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^{1}H, ^{15}N, and ^{13}C resonance assignments for a monomeric mutant of the HIV-1 capsid protein

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Abstract

The mature fullerene cone-shaped capsid of the human immunodeficiency virus 1 is composed of about 1,500 copies of the capsid protein (CA). The CA is 231 residues long, and consists of two distinct structural domains, the N-terminal domain and the C-terminal domain (CTD), joined by a flexible linker. The wild type CA exhibits monomer–dimer equilibrium in solution through the CTD–CTD dimerization. This CTD–CTD interaction, together with other intermolecular interdomain interactions, plays significant roles during the assembly of the mature capsid. In addition, CA–CA interactions also play a role in the assembly of the immature virion. The CA also interacts with some host cell proteins within the viral replication cycle. Thus, the capsid protein has been of significant interest as a target for designing inhibitors of assembly of immature virions and mature capsids and inhibitors of its interactions with host cell proteins. However, the equilibrium exhibited by the wild-type CA protein between the monomeric and dimeric states, along with the inherent flexibility from the interdomain linker, have hindered attempts at structural determination by solution NMR and X-ray crystallography methods. In this study, we have utilized a CA protein with W184A and M185A mutations that abolish the dimerization of CA protein as well as its infectivity, but preserve most of the remaining properties of the wild type CA. We have determined the detailed solution structure of the monomeric W184A/M185A-CA protein using 3D-NMR spectroscopy. Here, we present the detailed sequence-specific NMR assignments for this protein.

Keywords

HIV-1; Capsid protein; Monomeric mutant; 3D-NMR; Assignments

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Biological context

The mature capsid of HIV-1 is a fullerene cone formed by hexamers of the capsid protein (CA) with twelve pentamers of CA closing both ends of the cone (Ganser-Pornillos et al. 2007). HIV-1 CA is 231 residues long, with two distinct domains, the N-terminal domain (NTD) and the C-terminal domain (CTD) joined by a highly flexible linker. The capsid surface consists of NTD domains in hexagonal and pentagonal rings stabilized by NTD–NTD interactions, with each ring linked to neighboring hexamers through intermolecular CTD–CTD dimerization (Gamble et al. 1997). Additional inter-domain interactions further stabilize the fullerene lattice (Pornillos et al. 2009; Byeon et al. 2009). CA also plays a significant role, as part of the Gag polyprotein, in the assembly of the immature virus particle (Wright et al. 2007). HIV-1 CA also interacts with host cell proteins such as cyclophilin A and lysyl tRNA synthetase in its replication cycle. Thus, there has been a significant interest in CA as a target to develop inhibitors of the early and late stage events within the viral replication cycle (Adamson and Freed 2010).

Methods and experiments

Plasmid WISP98-85 (from Dr. Peter Prevelige, Jr) encoding W184A/M185A HIV-1 CA was used. Its coding sequence was amplified with PCR to introduce *NdeI*/*SalI* cutting sites at 5′ and 3′ ends, respectively, and ligated with a *NdeI* and *XhoI* cut pET20B (EMD). This construct, pET20bHIV1CA, which is expected to translate a HIV-1CA with a start codon Met and a C-terminal His Tag, was transformed into *E. coli* Rosetta2 (EMD). Auto-induction medium P5052N25, in which the ammonium chloride concentration was reduced to 25 mM from 50 mM in ^{15}N-HIV1CA. The reduction of ammonium chloride did not affect the yield of capsid protein production in *E. coli*. For 15N/13C labeling of HIV1CA, bacteria were grown in medium P040, with 25 mM ^{15}N NH_4Cl and 0.4% ^{13}C glucose and all the inorganic salts in P5052N.

Overexpression was induced with 0.8 mM ITPG at $OD_{600\ nm}$ 1.2–1.8 for 8 h at 37°C. *E. coli* adapted in 100% D_2O were transferred to P040 containing 75% D_2O for ^{2}H/^{15}N/^{13}C labeling of HIV-1 CA. Protein was induced as above, for 12 h. Cells were harvested by centrifugation at 6,000×*g* for 10 min, and lysed using sonication. The lysate was centrifuged at 20,000×*g* for 30 min, and the supernatant retained.

