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Subtype Polymorphisms Among HIV-1 Protease Variants Confer Altered Flap Conformations and Flexibility

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Human immunodeficiency virus type 1 protease (HIV-1PR), a 99 amino acid homodimeric aspartic protease, plays a fundamental role in the maturation and life cycle of the retrovirus HIV-1, as it functions in regulating post-translational processing of viral polyproteins *gag* and *gag-pol*. Consequently, this enzyme is a target of AIDS antiviral therapy given that its inhibition prevents viral maturation.[1] Accessibility of the substrate to the active site is mediated by two β-hairpins (aka *the flaps*), which undergo a conformational change during entry and catalysis. HIV-1 is categorized into different groups, subtypes, and circulating recombinant forms (CRFs), wherein groups refer to distinctive viral lineages, subtypes to taxonomic groups within a particular lineage, and CRFs to recombinant forms of the virus.[2] Each subtype exhibits a unique set of naturally occurring polymorphisms. Protease inhibitors used in the treatment of HIV-1 are often designed with respect to subtype B;[3] thus, it is of great importance to understand how subtype polymorphisms alter protein structure, flexibility, and inhibitor efficacy.[4−10]

Site-directed spin labeling (SDSL) double electron−electron resonance (DEER), a pulsed electron paramagnetic resonance (EPR) spectroscopy technique, provides a means to monitor the conformations of the flaps in HIV-1PR.[11−13] Results from subtype B provide detailed information of flap conformations sampled, with conformers described as curled/tucked, closed, semi-open, and wide-open detected in the distance profiles and modeled with molecular dynamics (MD) simualtions.[11,13−15] Distance measurements by SDSL DEER are based on the magnitude of the magnetic dipolar coupling of the unpaired spins, which scales as $1/r^3$, where r is the distance between the two spins.[16,17] For our studies here, we used six inactive (D25N) HIV-1PR constructs, with EPR-active spin labels incorporated into the flaps at the aqueous exposed sites K55C and K55C′ (Figure 1A).[11−13]

The flexibility and conformations of the flaps in HIV-1PR of the following subtypes from group M (main) were determined from SDSL DEER: B, C, F, and CRF01_A/E (a recombinant form of subtypes A and E), and also for two patient isolate constructs, V6 and MDR769.[18,19] Details of sample preparation, protein amino acid sequences, data collection, analyses, and error analyses are given as Supporting Information (SI). For all samples, experimental dipolar modulated echo curves were analyzed via Tikhonov regularization (TKR) with DeerAnalysis2008,[20] and as recently demonstrated, with high quality DEER echo curves, the TKR distance profiles provide rich information about the conformational ensemble structures, with profiles being regenerated with a series of Gaussian-shaped populations.[13] Data and analysis for subtype C are shown in panels C−E in Figure 1. For each construct

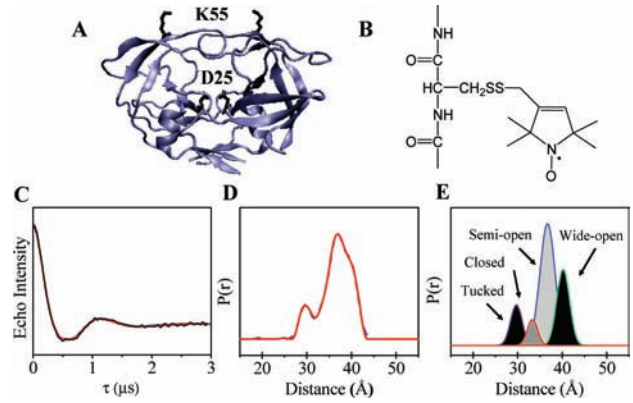

Figure 1. (A) Ribbon diagram of HIV-1 protease showing the active site and location of K55C and K55C′ sites. (B) Structure of MTSL spin-labeled cysteine side chain. (C). Background subtracted DEER echo curve for subtype C (black) with TKR and Gaussian regeneration fits (red and blue, respectively). (D) Distance profile from TKR analysis (red) overlain with summed Gaussian population profile (blue). (E) Gaussian populations used to regenerate the TKR distance profile.

