Journal of Molecular Graphics and Modelling 49 (2014) 99–109

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

Effect of ligand binding on the dynamics of trypsin. Comparison of different approaches

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ARTICLE INFO

Article history:
Received 2 January 2014
Received in revised form 7 February 2014
Accepted 8 February 2014
Available online 17 February 2014

Keywords:
Trypsin
Signal transduction
Molecular dynamics
GNM
PCA

ABSTRACT

The intramolecular signal transduction induced by the binding of ligands to trypsin was investigated by molecular dynamics simulations. Ligand binding changes the residue-residue interaction energies and suppresses the mobility of loops that are in direct contact with the ligand. The reduced mobility of these loops results in the altered flexibility of the nearby loops and thereby transmits the information from ligand binding site to the remote sites. The analysis of the flexibility of all residues confirmed the coupling between loops L1 (185–188) and L2 (221–224) and the residues in the active center. The significance of S1 pocket residues for the signal transduction from the active center to the substrate-binding site was confirmed by the dynamical network and covariance matrix analyses. Gaussian network model and principal component analysis demonstrated that the active center residues had zero amplitude in the slowest fluctuations acting as hinges or anchors. Overall, our results provide a new insight into protein–ligand interactions and show how the allosteric signaling may occur.

1. Introduction

Ligand binding is an important step in many biological processes. It can affect the structure and dynamics of a protein [1–3]. Although the process starts via local interactions with residues in direct contact, it often has an effect on remote protein regions. The mechanism, by which the information is passed from one site to another within a folded protein, is not clear. In some proteins, binding of ligand induces structural changes. However, in many proteins, ligand binding changes protein dynamics without inducing significant structural changes. It has been suggested that ligand binding initiates perturbations that propagate to the remote sites of protein [4–6]. Signal transduction induced by the ligand binding may affect the functional properties of protein, e.g. cause an allosteric effect. Initially, the allosteric effect was associated with oligomeric proteins. However, the allosteric behavior has been also observed within a single protein domain [5]. Recently it was suggested that all dynamic proteins could demonstrate allosteric properties [6].

The mechanisms of information transfer range from enthalpically driven conformational changes to merely entropically driven motions or can be a combination of both effects [7]. Inter-residue communication, the change of intrinsic dynamics of protein, and long-range correlated motions can contribute to signal transduction in proteins depending on the properties of a particular system. Dynamics, flexibility and ligand-induced conformational changes in biological macromolecules have become an active area of research [8–10]. The role of long-range correlated motions and the mechanism of signal transduction were studied for ubiquitin [11], calmodulin [3], maltose/maltodextrin-binding protein [12], lipase [8], and other proteins [13]. It has been recognized that the conformational and global dynamics correlates to enzymatic function [13–15]. Several theoretical and experimental studies have shown that the catalysis and specificity are not controlled by a few residues, but rather are the property of entire protein framework [16], and the enzymatic activity depends on a subtle interplay between chemical kinetics and molecular motions [13].

Here, we use molecular dynamics (MD) simulations to investigate the intramolecular signal transduction induced by the ligand binding to trypsin. Trypsin belongs to the family of serine proteases, which are intensively investigated experimentally and theoretically [17–22] and they are well suited for the detailed analysis of protein dynamics and for testing the new methods of analysis. Signal transduction is a key phenomenon of the allosteric regulation of protein activity. The allosteric effect was found in trypsin-like serine proteases, including factor IXa and thrombin [23–25]. For example, the binding of inhibitor alters the conformation of the 60-loop near the active site of thrombin that leads to enhanced

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http://dx.doi.org/10.1016/j.jmgm.2014.02.001
1093-3263/

reactivity with bovine pancreatic trypsin inhibitor (BPTI) [23]. Heparin modulates the 99-loop of factor IXa affecting its functional activity [24]. Allosteric inactivation of a trypsin-like serine protease points to new therapeutic strategies aimed at inhibiting or activating the enzyme [22,25].

Trypsin catalyzes the peptide or ester bonds hydrolysis of substrates containing Arg or Lys residues. Trypsin has 223 amino acid residues arranged in two six-stranded beta barrels. The residues H57, D102, and S195 form a catalytic triad, whereas the residues 189–195, 214–219, and 224–228 form a primary substrate-binding pocket called S1 binding pocket. The specificity of trypsin is determined by the amino acid residues at positions 189, 216, and 226. The residues 185–188 and 221–224 form two loops near the S1 pocket, called L1 and L2, respectively [16].

The dynamics of trypsin alone and in complex with ligands was studied before [19,22,26,27]. Experimental and theoretical studies have shown that the S1 substrate-binding pocket and the two loops outside the binding pocket (L1 and L2) significantly affect the enzyme activity and substrate specificity of trypsin [17,20]. Targeted molecular dynamics was used to demonstrate that the activation and deactivation of a-chymotrypsin begins with the movement of loop (215–225) pulling on loop (186–194) [20].

Here, we analyze the several components of signal transduction mechanism: how the binding of ligand changes the residue-residue interaction energy and the dynamics of protein and what role the long-range correlated motions play in the mechanism of signal transduction. These findings provide additional information for the development of new specific drugs.