The pellet was subjected to ammonium sulfate precipitation, and the pelleted material from the 65% fraction was retained. After ammonium sulfate precipitation, the pellet was solubilized in buffer containing 50 mM NaCl and 50 mM Tris (pH 8.0), and dialyzed in the same buffer. A Q-column (GE) was used for absorption of impurities from dialysate. Fractions of flow-through with purest HIV-1 CA content were pooled. His-tagged HIV-1 CA lysate was immobilized on colbalt-agarose beads (BD), and eluted using manufacturer's recommendations.

Purified HIV-1 CA was dialyzed against buffer containing 25 mM deuterated sodium acetate (pH 5.5), 25 mM sodium chloride, 1 mM deuterated EDTA, 10 mM deuterated DTT, 0.02% sodium azide, and 0.1 mM AEBSF. After concentration with Centricon ultrafiltration devices, samples were made 10% D_2O or lyophilized and resolubilized in 100% D_2O.

2D- and 3D-NMR measurements were performed at 30°C on a Bruker AVANCE 600 MHz NMR system equipped with a TCI-CryoProbe. Data were processed using NMRPipe (Delaglio et al. 1995). A squared sinebell with a 60° shift function was used in all dimensions, with no linear prediction. The processed spectra were analyzed using the program XEASY (Bartels et al. 1995). ^{1}HN, ^{15}N, $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ resonances were assigned

using sequential connectivities in the TROSY based HNCACB, CBCA (CO)NH, and HNCA spectra (Fig. 1). Side chain resonances were assigned using the spectra from the HCCH-TOCSY, HCCH-COSY, ^{15}N NOESY-HSQC (125 ms), and ^{13}C NOESY-HSQC (175 and 250 ms) experiments. Aromatic resonances were assigned using the spectra from the ^{15}N NOESY-HSQC and ^{13}C NOESY-HSQC experiments. ^{13}C carbonyl resonances were assigned using the TROSY-HNCO experiment. Exchangeable amide protons were identified using a ^{15}N SOFAST-HMQC experiment (Table 1).

This monomeric mutant CA assignments are consistent with previously published assignments of the isolated NTD and CTD domains (Tang et al. 2002; Wong et al. 2008), and solid state studies of the full-length protein (Chen and Tycko 2010; Han et al. 2010), and opens up new opportunities for full-length structure based design of antiviral inhibitors. The solution structure consists of a NTD domain with seven helices joined by a highly flexible five-residue linker to the CTD domain with five helices (including a short 3_{10} helix). The first and last residues of the interdomain linker are part of the unwound helices of the two domains on either side. The middle three residues of the linker do not display any sequential NOEs that would be indicative of a bent structure, and their chemical shifts are characterized by random coil values.

Assignments and data deposition

Backbone ^{1}H and^{15}N assignments were made for all non-proline residues of the protein (the residues in the C-terminal His-tag sequence were not assigned). All assignments are present in the HSQC experiment (Fig. 2). $^{13}C\alpha$ and $^{13}C_{\beta}$ assignments were made for all residues except for P122 and R143. Excluding aromatic residues, $^{13}C\gamma$, $^{13}C_{\delta}$, and $^{13}C\varepsilon$ resonance assignments were determined for 156/177, 85/90, and 13/19 nuclei, respectively. Carbonyl ^{13}C resonances were determined for all residues except S16, S33, I37, T48, M55, M68, H84, G89, A92, E98, R100, N121, P122, I124, R132, S146, G156, E159, N195, G206, G223, L231, 17 of which precede proline residues.

