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Journal of Molecular Graphics and Modelling 26 (2007) 720–727
Journal of Molecular Graphics and Modelling
www.elsevier.com/locate/JMGM

Insight into analysis of interactions of saquinavir with HIV-1 protease in comparison between the wild-type and G48V and G48V/L90M mutants based on QM and QM/MM calculations

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Received 12 December 2006; received in revised form 19 April 2007; accepted 28 April 2007
Available online 3 May 2007

Abstract

Saquinavir (SQV) was the first HIV-1 PR inhibitor licensed for clinical use and widely used for acquired immunodeficiency syndrome (AIDS) therapy. Its effectiveness, however, has been hindered by the emergence of resistant mutations. The two most important HIV-1 PR mutants are G48V and G48V/L90M. Inhibition studies of SQV on these mutants demonstrated 13.5- and 419-fold reductions of susceptibility, respectively. In this study, an analysis of energetic binding affinity between saquinavir and the HIV-1 PR wild-type and these two mutants has been performed in detail based on density functional theory and the hybrid quantum mechanical/molecular mechanical (QM/MM) calculations. We have found that the interaction of SQV with flap residue 48 of the mutants is significantly perturbed, as shown by the reduced stability of binding between SQV and residue 48 for the G48V and G48V/L90M mutants over the wild-type. This was associated with conformational changes of the inhibitor and the enzyme, leading to the loss of hydrogen bonding between the binding subsite P2 and the backbone carbonyl of residue 48. Moreover, the G48V/L90M mutations cause the repositioning of the residues close to residues 48 and 90, at important locations as a part of the flap and catalytic regions, respectively. The repositioning of these residues consequently perturbed the binding affinity of SQV in the pocket as indicated by the decreasing interaction energies. In addition to the loss of inhibitor/enzyme binding, it is interesting to observe that the mutation leads significantly to an increase of the stability of the enzyme.

Keywords: HIV-1 protease; Saquinavir; Molecular dynamics simulations; Mutation; ONIOM method

1. Introduction

The acquired immunodeficiency syndrome (AIDS) has become a serious global concern due to an increasing number of HIV patients and infected population all over the world. Since it was first recognized in 1981, the disease has never been completely cured. AIDS is caused by the human immunodeficiency virus (HIV). The replication cycle of the virus involves three essential enzymes, reverse transcriptase (RT), protease (PR) and integrase (IN). The three enzymes are become important targets for drug development. Effective drugs were developed against HIV-1 PR and HIV-1 RT; however, it was found that inhibitors at the first target are more potent [1]. Therefore, HIV-1 PR is an attractive target for antiviral therapy.

The HIV type I protease (HIV-1 PR) is a member of the aspartic protease family. The enzyme is a homodimer consisting of 99 amino acids. The active site contains two conserved active site residues, D25 and D25′. Interfacial contact between the subunits allows two crucial substrate binding clefts to be formed. They include the catalytic triad residues D25–T26–G27 on one side and the flap loop residues 46–54 on the other (Fig. 1). To date, ten HIV-1 PR inhibitors have been approved by the United Stated Food and Drug Administration (FDA), and are in clinical use. Saquinavir (SQV) is considered to be highly potent and selective to HIV-1 PR inhibitor [2]. It is classified as a peptidomimetic protease

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doi:10.1016/j.jmgm.2007.04.009

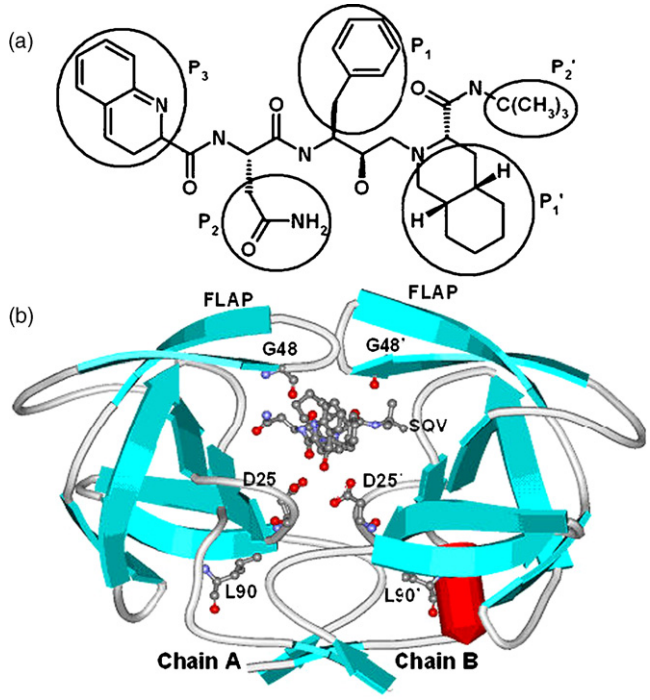

Fig. 1. Schematic representations of (a) saquinavir (SQV) and (b) the wild-type HIV-1 PR/SQV complex. According to the conventional classification, the protease subsites are designated by the inhibitor side chains P_1, P_2, P_3, P'_1 and P'_2. The catalytic dyad, D25 and D25′, as well as the major mutation residues, G48, G48′, L90 and L90′, are displayed as balls and sticks.

