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Structural and dynamical properties of different protonated states of mutant HIV-1 protease complexed with the saquinavir inhibitor studied by molecular dynamics simulations

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Abstract

To understand the basis of drug resistance, particularly of the HIV-1 PR, three molecular dynamics (MD) simulations of HIV-1 PR mutant species, G48V, complexed with saquinavir (SQV) in explicit aqueous solution with three protonation states, diprotonation on Asp25 and Asp25′ (Di-pro) and monoprotonation on each Asp residue (Mono-25 and Mono-25′). For all three states, H-bonds between saquinavir and HIV-1 PR were formed only in the two regions, flap and active site. It was found that conformation of P2 subsite of SQV in the Mono-25 state differs substantially from the other two states. The rotation about 177° from the optimal structure of the wild type was observed, the hydrogen bond between P2 and the flap residue (Val48) was broken and indirect hydrogen bonds with the three residues (Asp29, Gly27, and Asp30) were found instead. In terms of complexation energies, interaction energy of −37.3 kcal/mol for the Mono-25 state is significantly lower than those of −30.7 and −10.7 kcal/mol for the Mono-25′ and Di-pro states, respectively. It was found also that protonation at the Asp25 leads to a better arrangement in the catalytic dyad, i.e., the Asp25–Asp25′ interaction energy of −8.8 kcal/mol of the Mono-25 is significantly lower than that of −2.6 kcal/mol for the Mono-25′ state. The above data suggest us to conclude that interaction in the catalytic area should be used as criteria to enhance capability in drug designing and drug screening instead of using the total inhibitor/enzyme interaction.

Keywords: HIV-1 protease; Molecular dynamics simulations; Mutation; Protonation state; Saquinavir

1. Introduction

The human immunodeficiency virus type 1 (HIV-1) is the causative agent in acquired immunodeficiency syndrome (AIDS). This disease was recognized in the U.S., around 1981 [1]. There are three essential enzymes involved in the replication cycle of this virus which are reverse transcriptase (RT), protease (PR) and integrase (IN). They are therefore important targets for drug development. Although effective drugs have been developed against HIV-1 PR and HIV-1 RT, however, it was reported that inhibitors at the first target are more potent [2]. Therefore, HIV-1 PR is an attractive target for antiviral therapy. The HIV-1 PR consists of two identical polypeptides of 99 amino acids (Fig. 1), each chain contains an N-terminal Pro and C-terminal Phe. The active site of PR is formed at the dimer interface containing two conserved catalytic dyad, Asp25 and Asp25′ [3]. The substrate binding cleft is composed of equivalent residues from each subunit and is bound on one side by the active site aspartic acids and on the other by the flap region. To date, there are several FDA-approved PR inhibitors in clinical uses and saquinavir is considered to be a highly potent and selective transition state analog inhibitor of the HIV-1 PR [4].

A major problem for the clinical uses of PR inhibitors is the development of drug resistance due to substitutions observed in almost 50% of the residues and over 20 residues associated with resistance to clinically available inhibitors [5]. Recently, the correlation between the inhibitor structure of the HIV-1 PR target and the drug resistance was studied [6]. The kinetic experimental data show that decreased affinity of the

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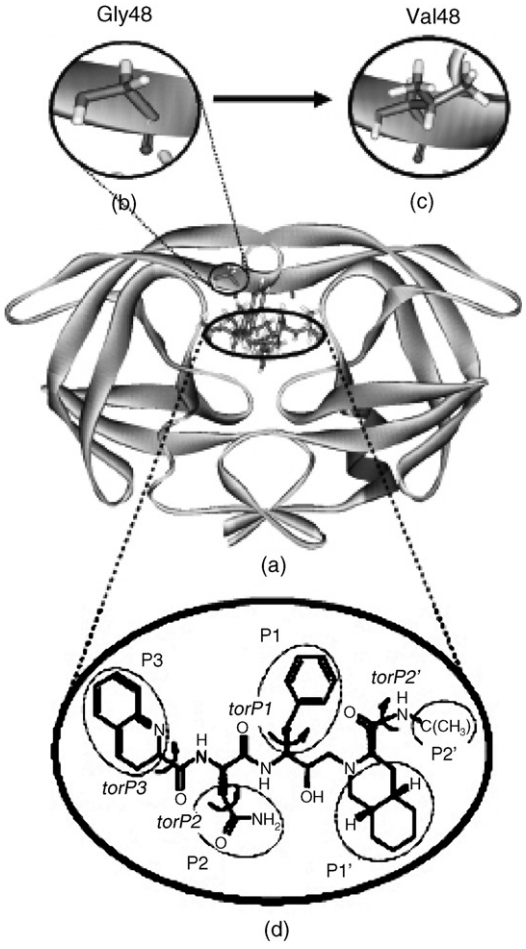

