Effect of Single Point Mutation (I63P) and Subtype Polymorphisms of HIV-1Protease on Backbone Dynamics

Huang, X.; Hu, L.; Dunn, B.M.; Liu, Z.L.; Fanucci, G.E. (U. of Florida, Chemistry); Rocca, J.R. (U. of Florida, AMRIS); Simmerling, C. (Stony Brook U., Chemistry)

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). Previous studies have shown that single point mutation (I63P in Subtype B) and several natural polymorphisms found in CRF_01 AE and PR5 are commonly associated with drug resistance; consequently, modified conformational sampling and backbone dynamics are generated. In order to elucidate how I63P and other natural polymorphisms alter the protease backbone dynamics, ^{15}N T_1 T_2 and NOE relaxation data have been collected and analyzed for subtype B, I63P, CRF_01 AE and PR5. Order parameters were generated by utilizing a 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 Cryoprobe 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 700 MHz in the Department of Chemistry and Biochemistry at Florida State University.

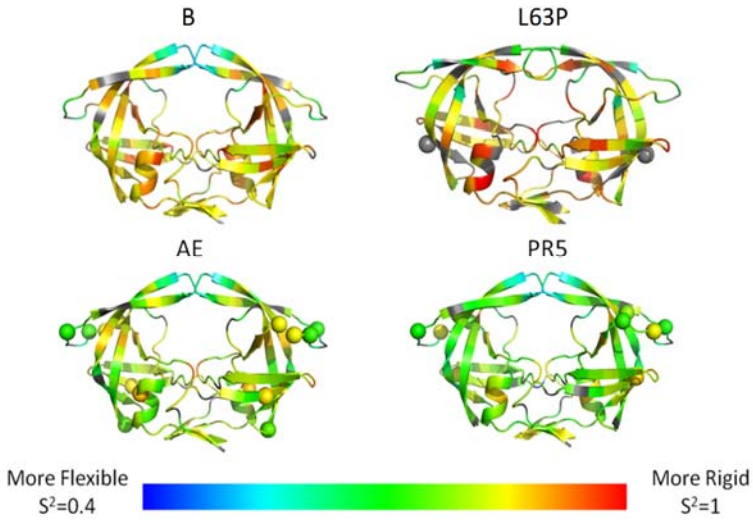

Figure 1. Thermograph of backbone dynamics for subtype B, I63P, CRF_01 AE and PR5

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, while the backbone is becoming more flexible for CRF_01 AE and PR5. The backbone dynamic changes agree with the DEER measurements on the conformation sampling in which more rigid backbone for I63P was achieved by having increased closed states and flexible CRF_01 AE and PR5 favor more percentage of curled open states. [1, 2] In figure 1, all mutations comparing with subtype B are indicated by the round spheres.

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References

[1] Huang, X., *et al.*, J. Bio. Chem., **289**, 17203-17214 (2014).
[2] Carter, J.D., *et al.*, FEBS Lett., **588**, 3123-3128 (2014).