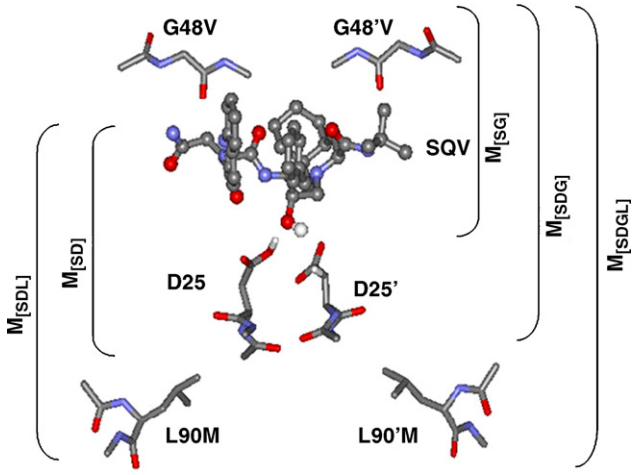

Fig. 2. Schematic structure representation of the quantum model for the interaction energy calculations, where saquinavir (SQV), the catalytic dyad (D25 and D25′) as well as the major mutations, G48V, G48′V, L90M and L90′M, are displayed (see text for more details).

inhibitor. The inhibitor contains a nonhydrolyzable hydroxyethylene isostere which was designed based on the transition state structure in the enzyme-substrate complex (Fig. 2).

There has been strong evidence that inappropriate treatment of HIV medications could result in sequential acquisition of drug resistant mutation. Genotypic and phenotypic resistance mutations of HIV-1 PR from the Stanford HIV database include almost 50% of the residues, and over 20 residues are associated with resistance to clinically available inhibitors [3], Recently, a correlation between inhibitor structure of the HIV-1 PR target and drug resistance was studied. Kinetic experiments show that the decreased affinity of drugs for many mutants is caused by an increase in dissociation rates [4]. The HIV-1 PR mutant species of the single mutation, G48V, and double mutation, G48V/L90M, are associated *in vivo* with saquinavir resistance by the enzyme [5] and kinetic studies on these mutants demonstrate 13.5- and 419-fold increases in K_i values, respectively, compared with the wild-type enzyme. Residue G48 is located in the flaps of the protease and contributes to the formation of the S_2/S'_2 and S_3/S'_3 binding sites the regions of the enzyme that bind with P_2/P'_2 and P_3/P'_3 of the inhibitor, respectively [6], but residue 90 does not directly make contact with the inhibitor.

Our previous molecular dynamics and quantum chemical calculations [7] showed that conformational change of the subsite P_2 of the G48V-SQV complex results in a decrease of interaction energy between this subsite and residue 48 [2]. This observation was supported by X-ray data where the authors stated that more space was needed in the P_3 subsite in order to accommodate the side chain of valine for the G48V mutation [2]. In this report, we employed extensive computations using density functional theory (DFT) and a hybrid quantum mechanical/molecular mechanical (QM/MM) method namely the Our own N-layered Integrated molecular Orbital and Molecular mechanics method (ONIOM) approach to calculate the interaction energy of an entire set and individual pairs of the systems. The studied systems included the HIV-1 PR wild-type and G48V and G48V/L90M mutants complexed with SQV. These mutations are more frequently found in patients treated with SQV. Viruses containing both G48V and L90M mutations result in an enzyme highly resistant to SQV. The inhibition constant (K_i) of the double mutant is 419 times less sensitive to SQV as compared with that of the wild-type [8].

2. Computational methods

A combination of different computational methodologies was applied in order to provide detailed insight into the structural, dynamical and energetic properties among the three complexes of HIV-1 PR/SQV which are (i) the wild-type (WT), (ii) a single mutation at residue 48 from glycine to valine (G48V) and (iii) the double mutation at residues 48 and 90 from glycine to valine and leucine to methionine, respectively (G48V/L90M). The molecular dynamics simulations were carried out and then followed by the quantum chemical calculations for extensively interaction energy analysis.

2.1. Molecular dynamics simulations

The WT, G48V single- and G48V/L90M double-mutant HIV-1 PR complexed with saquinavir inhibitor were constructed from the X-ray structure (Protein Data Bank code 1HXB; 2.3 Å resolutions). The protein was assigned to a monoprotonation state, in which the proton attaches to the carboxyl sidechain of D25. The TIP3P water molecules [9] were used to solvate the complexes. Sodium and chloride ions were added to neutralize the system. The force field parameters