doi:10.1016/j.jmb.2011.03.038 J. Mol. Biol. (2011) 410, 756–760

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

Accessory Mutations Maintain Stability in Drug-Resistant HIV-1 Protease

Max W. Chang and Bruce E. Torbett*

Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA

Received 29 January 2011; received in revised form 16 March 2011; accepted 17 March 2011

Edited by M. F. Summers

Keywords:
HIV protease;
drug resistance;
differential scanning calorimetry;
protein stability;
viral evolution

The underlying mechanisms driving the evolution of drug resistance in human immunodeficiency virus (HIV) are only partially understood. We investigated the evolutionary cost of the major resistance mutations in HIV-1 protease in terms of protein stability. The accumulation of resistance mutations destabilizes the protease, limiting further adaptation. From an analysis of clinical isolates, we identified specific accessory mutations that were able to restore the stability of the protease or even increase it beyond the wild-type baseline. Resistance mutations were also found to decrease the activity of HIV protease near neutral pH values, while incorporating stabilizing mutations improved the enzyme's pH tolerance. These findings help us to explain the prevalence of mutations located far from the active site and underscore the importance of protein stability during the evolution of drug resistance in HIV.

© 2011 Elsevier Ltd. All rights reserved.

Introduction

The evolution of drug resistance in response to antiretroviral therapies continues to be a major problem in the treatment of human immunodeficiency virus (HIV)/AIDS. HIV protease inhibitors have been an important component of these therapies since the 1990s, and the viral protease has been studied extensively in order to elucidate the mechanisms of resistance. Typically, the development of resistance involves multiple mutations in the enzyme's active site and periphery.[1] Mutations near the active site decrease inhibitor binding, often at the cost of substrate processing, which can be restored by distal mutations.[2–6]

Changes in an enzyme's active site are associated with protein destabilization, and successive mutations may destabilize the protein sufficiently to completely disrupt function.[7] The important role of "permissive" mutations that improve protein stability has been shown in the development of bacterial antibiotic resistance,[7] and a similar phenomenon has been noted with oseltamivir resistance in influenza.[8]

In protein engineering contexts, incorporation of stabilizing mutations has been used to promote the development of new enzyme functionality. The directed evolution of an enzyme may be accompanied by decreases in stability that require compensation by other mutations to proceed.[9,10] To complement such studies, we have used the application of neutral drift, evolution without external selection, to increase protein stability and "evolvability."[11] Similarly, viral replication in drug-naïve patients occurs without antiviral drug selection, and the polymorphisms that arise could similarly have consequences for protein stability.

Only a relatively small number of mutations in HIV protease are associated with major resistance against nearly all current protease inhibitors (positions shown in Fig. 1), and their consequences for protein stability are largely unknown. Some studies have noted the increased stability of protease after the introduction of multiple mutations but have not quantified their individual

*Corresponding author. 10550 North Torrey Pines Road, MEM-131, La Jolla, CA 92037, USA. E-mail address: betorbet@scripps.edu.

Abbreviations used: HIV, human immunodeficiency virus; DSC, differential scanning calorimetry.

0022-2836/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.

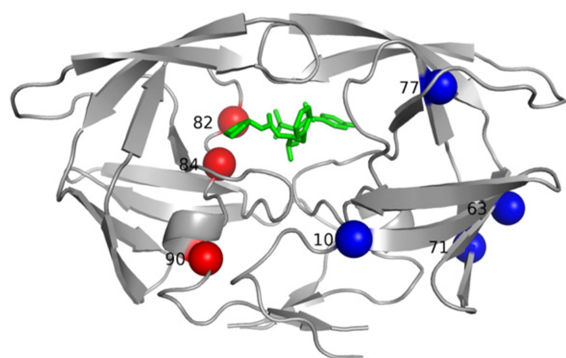

Fig. 1. The structure of HIV protease, a symmetric homodimer, with inhibitor (green) bound. Major resistance positions are shown in red on one subunit, and positions of candidate stabilizing mutations are shown in blue on the other subunit.

