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Correlating Conformational Shift Induction with Altered Inhibitor Potency in a Multidrug Resistant HIV-1 Protease Variant

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Supporting Information

ABSTRACT: Inhibitor-induced conformational ensemble shifts in a multidrug resistant HIV-1 protease variant, MDR769, are characterized by site-directed spin labeling double electron−electron resonance spectroscopy. For MDR769 compared to the native enzyme, changes in inhibitor IC_{50} values are related to a parameter defined as $|\Delta C|$, which is the relative change in the inhibitor-induced shift to the closed state. Specifically, a linear correlation is found between $|\Delta C|$ and the magnitude of the change in IC_{50}, provided that inhibitor binding is not too weak. Moreover, inhibitors that exhibit MDR769 resistance no longer induce a strong shift to a closed conformational ensemble as seen previously in the native enzyme.

HIV-1 protease (HIV-1 PR) is a 99-amino acid C2-symmetric homodimer responsible for HIV viral maturation,[1] and this protein is the target of therapies utilizing protease inhibitors (PIs). Residues 46−56 in each subunit of HIV-1 PR comprise a β-hairpin known as the flap, which together regulate access to the active site.[2] Cross-resistance to several inhibitors can arise from primary and compensatory amino acid changes,[3] which occur because of natural polymorphisms,[4] drug-pressure selected mutations,[5,6] or combinations of both. These substitutions have been shown to alter the HIV-1 PR flap conformational heterogeneity[7−9] and dynamics[10] in the free protease.

The failure of highly active antiretroviral therapy (HAART) in HIV/AIDS treatment is attributed to the emergence of drug-pressure selected mutations in the HIV genome after exposure to one or several inhibitors.[5,6] One particular multidrug resistant HIV-1 PR variant is the clinical isolate MDR769 (Figure 1). MDR769 is resistant to various inhibitors[11] and exhibits a higher level of resistance to most FDA-approved PIs, with the exception of darunavir (DRV), tipranavir (TPV), and lopinavir (LPV).[12] Crystal structures of this variant reveal an expanded active site pocket in the apo, substrate-bound, and inhibitor-bound forms.[12−15] Fewer H-bonding and van der Waals interactions result between PIs and the binding cleft, contributing to drug resistance in MDR769.[13]

Previous studies have shown that flap conformational sampling can be altered by drug-pressure selected mutations,[9] as well as sequence variations among subtypes.[7] For subtype B HIV-1 PR, we also demonstrated that PIs and the substrate mimic CA-p2 induce shifts in the conformational sampling ensemble toward the closed state.[17]

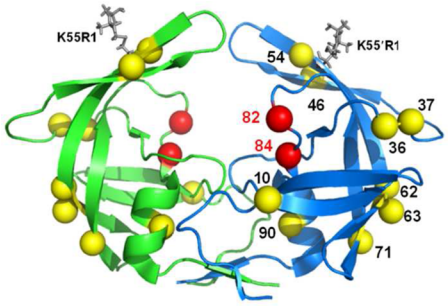

Figure 1. Ribbon diagram of MDR769 (PDB entry 1TW7) protease colored by subunit, rendered in PyMol 1.3. Primary mutations relative to the wild-type sequence (LAI) are shown as red spheres, while compensatory mutation sites are rendered as yellow spheres. MTSL spin probes (K55R1) are incorporated in silico via MMM 2011.1[16] and shown as gray capped sticks.

In this study, the relationship between changes in flap closure and drug potency was investigated by defining a parameter $|\Delta C|$, which is the magnitude of the difference in the inhibitor-induced conformational shift to the closed state between two HIV-1 protease variants. For instance, if a given protease inhibitor induced X% and Y% of the closed population (% closed) in variants A and B, respectively, $|\Delta C|$ can be calculated by using eq 1.

$$|\Delta C| = |\%\ \text{closed}_A - \%\ \text{closed}_B| = |X\% - Y\%| \quad (1)$$

This parameter is used to calculate the inhibitor-induced percentage change in flap closure between subtype B and MDR769. The values of $|\Delta C|$ are then compared to drug potency using previously reported half-maximal inhibitory concentration (IC_{50}) measurements.

