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Flap opening dynamics in HIV-1 protease explored with a coarse-grained model

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Abstract

We present a one-bead coarse-grained model that enables dynamical simulations of proteins on the time scale of tens of microseconds. The parameterization of the force field includes accurate conformational terms that allow for fast and reliable exploration of the configurational space. The model is applied to the dynamics of flap opening in HIV-1 protease. The experimental structure of the recently crystallized semi-open conformation of HIV-1 protease is well reproduced in the simulation, which supports the accuracy of our model. Thanks to very long simulations and extensive sampling of opening and closing events, we also investigate the thermodynamics and kinetics of the opening process. We have shown that the effect of the solvent slows down the dynamics to the experimentally observed time scales. The model is found to be reliable for application to substrate docking simulations, which are currently in progress.

Keywords: Coarse-grained models; HIV-1 protease; Molecular dynamics; Brownian dynamics; Langevin dynamics

1. Introduction

Human immunodeficiency virus type 1 protease (HIV-1 PR) plays a key role in processing the viral polypeptide precursors. Since it was observed that virions that lack HIV-1 PR are noninfectious (Kohl et al., 1988), inhibitors of HIV-1 PR have been sought for use in chemotherapy of AIDS. A few have been approved for clinical therapy (de Clercq, 2002; Wlodawer, 2002; Kurup et al., 2003) but despite their high selectivity they induce side effects and also drug-resistant strains of the virus emerge rapidly. Therefore, a deeper understanding of the events associated with binding of substrates and inhibitors is crucial for the design of more potent and selective inhibitors.

The initial phase of the reaction involves the association of a ligand with the enzyme together with its proper recognition. In case of HIV-1 PR, it is the movement of flexible flaps that controls the access to the binding site (see Fig. 1). The flaps serve as a gate for the approaching ligand (Chang et al., 2006). Substantial conformational changes of the flap region have been noted where the flap tips can separate up to several tens of Å (Hamelberg and McCammon, 2005; Tozzini and McCammon, 2005; Hornak et al., 2006).

A set of crystal structures of the bound and native states of the protease has been solved (Vondrasek and Wlodawer, 2002; Berman et al., 2000). The differences between the two forms in the crystal involve mainly the flap region. However, even in the free form the flaps are still relatively closed over the active site, in a conformation that was previously called "semi-open" (in the present paper we choose a different criterion to sort the conformations, which assigns this conformation to the "closed" class). NMR experiments for the free enzyme showed a substantial conformational change in the flap region and found that this large-scale motion occurs on a micro- to millisecond time scale with a faster

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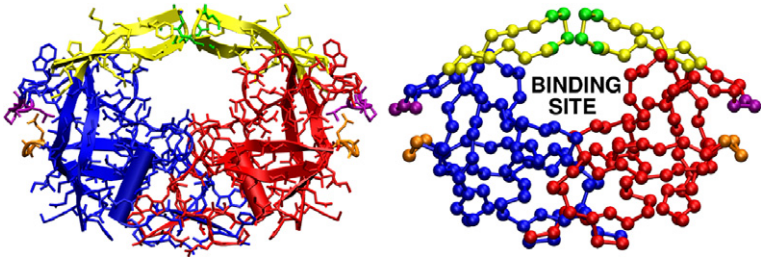

Fig. 1. Left: Heavy atom and ribbon representation of HIV-1 PR homo-dimer in the native form (PDB entry code—1HHP). Right: Coarse-grained Cα representation. Colors: monomers—blue and red, flaps (residues 43–57)—yellow, flap tips (49–52)—green, 17-turn (16–18)—orange, 39-turn (38–40)—purple.

movement on a subnanosecond time scale (Freedberg et al., 2002). In the inhibitor-bound protease, the flaps' flexibility was found to be very limited except for the flap tips residues Ile 50 and Gly 51 (Nicholson et al., 1995; Ishima et al., 1999). Overall, the NMR data suggest that the flaps stay in equilibrium between the closed and open forms, with the closed form being the dominant one.

The motion of the protease flaps has been also studied by all-atom molecular dynamics. These included an activated MD (Collins et al., 1995; Hamelberg and McCammon, 2005). A 10 ns MD led to flap opening, and curling of flap tips was proposed as a mechanism triggering the opening, but no re-closing was seen (Scott and Schiffer, 2000). A simulation of unbound V82F/I84V mutant was also reported but a complete flap opening was not seen (Perryman et al., 2004). Recently, an unconstrained all-atom simulation has been performed which led to a complete flap opening event together with re-closing (Hornak et al., 2006). However, all-atom approaches of the protease dynamics encountered difficulties due to the short simulation time scale. Therefore, there still is a need to understand the protease internal dynamics and especially its flap region mobility on longer time scales. In fact, this can reveal novel features of the flap opening mechanism, that might allow for better interfering with its movement. This might have profound implications on the design of new inhibitors of this enzyme.

In order to study the dynamics of the protease on a multiple microsecond time scale, we propose an extremely simplified coarse-grained model which allows for very long time scale simulations with modest computational costs. As we previously reported in a preliminary study (Tozzini and McCammon, 2005), this model is sophisticated enough to reproduce the flap opening dynamics. In this paper, we report extensive calculations in different statistical ensembles and their comparison with available experimental data which demonstrate the validity of the present model. We study the effect of the solvent by using stochastic dynamics and describe the kinetics and thermodynamics of the opening mechanism. The implications of our findings on the developments of novel anti-aids therapies are discussed.

2. Methods

2.1. Force field and its parameterization

The coarse graining procedure to pass from the all-atom representation to the one-bead representation is schematically described in Fig. 2. A whole amino acid is represented by a single bead placed on the Cα carbon. The force field (FF) potential energy U is the sum of the following terms

$$U = U_{\mathrm{b}} + U_{\theta} + U_{\alpha} + U_{\mathrm{nb}}^{\mathrm{loc}} + U_{\mathrm{nb}}^{\text{non-loc}} + U_{\mathrm{el}}.$$

The pseudo-bond term U_{b} is represented either as a sum of harmonic terms or of constraints (see Supplementary Information (SI) for details). The pseudo-bond angles (θ) and dihedrals (α) are primarily involved in determining the local conformational rearrangements. Most of the previously reported one and two-bead FFs use rather complex potentials for U_{α} imputing completely to this term the description of the conformational flexibility while U_{θ} is usually treated as harmonic (Tozzini, 2005). We have recently shown that this kind of description is not particularly adequate for highly mobile and flexible residues, such as glycine and alanine (Tozzini et al., 2006; Tozzini and McCammon, 2005) which are abundant in the flap region of HIV-1 PR. In these cases θ can sample more efficiently different local conformations, assuming two well separated values for contracted α-helix-like and for extended β-sheet-like conformations. Therefore, we used a quartic double well potential for U_{θ} (Tozzini and McCammon, 2005) whose relative shift and stability of the two minima are aminoacid-dependent (functional forms and parameters are given in the SI). The dihedral term U_{α} is treated here

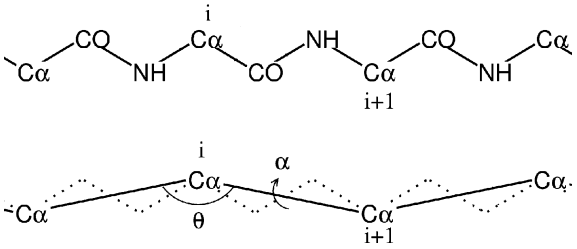

Fig. 2. Schematic representation of the coarse graining procedure. The internal coordinates are indicated.