investigated, similar analyses were performed, and populations assigned to flap conformations of curled/tucked, closed, semi-open, and wide-open were obtained. The average distances for the preceding populations are 25−30, 33, 36, and 40−45 Å, respectively. These assignments are based upon extensive characterization of subtype B apo-HIV-1PR.[11,13−15,21−24] Specifically, the closed state is defined as the population centered at 33 Å, which has been validated by MD simulations of inhibitor bound protease[14] and by the observation that this population is present in every Gaussian analysis of distance profiles of subtype B with various inhibitors or substrate analogues.[13] For all apo-constructs investigated here, a peak centered near 33 Å is required for regeneration of the TKR distance profile. The semi-open distance of 36 Å is assigned from MD simulations, and it is the conformation of highest percentage for apo-subtype B. This distance also represents the highest percentage population required to regenerate each of the distance profiles for the apo HIV-1PR constructs studied (Figure 2A and 2B). In addition, a third population centered at 25−30 Å is required for adequate fitting of the distance profiles. These distances are distinctly different than those obtained in the inhibitor/substrate closed state and are assigned to flap conformations that are either tucked or curled[21,22] toward one another or into the active site pocket, and variability in the average distance of this population was seen among the constructs. Finally, a population with an average distance of 40−45 Å is needed to fit each apoenzyme distance profile. This state is assigned to the wide-open conformation of the flaps, which was seen in MD simulations of subtype B.[14,23] Figure 2B plots the relative percentages of each of the

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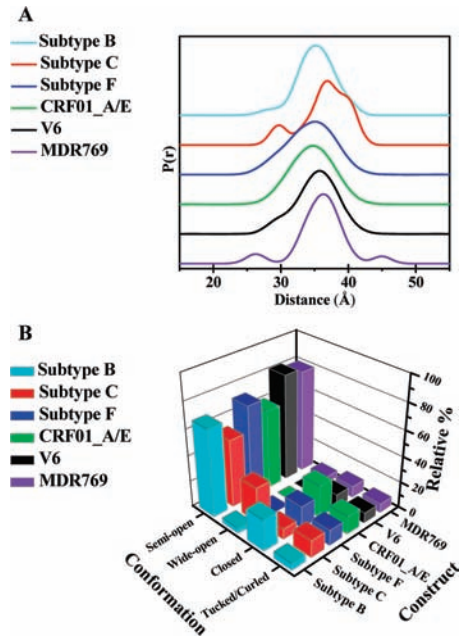

Figure 2. (A) Stack plot of distance profiles from analysis of DEER data of HIV-1PR variants. (B) Population distribution among tucked/curled, closed, semi-open and wide-open conformations determined via Gaussian regeneration of the DEER distance profiles for each construct. Error was approximated at ±5%. Two-dimensional plots for each data set and a table that summarizes results of the Gaussian regeneration including the average distances (center), breadths (fwhm), and relative percentages of each population are given as SI.

populations utilized in the Gaussian reconstruction of each of the TKR distance profiles.

The overall shape and breadth of the distance profiles in Figure 2A indicate that variations in the amino acid sequences among subtypes, CRFs, and patient isolates have a dramatic impact on average flap conformations. Table 1 lists values of the overall span, the most probable distance, and the average distance for each construct. Note, although the profiles for V6 and MDR769 differ slightly from those in our earlier report,[12] the findings here are consistent with the observation that MDR769 has a larger percentage of conformers more open than subtype B, whereas, for V6, although the average value for flap conformation matches within error that of B, a greater percentage of the V6 ensemble is seen in the tucked/curled conformation.

From analysis of the population relative percentages, the effects of the mutations on the average flap conformation can be understood as affecting the sampling of conformer populations and flap flexibility. Changes in flexibility are inferred from the breadth of each of the Gaussian-shaped populations. In particular, the breadths of the closed populations of subtype F, CR01_A/E, and V6 are wider than those seen for the other apo-constructs (SI), which may possibly indicate enhanced flap flexibility or, alternatively, flap instability for the closed conformation. For subtype C, a relatively large percentage of the wide-open conformer is observed. We hypothesize that the higher percentage of the wide-open conformation seen for subtype C may be attributed to the presence of four polymorphisms within the hydrophobic core, two of which, M36I and I93L, are thought to contribute to drug resistance. These core hydrophobic residues may facilitate the conformational changes required for substrate binding and catalysis via the hydrophobic sliding mechanism.[24] Similarly, MDR769 has three polymorphisms in the hydrophobic core that are also thought to contribute to drug resistance, which coincides with our report of greater than average flap distance when compared to subtype B.

Table 1. Summary of Distance Parameters Obtained from DEER Distance Profiles of HIV-1PR Constructs

construct	range (span) (±1 Å)	most prob. dist. (±0.2 Å)	avg. dist.(±0.2 Å)
B	24−45 (21)	35.2	35.2
C	25−45 (20)	36.9	36.5
F	24−45 (21)	35.1	34.3
CRF01_A/E	25−45 (20)	34.8	34.6
V6	25−45 (20)	35.8	35.2
MDR769	22−49 (27)	36.3	35.9

The DEER results reported in this work show that sequence variations within the subtypes of HIV-1 protease alter the average flap conformations. From detailed data analyses, these altered distance profiles can be understood as shifts in the conformational sampling of nominally four HIV-1PR conformations, with some states having enhanced flexibility or structural instability, which may play an important role in viral fitness and drug resistance.

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Supporting Information Available: Further experimental details, protein sequences, sample preparation, data and error analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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