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ScienceDirect
Journal of Molecular Graphics and Modelling 26 (2007) 558–570
Journal of Molecular Graphics and Modelling
www.elsevier.com/locate/JMGM

Structural analysis of lead fullerene-based inhibitor bound to human immunodeficiency virus type 1 protease in solution from molecular dynamics simulations

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Received 15 December 2006; received in revised form 16 March 2007; accepted 30 March 2007
Available online 4 April 2007

Abstract

Molecular dynamics (MD) simulations of the HIV-1 protease (HIVP) complexed with lead fullerene-based inhibitor (diphenyl C_{60} alcohol) in the three protonated states, unprotonated (Un-), monoprotonated (Mono-), and diprotonated (Di-) states at Asp25 and Asp25′ were performed. As the X-ray structure of the investigated complex is not available, it was built up starting with the X-ray crystallographic structure of the HIVP complexed with non-peptide inhibitor (PDB code: 1AID) and that of the diphenyl C_{60} alcohol optimized using the integrated ONIOM molecular orbital calculations. The inhibitor was, then, introduced into the enzyme pocket using a molecular docking method. Change of the HIVP binding cavity for all three states were evaluated in terms of distance between the two catalytic residues, Asp25 and Asp25′ as well as those between the catalytic residues and the flap regions. The torsional angles formed by the O–C–C–O of the two carboxyl groups of the catalytic dyad show the non-planar configuration with the most frequency at about −45° for the Un-, 35° and −95° for the Mono- and 60° for the Di-systems. At equilibrium, different orientations of the fullerene-based inhibitor in the three protonation states were observed. For the Di-state, the OH group of the inhibitor stably forms hydrogen bonds with the two aspartic residues. It turns to the flap region to form hydrogen bonding to the backbone N of Ile50′ for the Un-state. In contrast, the OH group turns to locate between the catalytic and the flap region for the Mono-states. Beside the molecular orientation, the rotation of the OH group of the inhibitor in the Un-state was also detected. In terms of solvation, the carboxylate oxygens of the aspartic residues in the Un- and Mono-states were solvated by one to three water molecules while the OH group in these two states was coordinated by one water molecule. This is in contrast to the Di-state in which no water molecule is available in the radius of 5–6 Å around the oxygen atoms of the carboxylate groups of enzyme and of the OH group of the inhibitor. The simulated results lead to the conclusion that the active site of the HIVP complexed with the diphenyl C_{60} alcohol is the diprotonation states on Asp25 and Asp25′.

Keywords: MD simulations; Fullerene-based inhibitors; HIV-1 protease; Molecular docking

1. Introduction

A number of potent and selective HIVP inhibitors have been developed and approved as drugs for the treatment of HIV infection. Most of these are peptidic such as saquinavir, ritonavir, etc. Efforts have been made in several laboratories to develop non-peptide-based HIVP inhibitors. One of these, which was a novel water-soluble fullerene derivative was proposed on the basis of modeling and then synthesized by Wudl, Friedman and coworkers in 1993 [1,2]. Although, the size of C_{60} fullerene looks very large for a drug, it is only one nanometer in diameter, roughly the size of many small pharmaceutical molecules. The ability of C_{60} fullerene derivatives to interact with the active site of HIV-1 protease has been examined through simple physical chemical analysis. Kinetic analysis of HIVP in the presence of various water-soluble C_{60} derivatives suggests a competitive

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doi:10.1016/j.jmgm.2007.03.013

mode of inhibition because of the ability to form bonds with the catalytic aspartic acids in addition to van der Waals contacts with the non-polar HIVP surface, thereby improving the binding.

Various functionalizations have been utilized both to increase the hydrophilicity (e.g. –OH, –COOH, $–NH_2$) and to prepare new compounds presenting biological and pharmacological activity. The inhibition values were promising with K_i in the range between 103 nM and 5.5 μM [3] and EC_{50} from 0.21 to 2.60 μM [4] in comparison with K_i of indinavir (0.14 nM), nelfinavir (0.28 nM), ritonavir (0.17 nM) and saquinavir (0.15 nM) [5]. The high level of solubility in water (34 mg/ml at pH 7.4) has been obtained by Hirsch and coworkers [6], who synthesized a dendrimeric fullerene derivative bearing 18 carboxylic groups ($EC_{50} = 0.22$ μM). Note that the solubility of amprenavir in water is 0.04 mg/ml. *In vitro* studies of a water-soluble fullerene derivative (diphenethylamino-succinate methano-C_{60}) with a peripheral blood mononuclear cell (PBMC) confirmed its inhibition ability with an EC_{50} of 7 μM. Such a compound has reached the same level of potency of the peptidomimetic inhibitors.