Pulsed electron paramagnetic resonance (EPR) spectroscopy, specifically site-directed spin labeling (SDSL) double electron−electron resonance (DEER), is a powerful tool for monitoring the conformational change in free and inhibitor-bound HIV-1 PR variants.[7,9,17,18] For DEER distance measurements in HIV-1 PR, nitroxide radical labels are attached to K55C/K55C′ (termed K55R1 after labeling) on solvent-exposed flap sites. Because HIV-1 PR is a homodimer, two labels are incorporated into the protease for a single cysteine substitution, and the dipolar interaction between these probes

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provides detailed distance and population distribution profiles that reflect various flap conformational ensembles, namely, the curled or tucked, closed, semiopen, and wide-open conformational states.[7,9]

Ligand-induced conformational shifts for MDR769 were acquired from DEER measurements, with methanethiosulfonate (MTSL) spin probes incorporated at sites K55R1/K55′R1. Figure 2 shows select DEER data and distance profiles

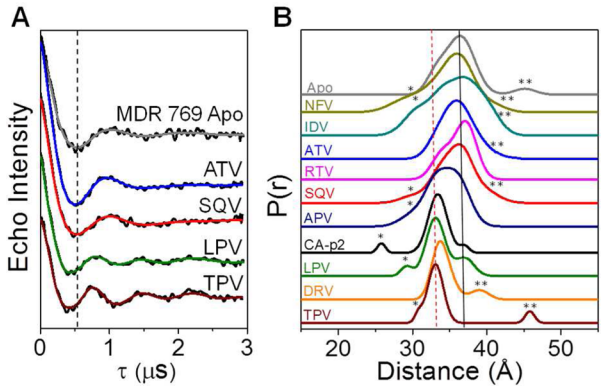

Figure 2. (A) Background-subtracted DEER dipolar evolution curves (black) with fits from Tikhonov regularization (TKR) analysis for MDR769. A vertical dashed line marks the local minimum for the apo form. (B) Stack plot of distance profiles for free MDR769 with FDA-approved inhibitors or substrate mimic CA-p2. Semiopen and closed populations have flap distances of ∼37 Å (solid black line) and ∼33 Å (dashed red line), respectively. The minor population at ∼26−30 Å corresponds to the curled or tucked flap conformation (asterisk), whereas those at ∼40−45 Å are assigned to the wide-open populations (two asterisks). Full details of data analyses are given in the Supporting Information.

(complete data in the Supporting Information). The effects of inhibitors on the average flap distance can clearly be seen in the DEER echo curves in Figure 2A. LPV and TPV shift the frequency with less dampening of the oscillations, generating distance profiles with most probable distances of 33 Å and narrower breadths. In Figure 2B, the solid line indicates a distance of ∼37 Å, which coincides with that expected for the semiopen conformational state, where the distance profile for apo MDR769 indicates a longer most probable flap distance (37 Å) relative to that of subtype B (36 Å), as seen previously.[9] The dashed line at 33 Å marks the distance expected for the inhibitor-induced closed conformation.[9,17]

In our method to ensure proper background subtraction, the Tikhonov regularization (TKR) distance profiles from DeerAnalysis2008 are deconstructed to a linear combination of Gaussian populations. Nominally, four Gaussian populations are required for sufficient regeneration of the TKR data, and these are assigned to curled or tucked, closed, semiopen, and wide-open HIV-1 PR conformational states.[7,9] Population assignments are based on assessing MTSL distances in HIV-1 PR models from molecular dynamics simulation and X-ray studies.[19−22] Minor populations located at 26−30 and 40−45 Å are assigned to the curled or tucked[23] and wide-open states,[20] respectively.

For apo subtype B HIV-1 PR, previous DEER studies reveal a distance profile containing a predominant semiopen conformation, where inhibitor binding shifts the conformational ensemble to increasing populations of the closed state.[17] For subtype B, the fractional occupancy of the closed state was >60% for 7 of 10 inhibitors, with a concomitant shift in the most probable distance from 36 to ∼33 Å. These PIs are ritonavir (RTV), saquinavir (SQV), amprenavir (APV), lopinavir (LPV), darunavir (DRV), tipranavir (TPV), and the CA-p2 substrate mimic. For the three remaining inhibitors, atazanavir (ATV) has ∼40% closed population and nelfinavir (NFV) and indinavir (IDV) have only <15% closed populations.

For MDR769, only 3 of 10 inhibitors, LPV, DRV, and TPV, shift the population to >60% closed. Six inhibitors retain the most probable distance of ∼35−37 Å (Figure 2B). For APV, RTV, or SQV, only 31−36% closed population is observed (Figure 3A), which is 40−60% less than that seen previously for subtype B (Figure 3B). Likewise, ATV exhibited a 30% decrease in the shift to the closed population. Because MDR769 represents a clinical isolate from a patient failing extensive antiretroviral therapy, it is not surprising that many of the inhibitors lose the ability to induce flap closure. Within error, IDV and NFV induced similar degrees of flap closure in subtype B and MDR769 (≤20%). CA-p2, a substrate mimic that serves as a positive control, was found to have flap closure comparable to that of subtype B.