Fig. 1. Schematic representation of the HIV-1 PR complexed with saquinavir (a) in which molecular structure of Gly48 (b) and Val48 (c) in the G48V and of saquinavir (d) were also displayed. Here, P1, P2, P3, P1′ and P2′ subsites are labeled by circle and torsional angle of each subsite was defined by *tor*.

drugs for many mutants is caused primarily by an increase in dissociation rates. The HIV-1 PR mutant species, G48V, is associated in vivo with saquinavir resistance [7]. Residue Gly48 locates in the flap region of the HIV-1 PR and is responsible for the formation of the S2/S2′ and S3/S3′ binding site—the regions of the enzyme that bind with P2/P2′ and P3/P3′ of the inhibitor, respectively [8]. In addition, it was found that Gly48 plays a role in shaping the binding pocket of the active site and stabilizing the enzyme–substrate complex [6]. Substitution of this residue by valine introduces a bulky side chain into the S3/S3′ binding pocket and results in resistance towards saquinavir leading to an increase of the inhibition constant (K_i) value by 13.5-fold [9]. However, this effect was not observed for other inhibitors.

As an aspartic protease, the protonation state of the catalytic aspartic acids, Asp25 and Asp25′, is the key to explain the catalytic mechanism of both wild type and mutant type. Plane wave-based ab initio molecular dynamics calculations [10] as well as NMR measurements [11] of the HIV-1 PR complexed with pepstatin lead to the conclusion that the system is, at least, monoprotonated. In addition, the ab initio calculation method was preformed on the active site of HIV-1 PR and the free energy perturbation (FEP) method was used to determine the binding free energy of four different protonated states of HIV-1 PR complexed with A74704 by Ky-Youb Nam et al. [12]. The results have the potentially significant implications that the complex is monoprotonated on Asp25.

In this study, molecular dynamics simulations of the G48V HIV-1 PR complexed with saquinavir in explicit aqueous solution were carried out. Due to an unclear detailed mechanism of the reaction catalyzed by HIV-1 PR [13], therefore, the simulations have to be performed for all three possible protonation states of the two aspartic residues, Asp25 and Asp25′.

2. Computational method

2.1. Preparation of the initial structures

In order to investigate the relative dynamics properties among different protonation states of the mutant HIV-1 PR complexed with the inhibitor (saquinavir), three MD simulations were preformed, monoprotonate on Asp25 (Mono-25), monoprotonate on Asp25′ (Mono-25′) and diprotonate on both aspartic acids (Di-pro). The crystal structure of saquinavir bound to wild-type protease was taken from the Protein Data Bank PDB (1HXB) [14] and used as the reference structure. Among the two available crystal structures, the first one which are commonly used in literatures [15] was applied for our simulation. All missing atoms of the protein were added using the LEaP module in the AMBER 7 software package [16]. The protonation state of the ionizable residues, the C- and the N-termini, except for D25/25′, was assigned based on the predicted pK_a values at pH 7. The pK_as of ionizable residues were calculated based on the Poisson–Boltzmann free energy calculations. Details of these calculations are given elsewhere [17]. Hydrogen atoms were then added to the two catalytic aspartic residues in order to generate the Mono-25, Mono-25′ and Di-pro states using the LEaP module in AMBER 7.0 software package [16].

It should be noted that the X-ray structure of the double mutant, G48V/L90MSQV complex (1FB7) could be considered as an alternative template [4]. However, the X-ray coordinates of the second monomer of the double mutant are not available. Thus, 1HXB is considered to be more appropriate as a template. The mutant protease enzyme was modeled from this structure replacing glycine by valine at residue 48 (G48V) using the Insight II molecular modeling software.

2.2. Molecular dynamics simulations

Three MD simulations were carried out for the mutant HIV-1 PR complexed with saquinavir in the above mentioned states. The Mono-25, Mono-25′ and Di-pro systems were solvated by 9633, 9627 and 9627 TIP3P water molecules [18], respectively. The crystallographic waters were also included in the simulations. The sodium and chloride ions were added to