$^{1}H_{\alpha}$ and $^{1}H_{\beta}$ assignments were made for all residues except for P49. Excluding aromatic residues, $^{1}H_{\gamma}$, $^{1}H_{\delta}$, and $^{1}H_{\varepsilon}$ assignments were made for 236/268, 103/127, and 25/28 nuclei, respectively.

The ^{1}H, ^{13}C, and ^{15}N chemical shifts have been deposited at the BioMagResBank (http://www.bmrb.wisc.edu) under accession code 17738.

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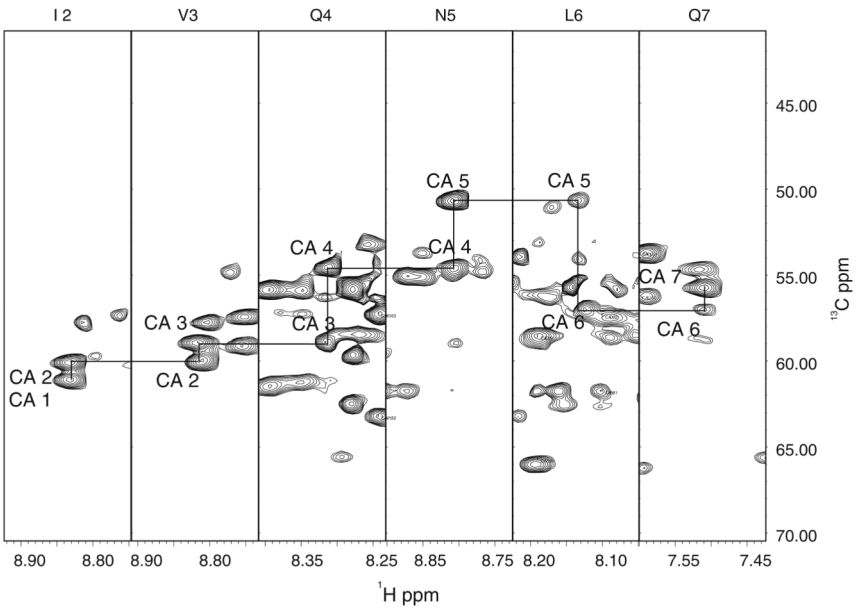

Fig. 1.

Strip plot from the HNCA spectrum of the double mutant HIV1-CA showing sequential $^{13}C\alpha$ connectivities for residues Ile 2–Gln 7. Connectivities between adjacent C_α nuclei are shown with *solid lines*

