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The analysis of water network for kinase selectivity based on the MD simulations

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ABSTRACT

We studied the effect of water molecules correlated with a hydrophobic environment by analyzing a hydrogen-bonded water network using molecular dynamics simulations to explain kinase selectivity within two example systems, a variant of Gleevec and a series of substituted JNK ligands. We carried out the analysis of the five-membered ring (R5) structure for water molecules, which is a dominant structure in aqueous solutions containing hydrophobic solutes due to hydrophobic effects. The patterns of water network using the R5 structure are well mapped to the selective activity profile for the kinase inhibitors.

1. Introduction

Protein kinase, which is one of the most important drug targets, having a 518-member gene family [1], is highly conserved in the ATP binding site, and consequently selectivity is a very important issue for generating kinase inhibitors. Despite the availability of many crystal structures, various features of ligand structure–activity relationships (SAR) could not be explained using only steric, electrostatic, and hydrogen bonding effects. So many researchers have considered additional factors such as solvation effects for a comprehensive interpretation of the available SAR. A prime example of tyrosine kinase inhibitor, Gleevec is an inhibitor of Bcr-Abl, as well as of PDGFR, and c-Kit. Clinical trials with Gleevec confirmed its validity against chronic myeloid leukemia (CML), where its target is the Bcr-Abl kinase, as well as a proportion of gastrointestinal stromal tumors (GISTs), where its target is the c-Kit kinase [2,3]. Fernández et al. analyzed the patterns of residence time of water molecules around the binding site of Gleevec in the above mentioned kinases to obtain information on selectivity. They reported that the propensity for water removal was different in the hinge site of Bcr-Abl and c-Kit. Thus, this local difference in de-wetting propensity could be targeted, and Gleevec could be modified by the addition of a methyl group to enhance its activity specifically on the c-Kit kinase, while suppressing Bcr-Abl inhibition and promoting JNK inhibition [4]. Barillari et al. analyzed a dataset consisting of 171 protein structures from PDB, representing 19 protein kinases from different branches of the kinome. They demonstrated that structurally similar ATP binding sites of kinases often have significantly different water patterns, and they showed that differences in conserved water patterns for the kinase could provide a useful tool to increase the selectivity of kinase inhibitors [5]. In another study, the methodology called *WaterMap* was developed using a statistical thermodynamic analysis of water molecules from an explicit solvent molecular dynamics simulation. The method has been successfully applied to understand binding profiles for a number of pharmaceutically relevant targets [6,7]. These researches focused on the importance of water molecules in determining the potency and selectivity of kinase inhibitors.

In our study, we describe the effect of water molecules correlated with a hydrophobic environment by analyzing a hydrogen-bonded water network from the results of molecular dynamics simulations to explain kinase selectivity within a variant of Gleevec [4] and a series of substituted JNK ligands [8].

2. Details of calculations

2.1. Molecular dynamics simulations

For this study, we have performed molecular dynamics simulations of three systems: c-Abl, c-Kit, and JNK isoforms (JNK1 and 3) in an aqueous solution. Crystal structures of c-Abl, c-Kit, and JNKs taken from the Protein Data Bank [9] were used in this study. The PDB codes for them are, 1IEP (c-Abl) [10], 1T46 (c-Kit) [11], 3V3V (JNK1) [12], and 1JNK (JNK3) [13]. All the MD simulations have been performed by using the CHARMM program (version 32.0) [14] with an all-hydrogen force field (PARAM22). We carried out NPT molecular dynamics simulations in an aqueous solution, using TIP3P water potential [15] at

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300 K. The Leap-frog Verlet algorithm with a time step of 1.0 fs was used to integrate the equation of motion. All interactions within 14.0 Å were calculated, and a shifted function and switching function were employed to smoothly reduce the energies and to avoid discontinuities in the energies for the van der Waals term and the electrostatic term, respectively. Before molecular dynamics simulations, they were relaxed sufficiently by the steepest descent and conjugate gradient minimization to eliminate the initial strains in systems. Heating was done from 50 K to 300 K for 10 ps and following the equilibration of 20 ps at the target temperature. Product simulations were done in 100 ps, and snapshots were saved every 1 ps for trajectory analysis.

2.2. Analysis of cyclic water ring

The water molecules were obtained within a 6.2 Å radius sphere of amino acids in the hinge region. Each pair of water molecules interact via the potential function of

$$\in_{mn} = \sum_{i}^{on\ m} \sum_{j}^{on\ n} \frac{q_i q_j e^2}{r_{ij}} + \frac{A}{r_{00}^{12}} - \frac{C}{r_{00}^{6}}$$

where q_i are the partial charges relative to the charge of the electron; A and C can be expressed in terms of Lennard–Jones as $A = 4\epsilon\sigma^{12}$ and $C = 4\epsilon\sigma^{6}$. To determine the hydrogen bond between water molecules, we chose the energy criterion of −2.25 kcal/mol [15]. Every 1000 steps, we calculated the frequency of a five-membered water ring for the target region.

