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^1H , ^{13}C and ^{15}N backbone resonance assignment of HIV-1 Gag (276–432) encompassing the C-terminal domain of the capsid protein, the spacer peptide 1 and the nucleocapsid protein

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Abstract

During the maturation of the HIV-1 particle, the Gag polyprotein is cleaved by the viral protease into several proteins: matrix (MA), capsid (CA), spacer peptide 1 (SP1), nucleocapsid (NC), spacer peptide 2 (SP2) and p6. After cleavage, these proteins rearrange to form infectious viral particles. The final

cleavage by the protease occurs between CA and SP1 and is the limiting step for the maturation of the particle. The CA–SP1 junction is the target of HIV-1 maturation inhibitors. CA is responsible for the formation of the viral capsid which protects the viral RNA inside. The SP1 domain is essential for viral assembly and infectivity, it is flexible and in helix-coil equilibrium. The presence of NC allows the SP1 domain to be less dynamic. The perturbation of the natural coil-helix equilibrium to helix interferes with protease cleavage and leads to non-completion of viral maturation. In this work, two mutations, W316A and M317A, that abolish the oligomerization of CA were introduced into the protein. The HIV-1 CA_{CTD}^{W316A, M317A}-SP1-NC which contains the C-terminal monomeric mutant of CA, SP1 and NC was produced to study the mechanism of action of HIV-1 maturation inhibitors. Here we report the backbone assignment of the protein CA_{CTD}^{W316A, M317A}-SP1-NC. These results will be useful to study the interaction between HIV-1 Gag and HIV-1 maturation inhibitors.

Keywords

HIV-1

Capsid protein

Spacer peptide 1

Nucleocapsid

NMR

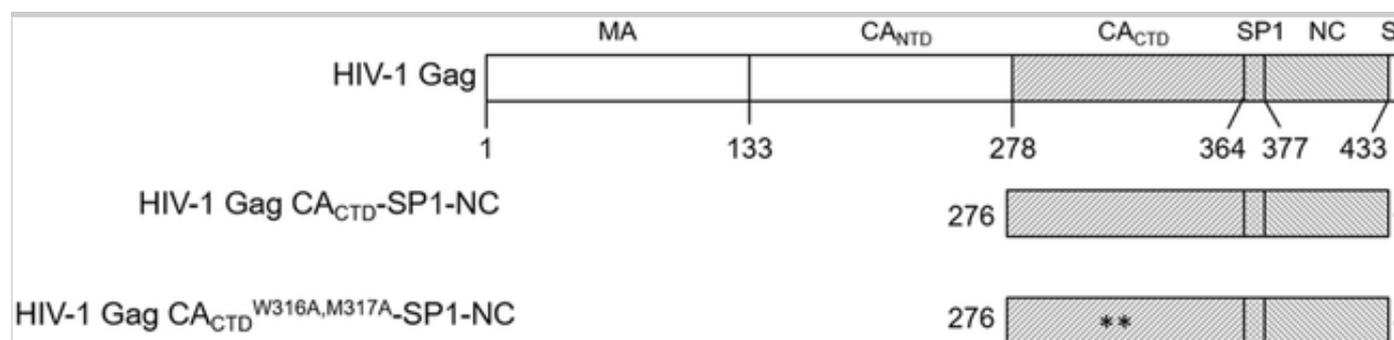
Biological context

Maturation of human immunodeficiency virus of type 1 (HIV-1) particle is the last and essential replication step for viral infectivity. During maturation, the HIV-1 Pr55^{Gag} (Gag) precursor (Fig. 1) is cleaved by the viral protease into several proteins (Wiegiers et al. 1998): matrix (MA) which binds to the plasma membrane, capsid (CA) which forms the viral capsid, spacer peptide 1 (SP1), nucleocapsid (NC) which binds to RNA and forms a ribonucleoprotein (RNP) complex, spacer peptide 2 (SP2) and p6 involved in Vpr encapsidation. Protease cleavages in Gag proceed at different rates following the order: SP1-NC > MA-CA/SP2-p6 > CA-SP1/NC-SP2 (Wiegiers et al. 1998; Coren et al. 2007; Lee et al. 2012). After cleavage, these proteins rearrange to form the mature HIV-1

particle.

