Effect of I63P on HIV-1 Protease Backbone Dynamics

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Introduction

HIV-1 protease (HIV-1 PR) is an essential enzyme for generating mature infectious virus particles. This protein plays an essential role in the replicative lifecycle of the virus, and hence it serves as one of the drug targets in the treatment of acquired immunodeficiency syndrome (AIDS). The I63P (in Subtype B background) is a commonly seen drug-pressure-selected mutation, and study has shown that the closed conformation is dominant in I63P, while semi-open states is more favored in subtype B. In order to elucidate how I63P alters protease backbone dynamics, ^{15}N T_1 T_2 and NOE relaxation data have been collected and analyzed for I63P. Order parameters are generated by utilizing model-free mechanism.

Experimental

Uniformly ^{15}N labeled HIV-1 PR constructs have been prepared for all relaxation experiments. 2D ^{1}H-^{15}N heteronuclear NMR relaxation experiments have been performed to obtain the parameters NOE, T_1 (CPMG version) and T_2. All NMR spectra were obtained at 293 K with a Bruker 5mm TXI Cryoprobe Avance II system operating at 600 MHz at the University of Florida AMRIS Facility, whereas NMR spectra for Subtype C were collected on a Bruker 5mm TXI Cyroprobe Avance III system operating at 800 MHz (Chemistry, University of Virginia). Another set of spectra for all constructs were obtained at 293 K with a Bruker 5mm TCI 700S4 h-C/N-D-05Z Cryoprobe Avance III system at 700MHz in the Department of Chemistry and Biochemistry at Florida State University.

Results and Discussion

^{15}N T_1 T_2 and NOE relaxation data were analyzed and the software relax (www.nmr-relax.com) was used to extract the order parameter S^2. As shown in Figure 1, the backbone for I63P is more rigid than subtype B, which indicates that closed conformation are more rigid than semi-open state. The round spheres indicate the location of I63P mutation.

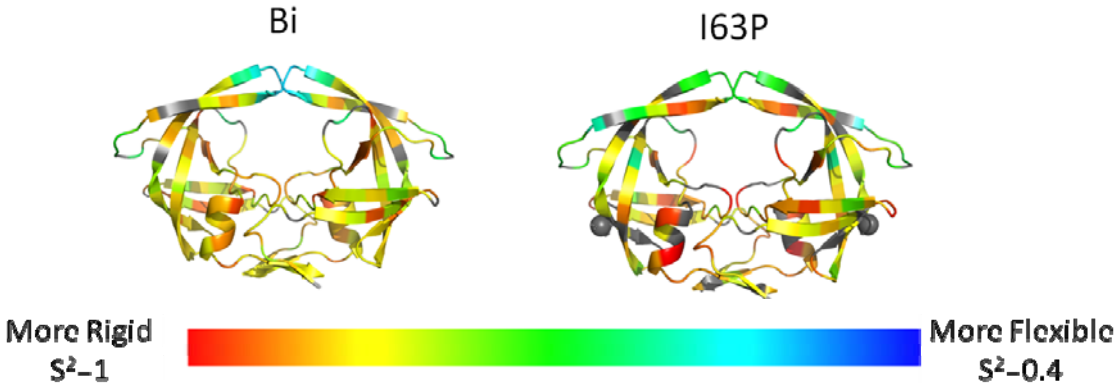

Figure 1. Thermograph of backbone dynamics for subtype B and I63P

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