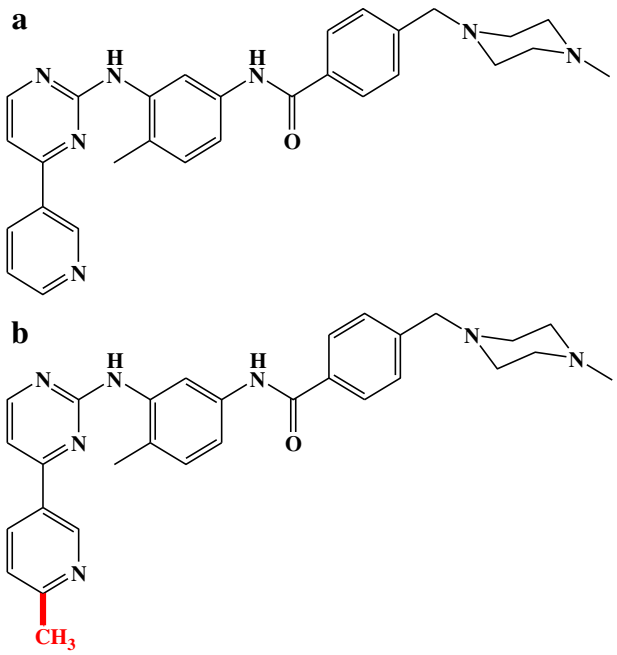

Fig. 1. (a) The structure of Gleevec. (b) The structure of a variant of Gleevec. The added methyl group is highlighted in bold and red.

3. Results and discussion

For the analysis of the water structure, we concentrated on the hydrogen-bonded circular network, five-membered ring (R5) structure, which is a dominant structure in aqueous solutions containing hydrophobic solutes due to hydrophobic effects [16,17]. First of all, we show that the method is able to describe the activity profile of Gleevec and a variant of Gleevec, which activity is otherwise difficult to explain using traditional techniques. To compare the affinity difference, we used three structures from the PDB; 1IEP, 1T46 and 3V3V, which are some of a number of structures with Gleevec in a complex with the kinase domain of Abl, c-Kit, and JNK1, respectively. c-Abl and c-Kit have similar binding modes against Gleevec. We thought that we could explain the affinity improvement of a ligand with a hydrophobic group, such as methyl, near the binding site, because in the hydrophobic environment water's five-membered ring distribution is superior [16,17].

An interesting site is proximal to the pyridine ring of Gleevec in Fig. 1a. This site is expressed in Fig. 1b. We analyzed the forming position and frequency of the five-membered ring structure at this binding site. Despite a 50% sequence identity within 6 Å of the active site, c-Abl and c-Kit showed differences in the five-membered ring pattern around the hinge region. In the c-Kit and JNK1, distribution of the five-membered ring structure was observed to have a similar pattern as shown in Fig. 2a. c-Ab1 had a 5-fold decreasing frequency at this site, in contrast to c-Kit and JNK1; also, the forming position showed differences from those of c-Kit and c-Abl (Fig. 2b). Fernández et al. showed that a small hydrophobic group, a methyl group, improves the activity of Gleevec against c-Kit and JNK1 but lowers it against Abl. As shown in Fig. 2, differences in water's five-membered ring pattern can explain this result well. The region in which the five-membered ring shows a high frequency can be seen to be a hydrophobic environment. So, if the hydrophobe is located near this region, the activity increases as the ligand binding energy increases due to the hydrophobic interaction. Because the five-membered ring patterns in c-Kit and JNK1 were shown to have a high frequency near the methyl group, the addition of the methyl group to the pyridine ring of Gleevec brought about an improvement of ligand affinity. In Fig. 3, we illustrate the methyl group of the Gleevec analogue which occupied the R5 position in the C-Kit hinge region. However, when a methyl group was added to the hinge region of the c-Abl on which the five-membered ring had slightly formed, affinity decreased 75%. Fernández et al. have shown that using the theory of 'de-hydrons' [18], a de-wetting hot spot can be discovered in the hinge region of the c-Kit and JNK1 due to water repulsion. However, a de-wetting site in c-Abl was not formed in the hinge region. For the de-wetting hot spots of the five-membered ring pattern that represents the hydrophobic environment, c-Kit and JNK1 are all the same, so the de-hydrons theory supports the idea of a difference of the five-membered ring pattern.

Next, the other example is the JNK isoforms, which share a more than 90% amino acid sequence identity and the ATP pocket is >98% homologous. Interestingly, for Gleevec and variants of Gleevec, adding methyl substituents, showed a different activity pattern against JNK isoforms in a high-throughput screening campaign. To look for the reason why this variant is more selective against JNK1 even though the sequence is similar to that in the isoform of JNK1, we performed an experiment that shows the difference of water network patterns. We also carried out molecular dynamics simulations in an aqueous solution for JNK1 (PDB, 3V3V) as mentioned earlier and JNK3 (PDB, 1JNK), and then analyzed the hydrogen-bonded circular network, R5, of water molecules. The whole sequence similarity of JNK1 and JNK3 was over 90%, but in the five-membered ring distribution that is formed near the hinge region there was an extensive difference (Fig. 4). In the case of JNK1, the five-membered ring sporadically formed around the entrance pocket of the extended hinge region. On the other hand, in JNK3, the five-membered ring extensively formed around the hinge region. We will explain the difference of the binding affinity of JNK1 and JNK3 with this five-membered ring pattern. Chamberlain et al. reported that the substitution of hydrophobic isopropyl piperazine in