were freely minimized without any restrictions. The steepest descent and conjugate gradient methods were combined to perform energy minimization in each stage. Then, all systems were slowly heated from 0 *K* to 300 *K* in 1 *ns*. After that, the dynamic equilibrium was made on each system at temperature of 300 *K* and constant pressure of 1 *atm*. Finally, 150 *ns* MD simulations were performed on three investigated systems without any restrictions. The Langevin thermostat with a collision frequency of 2.0 *ps*$^{-1}$ was utilized to regulate the temperature of the three systems. All the energy optimization and MD simulations were performed by applying PMEMD module in Amber. The SHAKE algorithm is used to restrain the chemical bonds involving hydrogen atoms, and the time step of dynamic simulation is set to 2 *fs*. The long-range electrostatic interactions were calculated by employing the particle mesh Ewald (PME) method. The electrostatic and van der Waals interactions were truncated at a suitable distance of 9.0 Å.

2.3 Principal component analysis

It has been demonstrated that PC analysis is a powerful tool to investigate the conformational change of protein induced by inhibitor bindings [49,50]. In this work, PC analysis was performed on MD trajectories to study the collective motions of A-FABP using the CPPTRAJ module in Amber 16 [51]. The collective motions were described by constructing the positional covariance matrix *C* based on the atomic coordinates, and the elements of the positional covariance matrix *C* can be calculated by the following equation:

$$C_{ij} = < (q_i - < q_i >)(q_j - < q_j >) > \quad (i, j = 1,2,3,3N), \quad (1)$$

where the q_i symbolizes the Cartesian coordinate of C_α atom

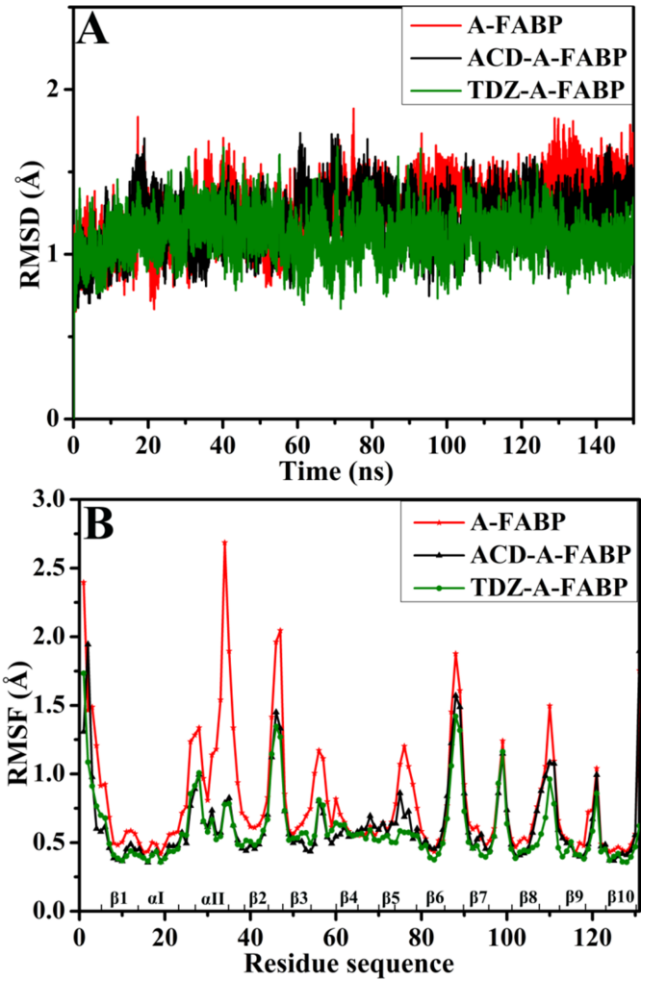

Figure 3.1.1: (A) The root-mean-square deviations (RMSDs) of the backbone atoms relative to the corresponding crystal structures as function of simulated time and (B) the root-mean-square fluctuations (RMSFs) of Cα atoms for A-FABP (red), ACD-A-FABP (black) and TDZ-A-FABP (green)

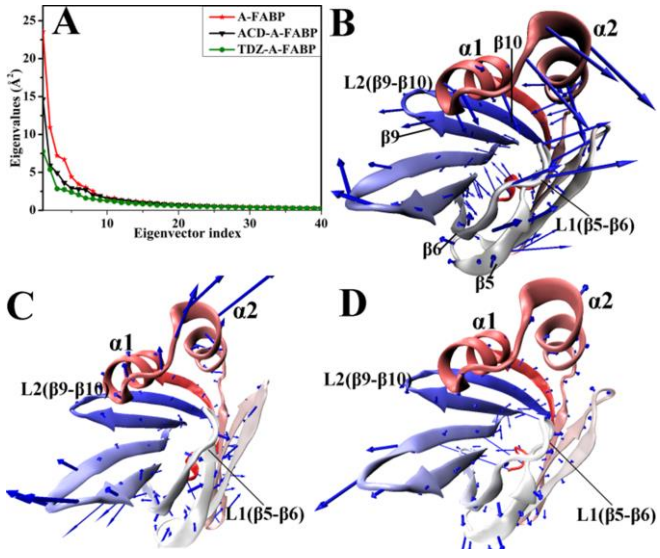

Figure 3.2.1: Results of collective motions in A-FABP from principal component analyses. (A) Eigenvalues of the total motions for A-FABP against the corresponding eigenvector indices. Concerted motions of domains along the first eigenvector stemming from principal component analysis: (B) the *apo* A-FABP, (C) the ACD-A-FABP complex and (D) the TDZ-A-FABP complex.