Fig. 1

Schematic representation of the HIV-1 Gag protein. CA_{CTD}-SP1-NC and mutant CA_{CTD}^{W316A, M317A}-SP1-NC start from residue 276 (Methionine) of CA_{CTD}. Mutations, W316A and M317A, are represented by *



The cleavage between CA and SP1 is the last step in the maturation of HIV-1. This cleavage site is the target of Bevirimat, an inhibitor of HIV-1 maturation (Li et al. 2003). To elucidate the mechanism of HIV-1 maturation inhibitors, many research groups have tried to determine the structure of CA-SP1 and the binding mode of maturation inhibitors to the hexameric structure of CA-SP1. In 2004, Newman et al. found that the SP1 domain is in helix-coil equilibrium and has a low propensity to form an α -helix (Newman et al. 2004). The crystal structure of CA_{CTD}-SP1 was determined in 2016 (Wagner et al. 2016). In this structure, CA_{CTD}-SP1 forms a hexamer and an α -helix is formed at the CA-SP1 junction encompassing the nine C-terminal residues of CA_{CTD} and the seven N-terminal residues of SP1. The NMR and cryo-EM structural studies of CA-SP1 have shown that SP1 is highly dynamic (Deshmukh et al. 2014; Wang et al. 2017). In 30% Trifluoroethanol (TFE) solution, the short peptide HIV-1 (345–392)Gag forms a long α -helix extending from CA³⁵⁸ to NC³⁸⁰ (Morellet et al. 2005). Thus, the presence of the NC domain could help the stability and proper folding of SP1 (Newman et al. 2004).

The NMR structure of CA has been published and was shown to form a dimer (Deshmukh et al. 2013). When the two mutations W316A and M317A are introduced in the CA_{CTD}, CA only forms a monomer that is easier to study by solution NMR. Thus, we produced the mutated protein HIV-1 Gag

CA_{CTD}^{W316A, M317A}-SP1-NC (Fig. 1) to eliminate the dimeric form of the protein and work only with the monomeric form by NMR in solution. We have published the structure of the protein CA_{CTD}^{W316A, M317A}-SP1-NC in the presence of the HIV-1 maturation inhibitor EP39, which has better solubility compared to Bevirimat (Coric et al. 2013; Chen et al. 2020). We report here the ¹H, ¹³C and ¹⁵N assignment of the backbone of the protein CA_{CTD}^{W316A, M317A}-SP1-NC which could serve as the basis for further studies on the mechanism of action of HIV-1 maturation inhibitors.

Methods and experiments

Protein expression and purification

¹⁵N, ¹³C-labeled HIV-1 CA_{CTD}^{W316A, M317A}-SP1-NC protein was expressed in *E. coli* BL21(DE3)pLysE using 1 g/L ¹⁵NH₄Cl and 2 g/L D-Glucose-¹³C₆ in M9 minimum medium. Protein purification was performed in three steps with Mono Q anion exchange, Resource S cation exchange and Superdex 75 gel filtration chromatographies (Guzman et al. 1998).

NMR sample preparation

NMR sample of ¹⁵N, ¹³C-labeled CA_{CTD}^{W316A, M317A}-SP1-NC was prepared at a concentration of 0.5 mM, in 25 mM deuterated acetate buffer, pH 6.5, containing 25 mM NaCl, 0.1 mM ZnCl₂, 0.1 mM β-mercaptoethanol and 10% D₂O.