Diphenyl C_{60} alcohol, the best potency of C_{60} derivative, in current form is probably too non-polar to be a drug. However, due to a large portion of the molecule that could be modified, it offers the opportunity to synthesize new derivatives to enhance its binding affinity, solubility, toxicity, and bioavailability. Indeed, many antiviral fullerene drugs show good in vitro antiviral activity and lack of toxicity at concentrations up to approximately 100 μM [7–9] in comparison with the commercially available drugs such as amprenavir, indinavir, ritonavir, atazanavir, saquinavir, nelfinavir and lopinavir ($CC_{50} \approx 4.49$–4.95 μM) [10]. The total surface area of diphenyl C_{60} alcohol of 485 $Å^2$ is directly comparable to that of typical clinically used HIVP inhibitors, e.g., indinavir, 544 $Å^2$ as reported by Friedman et al. [3]. Although it has a relatively high molecular weight and density of packing of the atoms in the fullerene core determines its interactions with solvent and protein. This is comparable to clinically relevant compounds.

X-ray studies of structures of HIVP complexed with peptide or peptide analog inhibitors indicate substantial conformational changes of an enzyme upon binding, i.e., a very large movement of the flaps upon the size of the inhibitor bound at the active site, is often reported from both theoretical and experimental studies [11–17]. These significant changes indicate a role of structural dynamics in inhibitor-enzyme binding. Ma et al. and Carlson and McCammon and references therein [18,19] reviewed the recent methods, which incorporate protein flexibility to understand the complex nature of ligand–receptor binding.

A number of potent fullerene-based inhibitors were studied and patented [4,20–25]. However, an X-ray crystal structure of HIVP complexed with fullerene derivatives is not yet available. The first microscopic level prediction of the tight closing of the flexible flaps, when a novel C_{60}-based inhibitor is docked into the Un-, Mono-, and Di-protonated states of the active site of HIVP, was investigated by Zhu et al. [26] using molecular dynamics simulations. The simulations addressed the exclusion of water density near the flap regions, around the active-site region and in the cavity as well as the changes in the shape of the cavity in order to accommodate the inhibitor. This reduction of water leads to an enhancement of the hydrophobic interaction between the C_{60} moiety and the interior of the cavity, including the flaps. This finding is consistent with that of Friedman et al. [3], that the activity of an inhibitor compound can be improved by maximizing the amount of hydrophobic surface area. The simulations indicated, also, that the most effective binding of fullerene-based inhibitors to the active site takes place when the Asp dyad is diprotonated. The suggestion agreed well with the ^{13}C NMR experiment of the pepstatin A/HIVP complex reported by Smith et al. [27] and the *ab initio* molecular dynamics calculations by Piana et al. [28].

Besides, the issue of the connection between flap motion, specifically flap-closing and favorable inhibition of the HIVP was also addressed by Simmerling and coworkers [29–31] using molecular dynamics simulations. In this study, cyclic urea inhibitor was embedded into the open state of free protease. It was found that the HIVP flaps were changed spontaneously to the closed conformation.

To investigated in more detail, we studied the binding of HIVP and the best potency of C_{60} derivative, diphenyl C_{60} alcohol, with $K_i = 103$ nM according to Friedman et al. [3]. The structure analysis parameters such as internal motion of the inhibitor's torsional angles as well as rotation of the inhibitor, itself, its location within the cavity and an analysis of the hydrogen bonding between the inhibitor and the active-site Asp residues were reported from our simulations. We performed 1200 ps molecular dynamics simulations of the complexes for the three protonated states, unprotonated (Un-), monoprotonated at Ash25 (Mono-) and diprotonated at both Ash25 and Ash25′ (Di-). The results revealed an insight into the structural origins of binding strength, binding orientation, the flap motion, the possible relationship between binding and catalytic efficiency, and the numbers as well as orientation of water molecules in the HIVP cavity site.

2. Computational methods

2.1. Preparation of HIVP

Since the X-ray crystallographic structure of the HIVP complexed with the fullerene-based inhibitors has never been published, the initial HIVP structure was taken from the HIVP complexed with haloperidol derivative at 2.2 Å resolutions (1AID entry in PDB database) [32]. This structure is a class of HIVP non-peptide inhibitor complex and has a large cavity site (about 10 Å), which can accommodate the fullerene spheroid (about 7 Å). Its structure is considered to be in the ''closed'' form according to Simmerling and coworkers [29–31] where the flaps are pulled in toward the bottom of the active site. The HIVP cavity was defined as the distance from the tip of the flap (O atom of Gly48) to the catalytic oxygen (O atom of Gly27) for both two chains of the dimer. The structural water as well as the inhibitor was, then, removed. All hydrogen atoms were added into the structure. Ionization states for all other ionizable residues (except for Asp25/25′) were assigned according to the