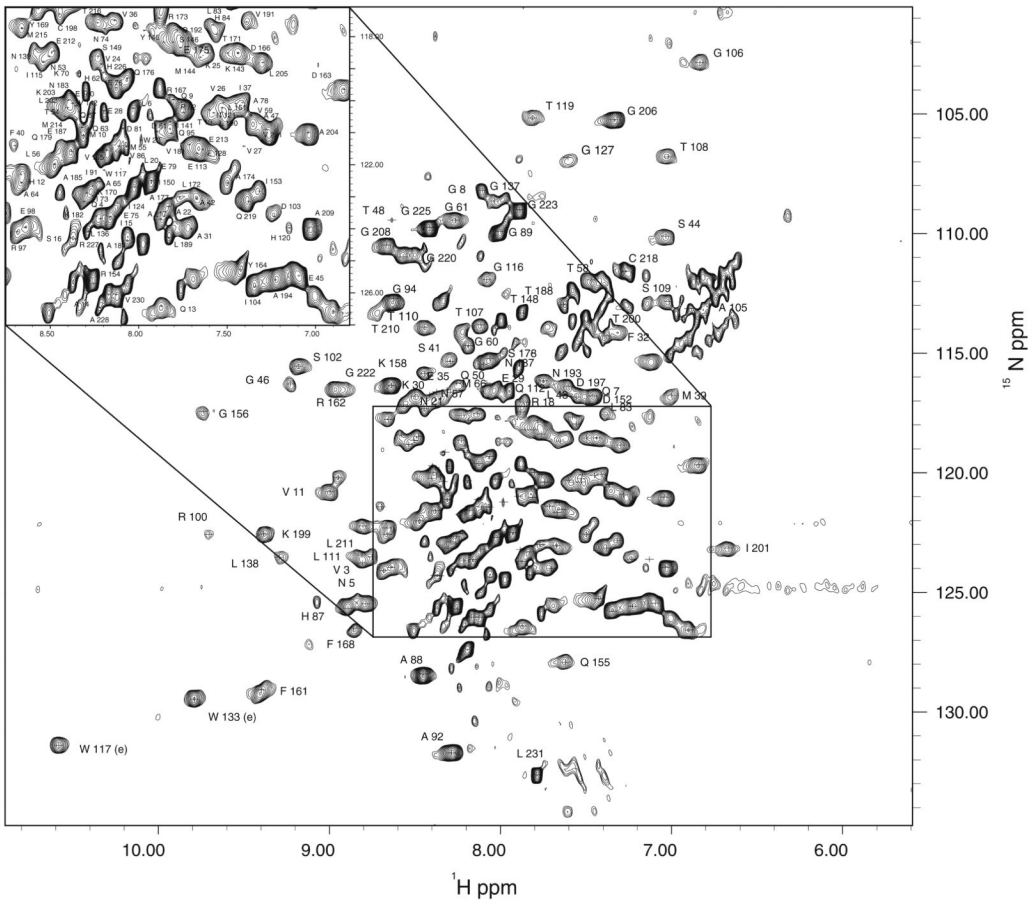

Fig. 2.

Assigned $^{1}H/^{15}N$ gHSQC spectrum for the full length monomeric mutant HIV1-CA protein. All non-proline residues are *labeled*. The *inset* at *upper left* shows the assignment in the crowded region (*box*) in the full spectrum standard P5052N to save ^{15}N label, was used to produce

Table 1

Summary of 2D/3D-NMR measurements

Sample	Experiment	Scans	F3	F2	F1
$^{1}H/^{15}N$ HIV1-CA DM, 10% D_2O	TROSY-^{15}N HSQC	16	2,048	200	
	^{15}N NOESY-HSQC, 125 ms mixing time	24	2,048	40	128
$^{1}H/^{15}N$ HIV1-CA DM, 100% D_2O	SOFAST^{15}N HMQC	8	2,048	128	
$^{1}H/^{13}C/^{15}N$ HIV1-CA DM, 10% D_2O	TROSY-HNCA	16	2,048	40	100
	TROSY-HNCACB	32	2,048	40	128
	TROSY-CBCA(CO)NH	32	2,048	40	128
	TROSY-HNCO	16	2,048	40	100
$^{1}H/^{13}C/^{15}N$ HIV1-CA DM, 100% D_2O	^{13}C HSQC	16	2,048	256	
	^{13}C NOESY-HSQC, 175 mixing time	24	2,048	64	128
	^{13}C NOESY-HSQC, 250 mixing time	24	2,048	64	128
	HCCH COSY	24	2,048	64	128
	HCCH TOCSY	24	2,048	64	128
$^{2}H/^{13}C/^{15}N$ HIV1-CA DM, 10% D_2O	TROSY-HNCACB	32	2,048	40	128
$^{2}H/^{13}C/^{15}N$ HIV1-CA DM, 100% D_2O	HCCH TOCSY	32	2,048	64	128

The list of 3D-NMR and 2D-NMR experiments (at 600 MHz) that were utilized in the sequence-specific assignment of backbone and sidechain nuclei, as well as identification of structural constraints. Sample conditions were: protein concentration 1.2 mM, 303 K, 25 mM perdeuterated acetate (pH 5.5), 25 mM NaCl, 0.02% azide, and 0.1 mM AEBSF, 10 mM DTT. ^{15}N-, $^{15}N/^{13}C$- and $^{15}N/^{13}C/^{2}H$ (70%)-labeled proteins were utilized as appropriate. DM refers to double mutant (W184A/M185A-CA)

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