NMR experiments

All NMR experiments were recorded at 303 K on a Bruker Avance III 600 MHz spectrometer equipped with a cryogenic probe. Acquisition and processing of the experiments were performed with Topspin®3.5pl7 and the analysis of the spectra and the assignment of the resonances were achieved with CCPNMR®2.4.2 (Vranken et al. 2005). Backbone assignments were obtained using 2D ¹⁵N-SOFAST-HMQC (Schanda et al. 2005) and 3D rapid BEST-TROSY versions (Lescop et al. 2007) of the 3D NMR experiments: HNCA, HNCOCA, HNCACB, HNCOCACB, HNCO, HNCACO (Table 1) to identify the ¹H^N, ¹⁵N, ¹³C^α and ¹³C^β chemical shifts of each residue and characterize the sequential assignment. TALOS-N software

(<http://spin.niddk.nih.gov/bax/software/TALOS-N>) (Shen and Bax 2013) was used to predict the secondary structures of the protein CA_{CTD}^{W316A, M317A}-SP1-NC.

Table 1

NMR experiments acquired for backbone resonance assignment of the mutated protein CA_{CTD}^{W316A, M317A}-SP1-NC

Experiment	Spectrometer frequency (MHz)	Number of points in each dimension			Scan number	Duration
		H	N	C		
2D SOFAST-HMQC	600	954	256	–	4	5 min
3D BT-HNCA	600	1020	120	120	16	24 h
3D BT-HN(CO)CA	600	1020	120	120	16	26 h
3D BT-HNCACB	600	1020	120	120	16	25 h
3D BT-HN(CO)CACB	600	1020	120	120	16	27 h
3D BT-HNCO	600	1020	120	120	4	6 h
3D BT-HN(CA)CO	600	1020	120	120	4	6 h

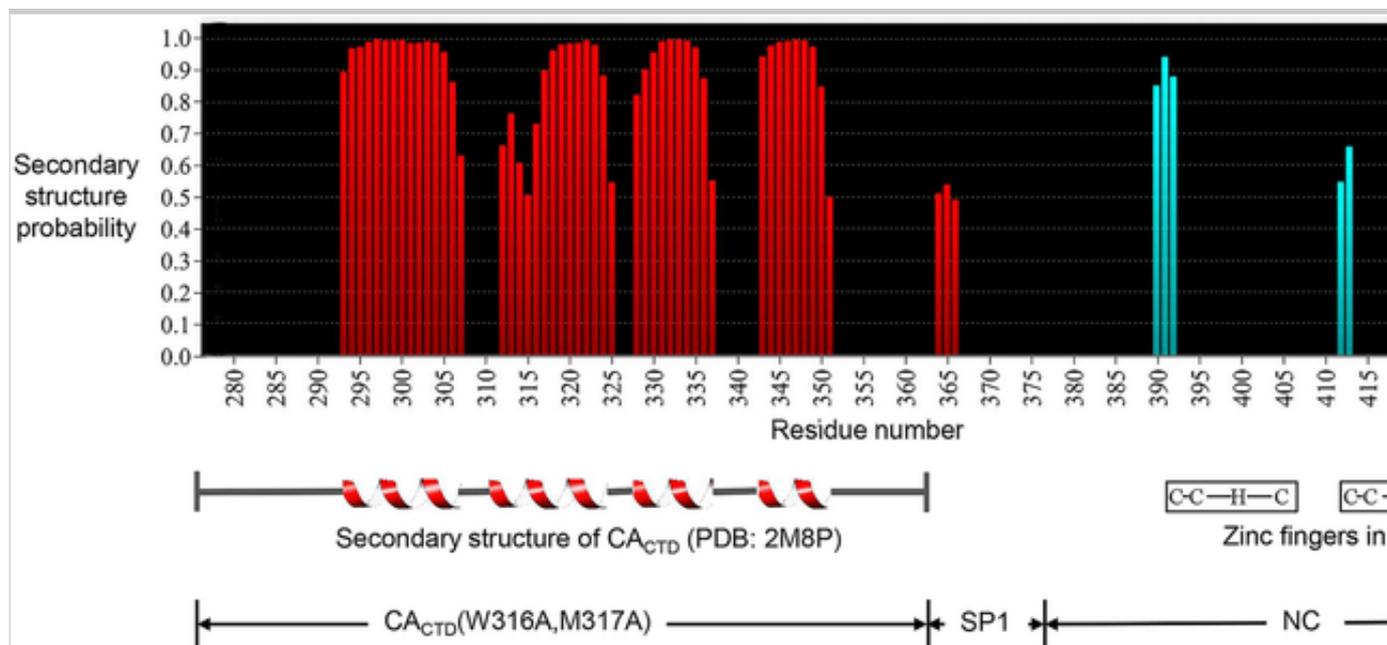
Resonance assignment and data deposition

