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Quantitative assessment of kinase selectivity based the water-ring network in protein binding sites using molecular dynamics simulations

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ABSTRACT

Protein kinases are implicated in a variety of diseases, such as cancer and inflammation, and are thus an important target for the pharmaceutical industry. However, the design of selective protein kinase inhibitors can prove challenging owing to the presence of a highly conserved binding site for ATP in kinases. Here we describe a novel method for analyzing the water-ring network that predicts kinase selectivity based on a molecular dynamics approach. To quantify the water-ring network in the binding site, we established a method to calculate the dipole moments of the water-ring network at specific positions in the ATP binding pocket. For two kinase systems (ZAP-70/Chk1 and MAP3K5/PDPK1), we found that the orientation of dipole moments plays a critical role in the protein-ligand binding mechanism. This solvent-centric approach complements current theoretical methods that consider only the steric and electrostatic properties of protein surfaces.

1. Introduction

Protein kinases, among the most important drug targets, have a 518-member gene family [1] and are highly conserved in the ATP binding site; consequently, selectivity is a highly important consideration in the design of kinase inhibitors. Although selective kinase inhibitors have been previously reported, achievement of the required selectivity remains challenging and therefore the sequence and structure of the ATP binding site common to the kinase family has been studied [2,3]. However, Viet et al. reported that kinase selectivity data are not strongly related to structural or sequential relationships between kinase targets [4]. This implies a challenge for discovering kinase-selective inhibitors, and that novel descriptors are needed to classify the kinase family according to selectivity data.

In addition to the sequence and structure of binding sites, water molecules play a crucial role in mediating protein-inhibitor interactions [5,6], and many studies show that water molecules that solvate protein binding sites contribute to the hydrophobic interaction considered to be a principal thermodynamic driving force [7,8]. Because of the importance of these water molecules, several studies have attempted demonstrate kinase selectivity using a solvent analysis approach.

Barillari et al. analyzed a dataset consisting of 171 protein kinase structures from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB), representing 19 protein kinases from different branches of the kinome [9]. They reported that structurally similar ATP binding sites of kinases often have significantly different conserved water molecules, and also showed that differences in conserved water patterns could provide useful information for the development of selective kinase inhibitors.

In another study, Fernandez et al. proposed that a quantifiable structure motif, called dehydron, is central to biomolecular interactions [10]. A dehydron is a defectively packed backbone hydrogen bond within proteins that is destabilized by exposure to bulk solvent. It has been demonstrated that re-engineering the tyrosine kinase inhibitor imatinib can repair dehydron shielding by positioning a hydrophobic group to obtain a selective c-Kit inhibitor [11].

Another way to account for solvent effects is through the thermodynamic analysis of water molecules from an explicit solvent molecular dynamics simulation, called WaterMap [7]. The successful application of this method has increased understanding of the binding profiles of a number of pharmaceutically relevant targets [8,12].

The method called Solvent Dipole Ordering (SDO) involves the treatment of water molecules with dipole moments [13]. It has been reported that SDO regions overlap significantly with the 3D structures of known inhibitors bound to the proteins [14].

Hydrogen-bonded water-ring networks are thought to play a critical role in kinase binding mechanisms. Several theoretical and crystallographic structure studies have therefore attempted to elucidate the properties of these water-ring networks [15–20]. In our previous study, we demonstrate correlations between water-ring networks and binding affinity in protein kinases [21,22].

Based on this background, we have investigated the structure and dipole moment of water-ring networks connected by the hydrogen-

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bonded cyclic network in active sites. The distribution of water-ring networks was observed, and its frequency was measured to statistically analyze the results. Furthermore, we calculated the dipole moments of water-ring networks and showed that their location and dipole in protein binding sites is related to kinase selectivity data. In summary, the aim of this study was to explain solvent effects in protein-ligand binding at an atomic level using on molecular dynamics simulations.

2. Details of calculations

2.1. Protein preparation

The protein kinase structures used in this study were obtained from PDB [23] under the following codes: 1U59 (ZAP-70) [24], 1NVR (Chk1) [25], 4BF2 (MAP3K5) [26], and 1OKY (PDPK1) [27]. All non-protein molecules including ligands and solvents were removed. The structures were prepared using the Prepare Protein module in Discovery Studio 4.5 (Accelrys, San Diego, CA, USA). This process included the identification of missing residues and the assignment of bond orders and formal charges in a protein, followed by the addition of hydrogen atoms. Protonation states were assigned under the assumption that systems had a pH of 7.0.

