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Analysis of ligand binding to proteins using molecular dynamics simulations

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ABSTRACT

This work aims to explore theoretically the molecular mechanisms of ligand binding to proteins through the use of molecular dynamics simulations. The binding of sodium dodecyl sulfate (SDS) to cobra cardio toxin A3 (CTX A3) and thiourea (TOU) to lysozyme have been chosen as the two model systems. Data acquisitions were made by Gromacs software. To begin with, the collisions of ligand molecules with every residue of CTX A3 and lysozyme were evaluated. With this information in hand, the average numbers of collisions with each residue was defined and then assessed. Next, a measure of the affinity of a residue, P_i, referred to as conformational factor, toward a ligand molecule was established. Based on the results provided, all site-making residues for CTX A3 and lysozyme were identified. The results are in good agreement with the experimental data. Finally, based on this method, all site-making residues of bovine carbonic anhydrase (BCA) toward the SDS ligand were predicted.

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1. Introduction

The interactions between proteins and other molecules are critical to many biological systems and processes. It has become increasingly clear that signal transduction, metabolic regulation, enzyme cooperativity, physiological response and other processes such as carrying oxygen by hemoglobin are all dependent upon non-covalent binding (Straatsma and McCammon, 1992). Therefore, great stride has been made and a range of techniques developed for studying such interactions.

The equilibrium dialysis, diffusion process, titrometry, calorimetry and various spectroscopic methods are among many techniques used for investigating the binding of a ligand to proteins. In equilibrium dialysis, a bag with a semipermeable wall and containing a protein is placed in the buffer solution. Equilibrium occurs across the membrane such that the free ligand concentration becomes the same both inside and outside the bag, although some of the ligand is bound to the protein; the fraction of ligand bound to the protein, then, can be calculated by making use of the total ligand concentration. This method has been used for studying a number of ligand-binding interactions (Housaindokht et al., 2005a, b; Housaindokht and Moosavi-Movahedi, 1994).

The method based on the diffusion process is, compared with the equilibrium dialysis, both simple and much less time consuming. Besides, it is more realistic, i.e. closer to *in vivo* condition. Therefore, it is quite suitable in the case of unstable proteins (Housaindokht et al., 2002).

Binding of ligand to proteins frequently causes change to their three-dimensional structures, leading to a change of absorption. Accordingly, spectroscopic techniques are valuable tools in studying conformational changes caused by ligand binding. Under these circumstances, however, it is not generally possible to assess the binding constant (Saboury et al., 2002).

Calorimetric methods are used to work out the thermodynamic parameters of binding processes (Housaindokht et al., 1993, 2005a, b). For instance, free-energy changes associated with binding of *n*-alkyl-sulfonates to insulin at various pH values and enthalpy changes of the interaction between glucose-oxidase and a number of *n*-alkyl tri-methyl ammonium bromides homologous at 25 °C were determined by these techniques (Moosavi-Movahedi and Housaindokht, 1990; Jones and Brass, 1991). Titrometric methods, on the other hand, can be used to study proton binding to proteins.

In spite of having many applications, experimental techniques have certain limitations. For example, equilibrium dialysis involves a great deal of time to reach the equilibrium state (ca 90 h). Moreover, ligands may bind to the membrane and this will affect the accuracy of analysis. Spectroscopic techniques are also limited to those situations where the ligand binds to chromophores or to the sites that have an effect on the chromophores. For instance, a fluorescence spectrum is only

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suitable to determine the binding sites that involve Trp, Tyr and Phe residues. Furthermore, there would be no easily accessible way to assess the contribution made by structural changes, which in most cases is associated with ligand-binding processes, to the overall experimental thermodynamics parameters. In addition, the common approaches in studying the ligand binding to macromolecules are unable to specify the binding sites (Scatchard, 1949; Hill, 1910; Wyman, 1965; Huang and Schroeder, 2006; Beutler and van Gunsteren, 1994; Gilson et al., 1997). For example, to study how new drugs bind to human serum albumin, competition experiments only enable us to determine whether or not the new drug binds to the known sites such as the warfarin binding site (Dufour and Dangles, 2005).

Using computational approaches to investigate and analyze the binding interactions of proteins with ligands can provide useful insights for drug design (Chou, 2004; Chou et al., 2003, 2006; Du et al., 2004, 2005a, b, 2007; Gao et al., 2007; Guo et al., 2007; Li et al., 2007a, b; Lubec et al., 2005; Sirois et al., 2004; Wang et al., 2007a, b, c, d, 2008; Wei et al., 2005, 2006a, b, 2007; Ye et al., 2007; Zhang et al., 2006; Zheng et al., 2007). Among the computational methods developed to predict and analyze protein–ligand binding sites are POCKET (Levitt and Banaszak, 1992), LIGSITE (Hendlich et al., 1997), SURFNET (Laskowski, 1995), CAST (Liang et al., 1998) and LIGSITE^{csc} (Huang and Schroeder, 2006). LIGSITE^{csc} is an extension of LIGSITE. Instead of defining protein–solvent–protein events on the basis of atom coordinates, it uses the Connolly surface and defines surface–solvent–surface events. In the Connolly algorithm, a hypothetical probe sphere (usual radius 1.4 Å) rolls over the protein.