NMR assignment was performed based on 3D heteronuclear NMR experiments on the ¹⁵N, ¹³C-labeled protein CA_{CTD}^{W316A, M317A}-SP1-NC. Assignment of ¹H–¹⁵N amide group resonances of CA_{CTD}^{W316A, M317A}-SP1-NC was achieved for 135 of 149 non-proline residues (91%). 13 residues (277Y, 357G, 358H, 359K, 372N, 381G, 382N, 383F, 384R, 385N, 386Q, 406R, 420G) could not be assigned due to their being highly dynamic. 92.4% of ¹³C, 91.1% of ¹³C^α and 90.4% of ¹³C^β resonances were assigned. No assignments could be achieved for ¹³C^α, ¹³C^β and ¹³C of the first residue methionine. ¹H–¹⁵N SOFAST-HMQC experiment with the assignment of the residue is shown (Fig. 2a) and a zoom of the most crowded part of the spectrum is shown to allow a better reading of the

The $^1\text{H}^{\text{N}}$, ^{15}N , $^{13}\text{C}^{\alpha}$, $^{13}\text{C}^{\beta}$ and ^{13}C chemical shifts of the different residues were analyzed and the regions of secondary structures were predicted by TALOS-N (Shen and Bax 2013) as shown in Fig. 3. In the TALOS-N prediction, there are four alpha helices in CA_{CTD} which is consistent with the NMR structure of CA_{CTD} (PDB code 2M8P). In SP1 domain, there is a weak probability of helix from residue 364 to 365. Previous research has shown that SP1 has a small propensity of forming an α -helix (Newman et al. 2004; Wagner et al. 2016; Wang et al. 2017), which is in agreement with the weak helical property of SP1 in this TALOS-N prediction. The isolated NC protein contains two zinc fingers and the linker between the two zinc fingers is highly flexible (Zargarian et al. 2014). Our results show that the NC domain has a short β -sheet structure at the beginning of the first zinc finger and a weak property of β -sheet and alpha helix at the beginning and end of the second zinc finger respectively. Most residues of NC do not have significant structure.

Fig. 3

Secondary structure of the protein $\text{CA}_{\text{CTD}}^{\text{W316A, M317A}}$ -SP1-NC predicted with TALOS-N. There are four alpha helices in CA_{CTD} , corresponding perfectly to those identified in the NMR structure of CA_{CTD} with PDB code 2M8P (Deshmukh et al. 2013). NC domain contains two zinc fingers of the consensus sequence $\text{CX}_2\text{CX}_4\text{HX}_4\text{C}$. Alpha helices are in red, beta-strands in blue and residues that are highly dynamic, predicted to be coil, or for which there is no consistent prediction are not shown



The backbone assignment of the protein $CA_{CTD}^{W316A, M317A}$ -SP1-NC will be useful for the study of the interaction between protein $CA_{CTD}^{W316A, M317A}$ -SP1-NC and HIV-1 maturation inhibitors.

BMRB accession number

Backbone resonance assignment of HIV-1 $CA_{CTD}^{W316A, M317A}$ -SP1-NC has been deposited to the BioMagResBank (<http://www.bmrwisc.edu>) under accession number 50712.

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Author contribution

XC expressed and purified the protein and wrote the manuscript, XC, PC and SB performed the NMR experiments and manuscript revision. XC and PC analyzed the NMR data.

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Data availability

The data generated and analyzed during the current study are available on the BMRB website (<http://www.bmrw.wisc.edu>) under accession number Entry 50,712.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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