Tubular Assembly of Rous Sarcoma Virus Capsid Proteins Probed by SsNMR and TEM

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Introduction

The capsid of retrovirus Rous Sarcoma Virus (RSV) is assembled by numerous copies of capsid proteins (CPs)[1]. Elucidation of the assembly mechanism will not only benefit the design of new antiviral drugs[2], but stimulate the construction of versatile biocompatible vehicles tunable for specific tasks[3-5]. However, due to the typical pronounced polymorphism of retroviral CP assemblies, these assemblies are not suitable for conventional structural biology techniques and the assembly mechanism remains elusive. Here we apply transmission electron microscopy (TEM) and solid state NMR (ssNMR) to study the tubular assembly of RSV CPs.

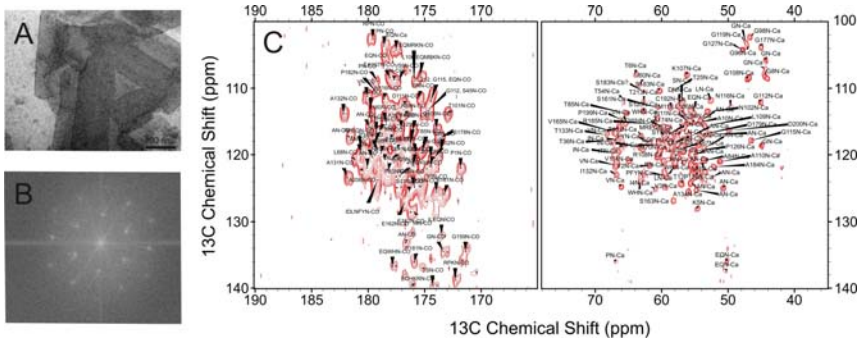

Figure 1. Tubular assembly of RSV capsid proteins probed by TEM and ssNMR.
A. Negatively stained TEM image of RSV capsid tubes.
B. FFT pattern of A exhibits six-folded symmetry.
C. Sequential assignments labeled in the 600 MHz 2D NCO (left) and NCA spectra.

Experimental

Tubes with diameter around 130 nm were assembled by RSV CPs as shown in Fig. 1A. In Fig. 1B, their FFT pattern shows six-folded symmetry and indicates that they are constructed by highly ordered CP hexamers. Multiple tubular assembly samples for NMR experiments were prepared with different isotopic labeling patterns, including uniformly ^{13}C, ^{15}N labeled samples and samples selectively labeled by 1,3 and 2-^{13}C glycerol[6]. Multiple spectra were acquired on 600 MHz and 900 MHz magnets at National High Magnetic Field Laboratory (NHMFL).

Results and Discussion

The highly uniform tubular assembly samples produced high quality NMR spectra. ^{13}C linewidths are generally ~ 0.5 ppm on 600 MHz. 135 out of 237 residues are sequentially assigned with 3D NCACX, NCOCX and CONCX spectra, as shown in the 2D NCO/A spectra in Fig. 1C. All assigned resonances are sharp and unique, suggesting a defined local structure. Since the assignment is still in process, we can't exclude the possibility that certain residues may involve in multiple conformations, which will either result in broad and weak resonances, or well-defined signals at distinct positions. In addition, 7 residues are detected in INEPT-TOBSY experiments[7]. They are assigned to the last 7 residues at the C terminus of the protein. Combined, our results indicate that the RSV CPs are mostly rigid, except its highly dynamic tail.

Conclusions

RSV CP is one of the largest proteins ever studied by ssNMR. It demonstrates that combining smart isotopic labeling strategy with high magnetic fields available at NHMFL, we are able to push the boundary of ssNMR. 60% of the residues in the protein are now sequentially assigned. Given the spectral quality, we are expected to resolve the majority of the signals. Additional measurements will be performed to characterize the dynamics and structural order of the protein. They will be invaluable to elucidate the mechanism of assembly polymorphism and curvature control of retroviral CPs.

Acknowledgements

Work was performed at the NHMFL, supported by NSF (DMR-1157490), and the State of Florida; additional support by Air Force Office of Scientific Research young investigator award (FA9550-13-1-0150).

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