2.2. Molecular dynamics simulations

Classical molecular dynamics simulations were performed using GROMACS version 4.5.3 [28]. The CHARMM27 all-atom force field [29] was used to generate the protein topology and the TIP3P [30] water model. Protein without ligand was solvated into a cubic box under periodic boundary conditions. The box size of each system was chosen to accommodate a minimum of 1.2 nm from the protein to the surface of the box walls. To neutralize the system, counter ions were added accordingly. Prior to molecular dynamics (MD) simulation, energy minimization of the system was performed with 500 steps of steepest descent. Following minimization, the system was equilibrated in two steps with position restraints on the heavy atoms of the protein. The first equilibration phase was conducted under NVT (canonical ensemble) for 100 ps at 300 K. Next, equilibration of pressure was performed under NPT (isothermal-isobaric ensemble) for 200 ps. Finally, a production molecular dynamics simulation was performed in the absence of any restraints. The temperature and pressure of the system were maintained using the Berendsen weak-coupling method. Short-range nonbonded interactions were cut-off at 1.2 nm with long-range electrostatics calculated using the particle-mesh Ewald (PME) [31,32]. We applied the dispersion correction due to the use of a cut-off for Lennard–Jones interactions. The V-rescale thermostat [33] was used to maintain temperature, and the Parrinello–Rahman barostat [34] was used for isotropic pressure coupling. The LINCS algorithm [35] was applied to all bonds involving hydrogen atoms, with a time step set to 1 fs. The product simulation at 300 K and 1 bar was continued for 10 ns. For this analysis, the trajectory from 8.5 ns to 10 ns was used and coordinates were saved every 15 ps.

2.3. Structure of water-ring network

Water molecules connected by hydrogen bonds form various kinds of hydrogen-bonded cyclic water-ring networks. The potential functions considered here involve a rigid water model, TIP3P. The classical form of the interactions between water molecules are often conveniently modeled through the Lennard–Jones (LJ) plus Coulomb interactions [30]. If v(a,b) is the interaction potential energy between water molecules a and b, then.

$$v(a,b) = \sum_{i}^{on\ a}\sum_{j}^{on\ b}\frac{q_i q_j e^2}{r_{ij}} + \frac{A}{r_{oo}{}^{12}} - \frac{C}{r_{oo}{}^{6}}$$

where r_{oo} represents the distance between oxygen atoms, and q_i is the partial charge on site *i*. The parameters *A* and *C* were chosen to yield reasonable structural and energetic results for liquid water. The values of parameters are as follows:

$$A = 582{,}000\ \text{kcal}\ \text{Å}^{12}\ \text{mol}^{-1}$$

$$C = 595\ \text{kcal}\ \text{Å}^{6}\ \text{mol}^{-1}$$

$$q_O = -0.834e,\quad q_H = 0.417e$$

To determine the hydrogen bond between water molecules, we chose the energy criterion of −2.25 kcal/mol as its value closely corresponds to the minimum of the pair-energy distribution of potential [30].

We focused on the small water-ring network such as the trimer, tetramer, pentamer and hexamer. Thus, water polygons larger than the water hexamer were not considered in this study. To determine the water-ring networks displaced by staurosporine, a kinase inhibitor, we superimposed the proteins in the trajectory onto the staurosporine-binding complex structure. Only water-ring networks within 1.5 Å from staurosporine were selected. There are no the crystallized water molecules found within 1.5 Å from staurosporine in all used X-ray crystal structures.

2.4. Dipole moment of water-ring network and staurosporine

The total dipole moment (μ) of the water-ring network was calculated as

$$\mu(t) = D\left(q_H\sum_{i}^{2N}(R_i(t)-C(t)) + q_O\sum_{i}^{N}(R_i(t)-C(t))\right)$$

where q_H is the charge on hydrogen and q_O is the charge on oxygen (0.417, and −0.834, respectively); $R_i(t)$ is the vector specifying the position of atom *i* at time *t*; *C* is the vector of center of the water-ring at time *t*; and D is a unit conversion factor, approximately equal to 4.8032. D is given by D = Debye / (*e* Å), and *N* is the number of atoms in the water-ring network [36,37]. Similarly, the dipole moment of staurosporine was calculated using the Dipole Moment module in Discovery Studio 4.5. The dipole moment (μ) was set in the form of

$$\mu = D\sum_{i}^{N}|q_i|(R_i-C)$$

where q_i is the charge on atom *i* derived from the Momany–Rone partial charge; R_i is the 3D coordinates of the position of atom *i*; *C* is the 3D coordinates of the center of charge; and D is a unit conversion factor as described previously. Next, we monitored angle correlation as a function of the dipole alignment between the water-ring network and staurosporine. The average dipole alignment is written as

$$\langle cos\theta\rangle = \frac{\langle \mu_{WAT}, \mu_{STU}\rangle}{(||\mu_{WAT}||\,||\mu_{STU}||)}$$

where μ_{WAT} is the dipole vector of the water-ring network; μ_{STU} is the dipole vector of staurosporine; and θ is the angle between the dipole vector of the water-ring network and staurosporine. The averaged cosine <cosθ> represents the degree of dipole alignment, and becomes 1 if the dipole of the water-ring network and staurosporine take the same orientation; it yields −1 if the dipole of the water-ring and staurosporine take exact opposite directions.

2.5. Molecular docking simulations

Molecular docking was carried out using the LigandFit module in Discovery Studio 4.5. Two stages comprise the LigandFit protocol: (1) docking, the conformational searching of flexible ligands using a