

Contents lists available at [ScienceDirect](http://ScienceDirect.com)

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Evolution of inhibitor-resistant natural mutant forms of HIV-1 protease probed by pre-steady state kinetic analysis



Maria Yu. Zakharova ^a, Alexandra A. Kuznetsova ^b, Elena N. Kaliberda ^a, Maria A. Dronina ^a, Alexander V. Kolesnikov ^{c,d}, Arina V. Kozyr ^c, Ivan V. Smirnov ^{a,e,f}, Lev D. Rumsh ^a, Olga S. Fedorova ^{b,g}, Dmitry G. Knorre ^b, Alexander G. Gabibov ^{a,e,f,**}, Nikita A. Kuznetsov ^{b,g,*}

^a Institute of Bioorganic Chemistry Russian Academy of Sciences, Moscow Region, 117997, Russian Federation

^b SB RAS Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, 630090, Russian Federation

^c State Research Center for Applied Microbiology and Biotechnology, Obolensk, Moscow Region, 142279, Russian Federation

^d Russian Institute of Immunological Engineering, Lyubuchany, Moscow Region, 142380, Russian Federation

^e Department of Chemistry, Lomonosov Moscow State University, Moscow Region, Russian Federation

^f Kazan Federal University, Kazan, Russian Federation

^g Department of Natural Sciences, Novosibirsk State University, Novosibirsk, 630090, Russian Federation

ARTICLE INFO

Article history:

Received 20 April 2017

Accepted 21 August 2017

Available online 24 August 2017

Keywords:

HIV-1 protease

Multidrug-resistant mutant

Active site evolution

Mechanism of protease action

Stopped-flow analysis

Pre-steady state kinetics

Enzyme-substrate interaction

FRET

ABSTRACT

Pre-steady state kinetic analysis of mechanistic features of substrate binding and processing is crucial for insight into the evolution of inhibitor-resistant forms of HIV-1 protease. These data may provide a correct vector for rational drug design assuming possible intrinsic dynamic effects. These data should also give some clues to the molecular mechanism of protease action and resistance to inhibitors. Here we report pre-steady state kinetics of the interaction of wild type or mutant forms of HIV-1 protease with a FRET-labeled peptide. The three-stage “minimal” kinetic scheme with first and second reversible steps of substrate binding and with following irreversible peptide cleavage step adequately described experimental data. For the first time, a set of “elementary” kinetic parameters of wild type HIV-1 protease and its natural mutant inhibitor-resistant forms MDR-HM, ANAM-11 and prDRV4 were compared. Inhibitors of the first and second generation were used to estimate the inhibitory effects on HIV-1 protease activity. The resulting set of kinetic data supported that the mutant forms are kinetically unaffected by inhibitors of the first generation, proving their functional resistance to these compounds. The second generation inhibitor darunavir inhibited mutant forms MDR-HM and ANAM-11, but was ineffective against prDRV4. Our kinetic data revealed that these inhibitors induced different conformational changes in the enzyme and, thereby they have different mode of binding in the enzyme active site. These data confirmed hypothesis that the driving force of the inhibitor-resistance evolution is disruption of enzyme-inhibitor complex by changing of the contact network in the inhibitor binding site.

© 2017 Elsevier B.V. and Société Française de Biochimie et Biologie Moléculaire (SFBBM). All rights reserved.

1. Introduction

HIV-1 protease (PR) remains an important pharmaceutical target. The enzyme is a member of the aspartic protease family and has an active site characterized by a deep substrate-binding pocket and two flexible gate loops, called FLAPs. The mechanistic events describing binding and processing of PR substrates are crucially important for the design of novel inhibitors. At present, numerous crystal structures of the substrate- and inhibitor-bound protease are available clarifying the molecular mechanism of drug resistance [1]. Alongside with static

Abbreviations: FRET, Förster resonance energy transfer; PR, HIV-1 protease; WT, wild type HIV-1 protease; MDR-HM, ANAM-11 and prDRV4, drug-resistant mutant protease forms; FLAP, flexible gate loops of the substrate-binding pocket; AM, Active-site Mutations; NAM, Non-Active site Mutations.

* Corresponding author. SB RAS Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, 630090, Russian Federation.

** Corresponding author. Institute of Bioorganic Chemistry Russian Academy of Sciences, Moscow Region, 117997, Russian Federation.

E-mail addresses: gabibov@mx.ibch.ru (A.G. Gabibov), nikita.kuznetsov@niboch.nsc.ru (N.A. Kuznetsov).

<http://dx.doi.org/10.1016/j.biochi.2017.08.014>

0300-9084/© 2017 Elsevier B.V. and Société Française de Biochimie et Biologie Moléculaire (SFBBM). All rights reserved.