Solid-State NMR Structural Studies of Viral Fusion Proteins in Membranes

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Enveloped viruses infect cells by joining their membrane with that of the target host cell. This process is catalyzed by a viral fusion protein and in particular by the ~20-residue N-terminal fusion peptide (FP) region which binds to the host cell membrane. Solid-state NMR (SSNMR) has been applied to probe the structures and membrane locations of domains of the HIV gp41 and the influenza virus HA2 membrane fusion proteins. Advantages of SSNMR include: (1) use of membrane bilayers without detergent and with lipid and cholesterol composition similar to that of host cells; and (2) preparation of samples under conditions very similar to those used for functional vesicle fusion assays.

The *N*-terminal ~20-residue influenza virus HA2 fusion peptide (IFP) domain has helix-turn-helix structure and in detergent there are reports of: (1) an open interhelical topology with a hydrophobic interhelical pocket and inverted V membrane insertion; and (2) a closed topology with tightly-packed antiparallel helices and membrane contact with a hydrophobic protein surface. SSNMR measurements in membranes lacking cholesterol show that the closed structure is predominant.

Both the IFP and the corresponding *N*-terminal HIV gp41 fusion peptide (HFP) have predominant oligomeric β sheet structure in membranes with 30 mole% cholesterol which is typical for viral and host cell membranes. SSNMR measurements for HFP show that there is antiparallel arrangement of adjacent stands with a broad distribution of antiparallel registries. The central regions of the antiparallel sheets are deeply inserted in the membrane and contact the methyl termini of the lipid acyl chains. This antiparallel distribution is also observed for a much larger segment of gp41 that includes the fusion peptide. A very different registry distribution is detected for the non-functional V2E mutant and suggests that the amino acid content of the antiparallel sheet is correlated to membrane fusion.