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Backbone ^1H , ^{13}C , and ^{15}N Chemical Shift Assignment for HIV-1 Protease Subtypes and Multi-Drug Resistant Variant MDR 769

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Abstract

HIV-1 protease (HIV-1PR) is an essential drug target in the treatment of patients infected with HIV-1. Mutations are found to arise in over 38 of 99 amino acid sites in this protein in response to drug therapy or natural selection, where many are found combinations that alter enzyme kinetics or inhibitor susceptibility without a clear structural mechanism. In efforts to understand how these mutations alter the flexibility and dynamics of HIV-1PR, we report the backbone ^1H , ^{13}C , and ^{15}N chemical shift assignments for subtypes C, circulating recombinant form CRF01_AE and a multi-drug resistant variant MDR 769. These assignments are essential for future work aimed at characterizing backbone dynamics, exchange dynamics and dynamics of protein/substrate or protein/inhibitor interactions.

Keywords

HIV-1 protease; naturally occurring polymorphism; multi-drug resistance; resonance assignments

Biological context

HIV-1PR is an aspartic protease consisting of two 99 amino acid subunits that is essential for viral replication. This protease cleaves the viral polyprotein precursors *gag* and *gag-pol* into individual components that then rearrange to form mature virus particles. Thus,

HIV-1PR is a primary target in antiviral therapy because its inhibition prevents viral maturation and extends the patient's life (Ashorn et al. 1990). Protease inhibitors (PIs), which have been developed since 1995, bind in a competitive manner to the active site pocket by interacting with the protease through hydrogen bonding and van der Waals interaction (Prabu-Jeyabalan et al. 2006). Despite remarkable success of HIV/AIDS treatments, rapid emergence of drug-pressure selected mutations results in failure of current drug regimes (Weber and Agniswamy 2009). The HIV-1PR clinical isolate MDR 769 was obtained from a patient who had received long-term antiretroviral therapy, and this protease has been found to be resistant to indinavir, saquinavir, nelfinavir and amprenavir based on in vitro drug susceptibility assays (Longsdon et al. 2004). In addition, the different HIV-1PR subtypes and circulating recombinant forms have naturally-occurring amino acid polymorphisms that have been shown to alter the protein conformation (Kear et al. 2009), flexibility (Sanches et al. 2007) and inhibitor efficacy (Velazquez-Campoy et al. 2002).

Results from pulsed electron paramagnetic resonance (EPR) studies have shown that the flap region, which controls substrate access to the binding pocket, sample various conformations including closed, semi-open and open-like states. Subtype polymorphisms as well as drug-selected mutations result in altered flap conformations and flexibility (Kear et al. 2009; Galiano et al. 2009). In order to elucidate the role of the polymorphisms on protease dynamics and inhibitor binding via NMR methods, the resonance assignments of these proteins are necessary. Over 10% of the amino acid residues differ among the variants studied here and the consensus subtype B LAI (Spinelli, et al. 1991) sequence; thus, many of the peaks in the ^1H - ^{15}N HSQC spectra are shifted in position, as shown in Fig. 1A. Chemical shifts differences of CRF01_AE compare to subtype B are plotted in Fig. 1B and presented in the protease ribbon graph Fig. 1C. Noticed that, not only the chemical shifts of the mutation neighboring residues are perturbed, but also the residues far away from mutation sites such as I54 and V75 - I84. Overlay of individual HSQC spectra and chemical shift perturbation plots of other variants can be found in Supplementary Information. The degree of chemical shift changes are severe enough that we were unable to assign subtype C, CRF01_AE and MDR 769 based upon the previously made assignments of subtype B triple mutant protease (TMPR) assignment (Freedberg et al. 2002). In this paper, we report the backbone assignment of ^1H , ^{15}N and ^{13}C -labeled subtypes C, F and CRF01_AE. These data will provide the basis for future relaxation studies and ligand titration experiments, which will be compared to results of molecular dynamics simulations.

Methods and experiments

Sample preparation

Codon- and expression-optimized DNAs encoding the desired HIV-1PR amino acid sequences were purchased from DNA2.0 (Menlo Park, CA). Each gene was cloned into the pET-23a vector (Novagen, Madison, WI) under the control of T7 promoter. The sequences of our variants are listed in Table 1. The sequences of subtype C and CRF01_AE contain the three protease-stabilizing amino acid substitutions Q7K, L33I and L63I (Mildner et al. 1994). These mutations were not incorporated into the MDR769 sequence. For all sequences, the two native cysteine residues were substituted with alanine (C67A and C95A) to prevent non-specific disulfide bridge formation and the inactivating D25N mutation was incorporated to protect the protease from autolysis. For those amino acid changes not originally purchased in the DNA, site-specific mutations were incorporated into the sequences using the QuikChange site-directed mutagenesis kit (Stratagene, Cedar Creek, TX). $^{13}\text{C}/^{15}\text{N}$ -labeled HIV-1 PR was expressed in *E. coli*, BL21*(DE3)pLysS cells (Invitrogen, Carlsbad, CA), cultured in modified minimal medium using $^{15}\text{NH}_4\text{Cl}$ and $^{13}\text{C}_6$ -glucose as the sole nitrogen and carbon sources, respectively, and then induced by adding 1 mM isopropyl- β -D-thiogalactoside (IPTG) at 37 °C for 5–6 h when the culture density (as

determined by absorbance at 600 nm) was 1.0 or greater. The three different protein constructs were purified as described previously (Kear et al. 2009). The NMR sample contained 0.1 mM $^{13}\text{C}/^{15}\text{N}$ -labeled HIV-1 PR in 2 mM $\text{D}_3\text{-NaOAc}$ buffer, pH 5.0, with 10% D_2O and 0.1 mM DSS.