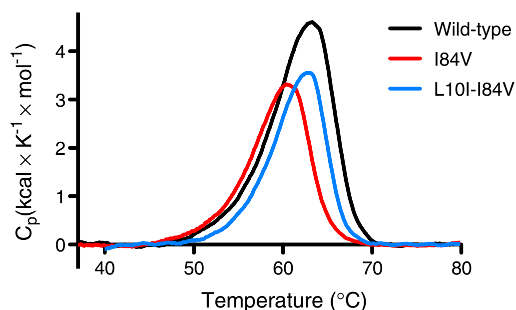

Fig. 2. Structural stability of wild-type, I84V, and L10I–I84V HIV proteases, as determined by DSC. Protease samples were measured using a Nano II DSC (Calorimetry Sciences Corporation) in a buffer containing 20 mM NaCl and 20 mM sodium acetate at pH 5.0. The protease (dimer) concentration for all samples was 30 μM, except for I84V–L90M, which was 24 μM.

effects.[6,12] One group found that the drug resistance mutation I84V lowered the melting temperature (T_m) of HIV protease, while the presence of 10 other mutations raised the T_m above the wild-type baseline.[13] These findings are consistent with the hypothesis that changes in the active site caused by resistance mutations negatively impact protease stability, leading to the development of other mutations that restabilize the enzyme. To further investigate this phenomenon, we studied the contributions of individual resistance mutations to HIV protease stability and identified compensatory mutations that were able to restore stability.

Major drug resistance mutations destabilize HIV protease

The V82A, I84V, and L90M mutations in protease are each capable of providing major resistance against several clinically approved inhibitors.[14] Positions 82 and 84 lie inside the active site, and mutations at these points directly affect the binding of substrate and inhibitors. Changes at position 90 affect the dimer interface, consequently altering the binding site. Using the NL4-3 strain as a template, we constructed protease mutants containing substitutions at these positions, then expressed and purified as described previously.[15]

Subsequently, the melting temperature of these mutants was determined using differential scanning calorimetry (DSC). As shown in Figs. 2 and 3a, the T_m of each mutant was at least 2.8 °C lower than the wild-type NL4-3 protease. Additionally, a double mutant containing both I84V and L90M mutations showed a large T_m decrease roughly equivalent to the sum of the individual mutations. In absolute terms, the measured T_m values for the wild-type and I84V proteases were roughly 10 °C higher than those reported by Muzammil *et al.*,[13] an inconsistency likely due to differences in experimental pH. However, the relative T_m change between the two proteases was fairly consistent, approximately 4 °C in the previous study *versus* 2.8 ° C in our study.

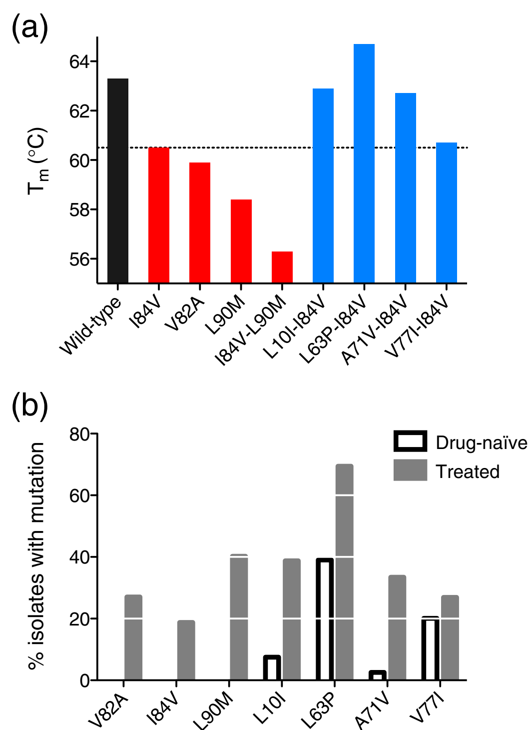

Fig. 3. Effect of drug resistance and accessory mutations on the stability of HIV protease. (a) The melting temperatures of HIV protease and mutants, as determined by DSC. (b) The prevalence of specific mutations in drug-naïve and treated patient isolates. Data from the Stanford HIV Drug Resistance Database.

Download English Version:

https://daneshyari.com/en/article/2185120

Download Persian Version:

https://daneshyari.com/article/2185120

Daneshyari.com