Site-Specific Signature of Rous Sarcoma Virus Capsid Protein to Switch Between Pentameric and Hexameric Assembly

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Introduction

The molecular basis of the versatile assemblies of Rous sarcoma virus (RSV) capsid protein (CA) is not yet clear due to the intrinsic polymorphism. It is assumed that different shapes are constructed by inserting twelve CA pentamers into its hexameric lattice. We established the first atomic resolution model of the RSV CA tubular assembly comprising entirely of hexamers by combining solid state NMR (ssNMR) constraints with cryo-electron microscopy reconstruction images.[1] In the past year, we acquired high resolution 2D and 3D ssNMR spectra of the RSV CA spherical assembly consisting entirely of pentamers, which manifest for the first time, site-specific signature of the CA to switch between pentameric and hexameric assembly.

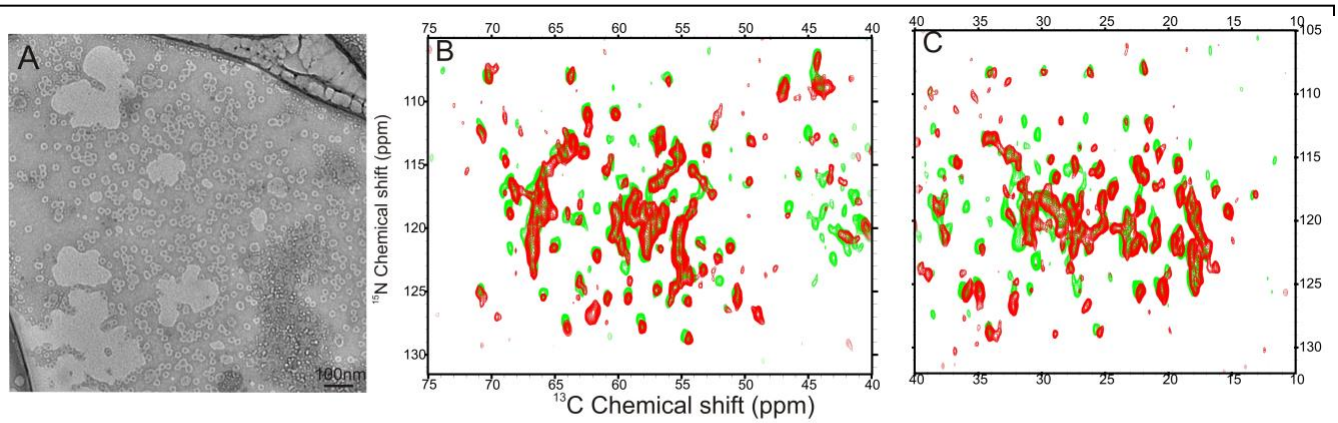

Figure 1. RSV CA exhibits different backbone and side-chain structures in pentameric and hexameric lattices. (A) The transmission electron microscopy image of our RSV CA spherical assembly sample, negatively stained by 1.5% uranyl acetate. The sample consists predominantly of small spheres near 20 nm, corresponding to T=1 capsids comprised entirely of 12 pentamers. (B) and (C) are different regions of the overlay 2D NCACX spectra of RSV CA spherical assembly (red) and tubular assembly (green). The 2D NCACX spectra of both samples are acquired with the same experimental setup: 50 ms DARR mixing, 13.5 kHz magic angle spinning, at 600 MHZ field.

Experimental

Highly uniform spherical assembly of RSV CA was prepared and screened by transmission electron microscopy (Fig. 1A). 2D and 3D spectra were acquired on the 600 MHz ssNMR system at NHMFL.

Results and Discussion

All spectra of RSV CA spherical assembly exhibit quality comparable to those of RSV tubular assembly,[1] with ~ 0.5-0.6 ppm linewidth along the ^{13}C dimension and 0.8-1.0 ppm along the ^{15}N dimension. Complete assignments of all signals are expected, as demonstrated in our prior work.[1]

In our published work,[1] we observed that RSV CA adopts the same backbone structure, but varies only in the side-chain conformation to form tubes of different diameter, indicating that only side-chain variation in CA is necessary to adjust curvature in the hexameric lattice. As shown in Fig. 1B and C, we find that not only side-chain (Fig. 1C), but backbone sites display clear shifted and mismatched resonances.

Conclusions

To our knowledge, we believe this is the first direct observation of site-specific signature of retroviral CA to form pentameric then hexameric assembly. This observation proves that RSV CA re-arranges both side-chain and backbone conformation to switch from hexamer to pentamer in assemblies, to incorporate sharp curvature. Efforts are underway to finalize the assignments of the observed site-specific signature for retroviral capsid assembly controlling pentameric and hexameric subunits.

Acknowledgements

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References

[1] Jeon, J., *et al.*, Journal of the American Chemical Society, ***139***, 2006-2013 (2017).