NMR spectroscopy

All NMR spectra for CRF01_AE and MDR769 were obtained at 293 K with a Bruker 5 mm 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 5 mm TXI Cryoprobe Avance III system operating at 800 MHz (Chemistry, University of Virginia). Backbone resonance assignments were carried out using 2D ^1H - ^{15}N HSQC and 3D HNCACB, CBCA(CO)NH, HNCA and HN(CO)CA experiments. DSS was used as an internal standard for referencing all proton chemical shifts and as an external standard for referencing nitrogen and carbon chemical shifts. The NMR data were processed using NMRPipe (Delaglio et al. 1995), and analyzed with Sparky (Goddard and Kneller, Sparky 3, UCSF, San Francisco).

Assignments and data deposition

HIV-1 PR is a symmetric 99 amino acid homodimer with six proline residues in the sequences of subtype C and CR01_A/E (P1, P9, P39, P44, P79 and P81) with a seventh (P63) in MDR769, giving rise to a predicted chemical shift degeneracy for each monomer and resulting in 93/92 cross-peaks expected to be seen in the ^1H - ^{15}N HSQC spectra. Fig. 2 shows the ^1H - ^{15}N HSQC spectrum of HIV-1 PR CRF01_AE, where analysis of the triple resonance experiments enabled us to identify the assignments for 90 out of 93 amino acids backbone correlation peaks. Amide groups L5, L24, K45 remained unassigned probably due to overlap of the peaks. Similarly, the backbone chemical shift assignments for subtype C and MDR 769 are 88/93 and 88/92, respectively. The ^1H - ^{15}N HSQC spectra of Subtype C and MDR 769 can be found in the supporting material. All assignments for the three variants have been deposited in the BMRB database at Madison, WI (<http://www.bmrbl.wisc.edu/>), with accession numbers 17964 (CRF01_AE), 17996 (subtype C) and 18138 (MDR 769).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. (A) Overlay of 2D ^1H - ^{15}N HSQC spectra of subtype B (blue) and CRF01_AE (red). (B) Differences in the backbone amide chemical shifts of HIV-1 PR (CRF01_AE *versus* Subtype B). ΔH and ΔN are the differences in chemical shifts (in ppm) for the individual ^1H and ^{15}N atoms, respectively. Sites of mutations are labeled in red open circle. (C) The protease molecule is colored according to the chemical shifts differences. Mutation sites are labeled as spheres.

Fig. 2. $[^1\text{H}-^{15}\text{N}]$ HSQC spectrum of ^{13}C , ^{15}N labeled HIV-1 PR CRF01_AE in 2mM $\text{D}_3\text{-NaOAc}$ in $\text{H}_2\text{O}/\text{D}_2\text{O}$ 90/10 (v/v), acquired at and 293 K and 600 MHz. Peaks are labeled according to one-letter amino acid codes and to position in the protein sequence. Cross-peaks assigned by asterisks (*) are side-chain amide resonances.

Table 1

HIV-1 Protease Variant Amino Acid Sequences Utilized in our Studies

Residue	Number	1-10	11-20	21-30	31-40	41-50
Subtype B LAI ^a		PQITLWQRPL	VTIKIGGQLK	EALLDTGADD	TVLEEMSLPG	RWKPKMIGGI
Subtype B TMR ^b		PQITLWKRPL	VTIKIGGQLK	EALLDTGADD	TVLEEMSLPG	RWKPKMIGGI
Subtype B (PMPRI) ^c		PQITLWKRPL	VTIKIGGQLK	EALLNTGADD	TVLEEMSLPG	RWKPKMIGGI
Subtype C (PMPRI) ^c		PQITLWKRPL	VSIVGGQIK	EALLNTGADD	TVLEEMSLPG	RWKPKMIGGI
CRF01_AE (PMPRI) ^c		PQITLWKRPL	VTIKIGGQLK	EALLNTGADD	TVLEEMSLPG	RWKPKMIGGI
MDR 769 (DMPRI) ^d		PQITLWQRPL	VTIKIGGQLK	EALLNTGADD	TVLEEMSLPG	RWKPKMIGGI
Residue number		51-60	61-70	71-80	81-90	91-99
Subtype B LAI		GGFIKVRQYD	QILIEICGHK	AIGTVLVGPT	PVNIIGRNLL	TQIGCTLNF
Subtype B TMR		GGFIKVRQYD	QILIEICGHK	AIGTVLVGPT	PVNIIGRNLL	TQIGCTLNF
Subtype B (PMPRI) ^c		GGFIKVRQYD	QILIEIAGHK	AIGTVLVGPT	PVNIIGRNLL	TQIGATLNF
Subtype C (PMPRI) ^c		GGFIKVRQYD	QILIEIAGKK	AIGTVLVGPT	PVNIIGRNML	TQIGATLNF
CRF01_AE (PMPRI) ^c		GGFIKVRQYD	QILIEIAGKK	AIGTVLVGPT	PVNIIGRNML	TQIGATLNF
MDR 769 (DMPRI) ^d		GGFVKVRQYD	QVPIEIAAGHK	VIGTVLVGPT	PANVIGRNIM	TQIGATLNF

* The residues highlighted in black with white text indicate sites of amino acid mutations relative to Subtype B LAI that arise as natural polymorphisms or drug-pressure selected mutations. Those in grey indicate the locations of mutations that we introduce either to stabilize the protease (grey with black text) or substitution of cysteine to alanine to help prevent crosslinking and side-chain oxidation. The active site D25N mutation is underlined.

^a Spinelli et al. 1991.

^b Freedberg et al. 2002

^c Inactive pentamutated protease (PMPRI)

^d Inactive dimutated protease (DMPRI)