Molecular dynamics simulations, generally, enable investigations of dynamics and conformational changes of biological macromolecules to be conducted and can yield information that would not be available through any other means (Lemaitre et al., 2004; Fraternali et al., 2003; Monticelli et al., 2004; Dairou et al., 2006; Shirts et al., 2003).

In the study of ligand-binding simulations, the free energy of binding is a measure of the complex stability and generally is used in the study of all binding processes (Stewart and McCammon, 2006). Various approaches have been developed to estimate the relative free energy of binding. Among these, free-energy perturbation (FEP) and thermodynamics integration (TI) techniques have been applied in order to predict the accurate free energy of binding a ligand to macromolecules. Among the other methods, one can point out to linear interaction energy (LIE) (Almlöf et al., 1994), molecular mechanics/Poisson Boltzmann surface area (MM/PBSA) (Gilson et al., 1997), chemical Monte Carlo/molecular dynamics (CMC/MD) (Eriksson et al., 1999), four dimensional-potential of mean-force (4D-PMF) (Beutler and van Gunsteren, 1994) and one-window free-energy grid (OWFEG) (Pearlman, 1999). Each one of these methods has been employed to deal with a single kind of ligand binding to macromolecules (Kollman, 1993; Gilson et al., 1997; Eriksson et al., 1999; Randmer and Kollman, 1998).These techniques have their own limitations too. For instance, in spite of being more accurate, TI and FEP methods have major restrictions in that they only allow us to compare binding of two different ligands to one site or binding of one ligand to different sites. Besides, running the calculations is quite more costly (Lindahl et al., 1995).

In this work, a new technique is established to assess the affinity of a residue toward a given ligand in terms of the number of collisions between them, which is obtained by means of dynamics simulations. The interactions of SDS with CTXA3 and thiourea (TOU) with lysozyme have been chosen as the model systems. Based on the presented method all site-making residues of bovine carbonic anhydrase (BCA) toward the SDS ligand were most likely predicted.

1.1. Simulation details

All calculations were carried out using gromacs3.3.1 package (Lindahl et al., 1995) and gromos96 force field (van Gunsteren et al., 1996). The components of simulation boxes for the given systems are listed in Table 1. It should be noted that the number of water molecules, ligands and all other components related to each simulation box have been obtained on the basis of the experimental values reported in references Forouhar et al. (2003), Salem et al. (2006) and Katherine et al. (2005) for CTXA3, lysozyme and BCA, respectively.

A steepest-descent algorithm was performed to minimize the energy of each system and to relax the water molecules.

Molecular dynamics simulation for each system was carried out in two stages. In the first stage, position-restrain simulation was conducted, in which the atoms of the protein molecule were held fixed whereas the water molecules were free to move around so that they would reach the equilibrium state. In the second stage, each system was simulated with a time step of 0.2 fs. LINCS algorithm (Berendsen et al., 1981) was employed to fix the chemical bonds between the atoms of the protein and SETTLE algorithm (Darden et al., 1993) in the case of water molecules. The atoms in the system were given initial velocities according to Maxwell–Boltzmann distribution at 300 °C. To maintain a constant temperature and pressure for various components during simulations, the Brendsen coupling algorithm was used (Hess et al., 1997; Berendsen et al., 1984) for each component of the system with relaxation times of 0.1 and 0.5 ps, respectively. As the systems involve many positive and negative charges, PME algorithm was applied to estimate the electrostatic interactions. In this algorithm every atom interacts with all atoms in the simulation box and all of their images in an infinite number of identical copies surrounding the main box; so satisfactory results are produced from the electrostatic interactions (Danciulescu et al., 2004).

2. Results and discussion

MD simulations make it possible to study the behavior of a protein and a ligand over a specific time span. Due to the motions of the ligand and protein molecule, the ligand could collide with different parts of the protein structure. It can also be expected that the ligand will collide more frequently with binding sites on the protein structure. With this in mind, the following points have

Table 1
Overview of studied systems and simulation detail

Protein	PDB entry	dimension of box	No. of water molecules	Ligand	No. of ligands	Span time of simulation (ns)
CtxA3	1H0J	$10 \times 10 \times 10$	30000	SDS	14	2
Lysozyme	2F4A	$4.6 \times 4.6 \times 4.6$	2602	TOU	100	2
BCA	1V9E	$7 \times 7 \times 7$	10000	SDS	32	5

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