MD simulation is a powerful instrument for studying the dynamics of different systems. However, the analysis of MD trajectories is a complicated and time-consuming procedure. We use several different computational approaches to analyze protein motions and compare their advantages and shortcomings. We use the Gaussian network model (GNM) [13,14,28] and anisotropic network model (ANM) [29,30] to determine the motions occurring at low-frequency modes. The dynamic cross-correlation matrix (DCCM) and principal component analysis (PCA) [31,32] based on the analysis of covariance matrix obtained from MD trajectory are used to investigate long-range correlated motions in protein. The analysis of residue-residue interaction energies and their correlation [33,34] provides new insights into the pathway of signal transduction.

2. Materials & methods

2.1. *MD simulations*

The coordinates of trypsin in complex with bovine pancreatic trypsin inhibitor (BPTI) were obtained from the Protein Data Bank (PDB ID: 3OTJ.pdb) [35]. The structure of free trypsin was obtained by removing the BPTI molecule from the complex. Comparison of the structures of unbound trypsin (PDB entry code 1S0Q.pdb) and trypsin in the complexes with BPTI (3OTJ.pdb) and with small inhibitor (1O2T.pdb) reveals that change of the protein backbone is very little. The root-mean square deviation (RMSD) of backbone atoms of ligand-bound trypsin with respect to unbound protein is about 0.3–0.4 Å. BPTI is a native inhibitor of trypsin and N^{α}-benzoyl-L-arginine ethyl ester (BAEE) is a specific trypsin substrate, which is widely used for experimental study of trypsin activity.

The structure of substrate N^{α}-benzoyl-L-arginine ethyl ester (Figure S1) was constructed and was optimized using PM3 method [36]. The structure of the complex of trypsin and BAEE was constructed by manual substitution of the BPTI molecule with BAEE so that N, Cα, C atoms of backbone and Cβ, Cγ, Cδ atoms of the side chain of K15 residue of BPTI were superimposed with corresponding atoms of Arg residue of BAEE. In that case, carbonyl oxygen atom of the substrate is fixed at the oxyanion hole, the guanidine group is located near to D189 and the ester C—O bond is in vicinity of S195.

MD simulations were performed using NAMD 2.6 [37] software with CHARMM27 force field parameters for the proteins [38], CHARMM36 all-atom carbohydrate force field [39] for BAEE and TIP3P for water molecules. Simulations were carried out under NPT ensemble conditions with periodic boundary conditions and Particle Mesh Ewald (PME) electrostatics using a 2 fs time step. The Langevin piston method was used to maintain a constant pressure of one atm with a piston period of 100 fs and a piston decay of 50 fs. The temperature was maintained at 300 K. Langevin dynamics maintained the temperature at 300 K with a damping coefficient of 1.0 ps^{-1}. The trypsin alone or in the complex with ligands was solvated in a box of $80 \times 94 \times 74$ $Å^3$. Following the initial minimization, heating, and equilibration stages for 2 ns, the production phase of each MD simulation ran for 20 ns. Coordinates were saved every 10 ps for further analysis. The overall stability of each simulation system was evaluated by monitoring the time evolution of the root-mean square deviation (RMSD) of backbone atoms with respect to the initial structure (Figure S2). Trajectories were analyzed using the VMD [40] and CHARMM [41] program packages. GNM, ANM and PCA were carried out with ProDy procedure [42] implemented in VMD program. Default values of force constants and distance cut-offs were used for the ANM (15 Å), for the GNM (10 Å) analyses. All molecular images were prepared using the VMD.

2.2. *Binding energy calculation*

The binding energy was calculated as the difference between the energy of the complex and the energy of the individual components from a single trajectory of the complex according to:

$$< \Delta E_{\text{bind}} > = < \Delta E_{\text{VdW}} + < \Delta E_{\text{elec}} + \Delta E_{\text{polar}} >$$

where < > denotes an MD-averaged quantities; E_{VdW} stands for the van der Waals interaction energy; E_{elec} stands for the electrostatic interaction energy and G_{polar}, the polar contribution of solvation energy, is the energy required to move the system from a vacuum dielectric ($\epsilon_{\text{in}} = 1.0$) to an aqueous dielectric ($\epsilon_{\text{out}} = 78$). The sum of electrostatic and polar solvation energies (PB)

$$\Delta E_{\text{PB}} = \Delta E_{\text{elect}} + \Delta G_{\text{polar}}$$

was calculated using a Poisson–Boltzmann implicit continuum solvent model [43] and the CHARMM [41] program. In CHARMM calculations, the grid spacing was set to 0.45 Å, and the longest linear dimension of the grid was extended at least 20% beyond the protein. Despite the MM/PBSA method is sensitive to simulation protocols, such as charge models, force fields, the solute dielectric constant it is widespread used for the study of protein–ligand interaction. Applicability of the method is intensively discussed in the literature [44,45]. Entropy analysis was not included in this work.

2.3. *Dynamic cross-correlation matrix*

Motional correlations between/among residues were analyzed by examining the dynamic cross-correlation map (DCCM) of Cα atoms for trypsin in the ligand-bound and unbound states. Protein structures from the trajectory were superimposed onto the reference structure in order to remove an overall translational and rotational motion of protein.