Membrane-bound Structure and Oligomeric Assembly of Viral Fusion Proteins by Solid-State NMR

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Enveloped viruses enter cells by using their fusion proteins to merge the viral and cell membranes. Although extensive structural studies have been conducted on the soluble ectodomain by crystallography and the N-terminal fusion peptide by NMR, the structure and oligomeric assembly of the hydrophobic C-terminal transmembrane domain (TMD) have been poorly understood. Here we use solid-state NMR to determine the full secondary structure and oligomeric assembly of the TMD of two viral fusion proteins, the parainfluenza virus 5 (PIV5) fusion protein F and the HIV fusion protein gp41. For the PIV5 TMD, ^{13}C and ^{15}N chemical shifts from 2D ^{13}C-^{13}C and ^{13}C-^{15}N correlation spectra indicate that the backbone conformation is membrane-dependent [1]: the central Leu-rich segment is robustly α-helical, while the N- and C-termini, rich in Val and Ile residues, acquire β-strand conformation in negative-curvature POPE membranes while remaining helical in lamellar POPC/cholesterol membranes. The β-sheet rich conformation in the POPE membrane shows stronger lipid mixing activities and cause higher membrane curvature, indicating that the β-strand conformation is fusogenic. To determine the oligomeric structure, we conducted ^{19}F spin diffusion CODEX experiments using peptides containing fluorinated Phe residues in the α-helical core. The observed CODEX intensity decays indicates that the TMD trimerizes in both lamellar and negative-curvature membranes, with interhelical distances of 8.2 Å to 10.5 Å for different residues (**Fig 1a, b**) [2]. These results indicate that the Leu-rich central segment is the core of the α-helical coiled coil, while the Ile- and Val-rich termini adopt the β-strand conformation to induce membrane curvature and fusion.

We also investigated the structural topology and oligomeric state of HIV gp41, using a construct that spans the membrane-proximal external region (MPER) and the C-terminal TMD. Solution NMR studies of micelle-bound full-length gp41 could not detect the signals of these two domains, indicating complex motion of this region, while a high-resolution study of the TMD showed a trimeric structure but without the important antibody-targeted MPER. We measured ^{13}C and ^{15}N chemical shifts of membrane-bound MPER-TMD using 2D correlation experiments, and found that unlike the PIV5 fusion protein, gp41 MPER-TMD is α-helical in both negative-curvature POPE membranes and lamellar virus-mimetic complex membranes. Lipid-protein 2D ^{1}H-^{13}C correlation spectra and water-edited 2D correlation spectra of the protein allowed us to probe the depth of insertion and hydration of the protein. The results show that the TMD is well inserted into the lipid bilayer while the MPER lies on the membrane surface. ^{19}F CODEX spin diffusion experiments indicate that the MPER-TMD associates as a trimer, with interhelical distances of 11.5 Å to 12 Å for selected fluorinated Phe and Trp residues in the TMD and the MPER. We also measured intramolecular ^{13}C-^{19}F distances between MPER and TMD residues to investigate how these two domains are oriented relative to each other. The resulting distances of 9.7 – 10.6 Å (**Fig.1c**) indicate a significant kink between the MPER and TMD (**Fig. 1d**). These interhelical and intrahelical distance constraints, together with membrane depth measurements, led to the first SSNMR structural model of the oligomeric structure of the C-terminal region of gp41 in lipid bilayers, providing valuable insight into the pre-fusion state of the protein.

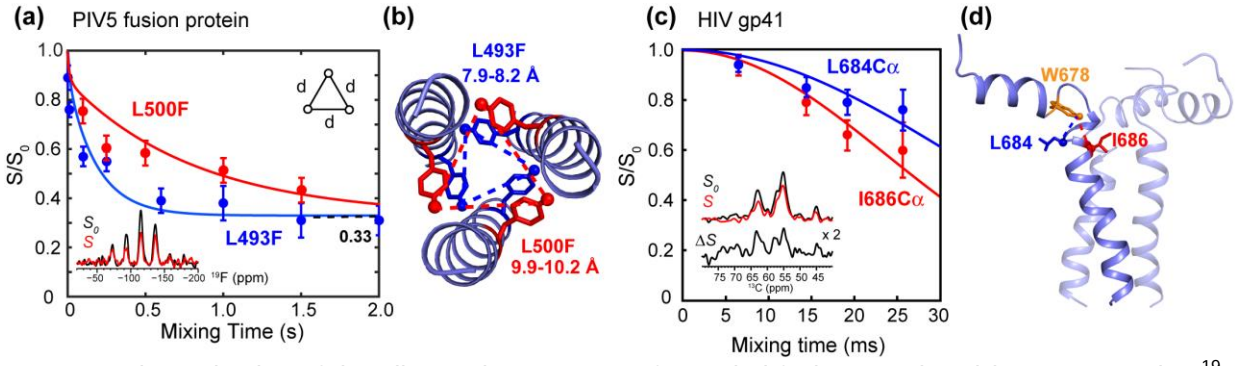

Figure 1. SSNMR determination of the oligomeric structures of two viral fusion proteins. (a) Representative ^{19}F CODEX data of PIV5 TMD at L493 (blue) and L500 (red). Echo intensities decay to 0.33, indicating trimer formation. A representative pair of CODEX S_0 and S spectra is shown. (b) Proposed trimeric structure of PIV5 TMD, showing interhelical ^{19}F-^{19}F distances of L493F and L500F. (c) Frequency-selective ^{13}C-^{19}F REDOR data of gp41 MPER-TMD with a representative pair of REDOR S_0 and S spectra. (d) Proposed trimer structural model of MPER-TMD.

References:

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2. M. Lee, H. Yao, B. Kwon, A.J. Waring, P. Ruchala, C. Singh, and M. Hong, ***J. Mol. Biol***., in press (2018).