Solid-State NMR Structural and Dynamics Studies of HIV-1 Protein Assemblies

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Introduction

Gag polyprotein from HIV-1 virus is responsible for assembly of virions from infected cells. Gag and its two products, capsid CA protein and capsid-spacer peptide 1 (CA-SP1) are the focus of this research. CA organizes and protects the viral genome by assembling into conical capsids. Following the viral entry into the host, CA disassembles to allow release of the viral genetic material into the host cytoplasmic compartment (uncoating). CA and the Gag processing intermediate CA-SP1 have recently become attractive targets of HIV-1 uncoating and capsid maturation inhibitors. Despite the promise of targeting CA maturation and uncoating processes by novel inhibitors, the current research is hampered by lack of understanding of the molecular mechanisms of the maturation and uncoating and of their temporal regulation, and detailed atomic-resolution structural and dynamics information of the assembled Gag, CA, and CA-SP1 is still lacking. The objective of our ongoing work is to gain atomic-level insight on structure and dynamics of these HIV-1 protein assemblies and their interactions with host proteins and small-molecule inhibitors, through state-of-the-art solid-state NMR spectroscopy [1-3].

Experimental

MAS NMR spectra were acquired at 21.1 T (900 MHz) on the ultra-wide bore 105 mm NMR magnet, outfitted with a 3.2 mm Low-E triple- resonance HXY probe developed and built at NHMFL. 2D (DARR, INADEQUATE, NCA, and NCACX) spectra were acquired on U-^{13}C, ^{15}N-labeled CA and CA-SP1 assemblies of tubular morphology. All spectra were processed in NMRPipe and analyzed in Sparky.

Results and Discussion

We have analyzed the structure of HIV-1 CA and CA-SP1 assemblies of tubular morphologies from two sequence variants (Fig. 1). These assemblies yield unprecedented high-resolution MAS NMR spectra at high magnetic fields, permitting resonance assignments of these systems. We have demonstrated that CA assemblies from two sequence variants exhibit surprisingly large conformational differences in two functionally important loops. Remarkably, we discovered that the SP1 peptide is dynamically disordered and exists in a random coil conformation in the assembled CA- SP1 from both constructs (Fig. 2). Our results published in JACS [3] provide support for the hypothesis that capsid maturation proceed via *de novo* reassembly and that the role of SP1 cleavage is to mediate the disassembly of the immature lattice for maturation. Our data highlight the importance of conformational plasticity in HIV-1 capsid protein and call for atomic-level scrutiny of the structures and dynamics of the maturation intermediates.

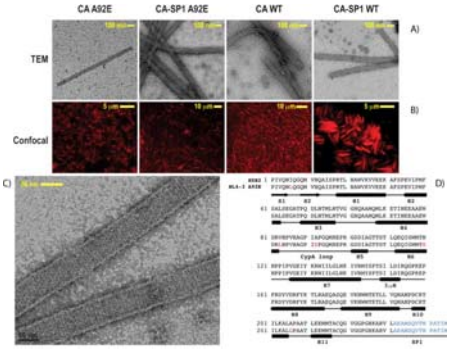

Figure 1. From left to right: TEM (A) and confocal (B) images of CA A92E, CA-SP1 A92E, CA WT and CA-SP1 WT tubular assemblies. (C) TEM image of the CA-SP1 WT tubular assemblies acquired at higher magnification and illustrating that highly regular hexameric lattice is clearly observed. (D) Primary sequences of the HXB2 and NL4-3 (A92E mutant) strains.

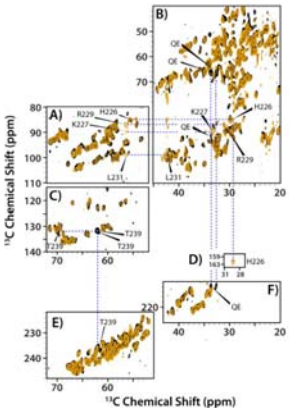

Figure 2. The superposition of the INADEQUATE spectra of the tubular assemblies of CA (orange) and CA-SP1 (black) HXB2 acquired at 21.1 T and 4 °C. A)-C) the aliphatic region; D) the Cβ–Cγ region of His residues; E) and F) the Cα-C' region.

Conclusions

The current work sets the stage for mechanisms of HIV-1 maturation and into evaluation the interactions of HIV-1 protein assemblies with maturation inhibitors at the detailed structural level. MAS NMR methods are envisioned to become a central tool in these studies as they offer unprecedented atomic-resolution insight into the structure and dynamics of the various HIV-1 protein complexes.

Acknowledgements

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References

[1] Han, Y., *et al*., J. Am. Chem. Soc., **132**, 1976-1987 (2010). [2] Byeon, I.-J.L., et al., J. Am. Chem. Soc., **134**, 6455-6466 (2012). [3] Han, Y., *et al*., J. Am. Chem. Soc., **135**, 17793-17803 (2013).