6

Studies of drug resistance and the dynamic behavior of HIV-1 protease through molecular dynamics simulations

Fangyu Ding and Carlos Simmerling

INTRODUCTION

The human immunodeficiency virus (HIV) was first discovered to be the causative agent of acquired immunodeficiency syndrome (AIDS) in the early 1980s.[1,2] There are currently three main avenues for preventing virus replication. The first is to block attachment of virus to the host cell surface by inhibitors of binding to coreceptors, such as CCR5.[3,4] The second is to block the process of reverse transcription,[5] an approach taken by a major class of anti-AIDS drugs, including, for example, azidothymidine (AZT), delavirdine, nevirapin, and so on. The third way is to disrupt the function of the viral protease (HIV-PR) that cleaves the gag-pol polyproteins required to assemble an active virus by binding an inhibitor to the center of the protease and freezing it closed; this is described in more detail in this review. At present there are eleven FDA-approved protease inhibitors in clinical use: Agenerase (amprenavir), Aptivus (tipranavir), Crixivan (indinavir), Fortovase (saquinavir soft gel), Invirase (saquinavir hard gel), Kaletra (lopinavir-ritronavir), Lexiva (Fosamprenavir), Norvir (ritonavir), Prezista (darunavir), Reyataz (atazanavir), and Viracept.[6] All these inhibitors can lose most of their potency when confronted with mutations associated with drug resistance.[7] Therefore, a thorough understanding of the mechanistic events associated with binding of HIV-PR substrates and inhibitors is pharmacologically critical for the design of novel inhibitors of the enzyme. There is evidence that flexibility of the enzyme plays an important role in inhibitor binding and resistance.[8,9]

This chapter focuses on recent advances and challenges in understanding protease dynamics and its potential for revealing new approaches to HIV-PR drug design. A particular focus is the application of computational techniques that can provide detailed insight into the dynamic aspects of HIV-PR behavior. Because recent molecular dynamics simulations of HIV-PR have suggested that the dynamics of this enzyme is crucial for its function, affecting flexibility of the protease by, for example, allosteric inhibitors could provide new opportunities to design a more potent inhibitor.

Experimental data on structure of HIV-1 protease: Large structural rearrangement on binding

An extensive set of x-ray crystal structures of HIV-1 protease in both bound and unbound forms has been solved,[10] revealing a C_2 symmetric homodimer with a large substrate binding pocket covered by two glycine-rich beta-hairpins or flaps.[11–13] In almost all of the liganded forms, both flaps are pulled in toward the bottom of the active site ["closed" form, Figure 6.1(a)]. However, there are several crystal structures that have been resolved in an unusual "flap-intermediate" conformation with one flap partly and the other flap fully closed.[14,15] These observations provide experimental supports to the hypothesis that the substrate enters the protease binding site through the flaps and the subsequent flap motion is asynchronous with one flap closing first. In contrast to the bound structures, crystal structures of the ligand-free protease are more heterogeneous[16]; three conformations of the flap domains have been captured: "closed," "semiopen," and "wide-open" forms. Although the relationship between the conformational flexibility and catalytic activity is still unclear, it has been suggested that mutations might affect the flexibility of the unbound enzyme; for example, the M46I mutation appears to stabilize the closed form of the flaps.[17] Most ligand-free HIV-PR adopt the semiopen form [Figure 6.1(b)], in which the flaps are pulled up and shifted away from the active site, but still substantially cover the active site. A more striking difference between the semiopen and closed form of unbound enzyme is that the relative orientation of the flaps is reversed (top views in Figure 6.1). Despite the observation of semiopen conformation in five of the nine available crystal structures of unbound HIV-PR,[11–13,18–23] it was not entirely clear whether this reflects the preferred flap conformation in solution or is a result of crystal-packing effects.[24–26] Although a large-scale flap opening is presumably required for normal substrate access to the active site [Figure 6.1(c)], a transient open form was observed only in molecular dynamics (MD) studies[27,28] [Figure 6.1(c)], and an x-ray "wide-open" structure[18,21,22] is more likely an artifact due to the crystal-packing contact,[29] in which each flap