The relationship between induced conformational shifts detected with DEER spectroscopy and underlying biological implications was examined by plotting previously reported half-maximal inhibitory concentrations (IC_{50}) for MDR769[12] on a logarithmic scale against the percentage of the closed state (Figure 3C). The correlation between induced conformational shifts and IC_{50} measurements agrees with X-ray models,[12,13]

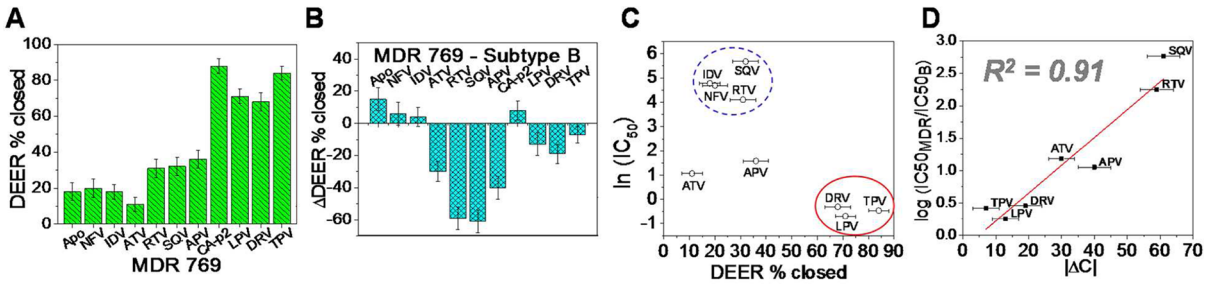

Figure 3. (A) DEER percentage closed (% closed) for MDR769 protease. (B) DEER % difference between MDR769 and subtype B.[17] (C) Logarithmic plot of the half-maximal inhibitory concentration (IC_{50})[12] vs DEER % closed for MDR769. Inhibitors clustered into two distinct groups, but ATV and APV appear to be outliers. (D) A plot of the log IC_{50} fold change[12] vs the magnitude of the % difference in the DEER closed population (|ΔCl) between MDR769 and subtype B reveals a linear correlation. Inhibitors that exhibited low % closed populations in both MDR769 and subtype B PR (IDV and NFV) are excluded from analysis.

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where drugs that bind the HIV-1 PR active site pocket and inhibit viral propagation have multiple interactions with HIV-1 PR that stabilize the closed conformation. Results show that inhibitors with a percentage closed (% closed) of ≥68%, namely, LPV, DRV, and TPV, have excellent drug potency ($IC_{50} \leq 0.7$ nM) with respect to the HIV virus. DEER populations indicated that the distance distribution widths are also narrower for these PIs, suggesting conformationally rigid flaps that are locked-in onto the PI in the active site pocket, consistent with inhibitor-bound MDR769 PR crystal structures that reveal multiple inhibitor−flap interactions.[12,13] In contrast, most PIs with a % closed of ≤32% have IC_{50} values in the range of 60−300 nM. Currently, there is no available crystal structure for MDR769 bound to the inhibitors NFV, IDV, RTV, SQV, APV, and ATV; interestingly, the DEER data for these inhibitors reveal conformational flexibility, which may complicate crystallographic resolution or even interfere with crystallization.

Even though APV and ATV have IC_{50} values of 3−5 nM, DEER data suggest that these inhibitors may bind to MDR769 without promoting substantial flap closure. The presence of outliers, APV and ATV, in Figure 3C may arise because of the active site mutation, D25N, that was introduced into the DEER constructs. This substitution is often incorporated in spectroscopic investigations[24,25] because it imparts sample stability and homogeneity. Without inhibitor-bound crystal structures, it is difficult to understand why APV and ATV are affected by the D25N substitution, but we speculate that the mode of binding may be altered by this substitution.

Nevertheless, when the logarithmic fold change of IC_{50} (MDR769 relative to wild type), a measure of drug resistance, is plotted versus the magnitude of $|\Delta C|$, defined earlier as the % change in flap closure, ATV and APV fit within a linear trend (Figure 3D) with a coefficient of determination (R^2) equal to 0.91. Here, however, NFV and IDV were excluded because of their weak ability to induce flap closure in both subtype B and MDR769. The weak effects of these two inhibitors are not surprising, given they have the largest wild-type K_d values, ∼60−67 times the K_d of DRV (10 pM).[17] Finally, the linear trend in Figure 3D suggests that aside from IC_{50}, the parameter $|\Delta C|$ can be used to evaluate inhibitor effectiveness against emerging drug resistant constructs or the effectiveness of novel inhibitors for drug potency against various HIV-1 PR variants. Other drug resistant variants or inhibitors can be used to further test and validate the proposed method.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, sample preparation, time domain echo curves, and data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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