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NMR structures and localization of the potential fusion peptides and the pre-transmembrane region of SARS-CoV: Implications in membrane fusion

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ABSTRACT

Severe acute respiratory syndrome-associated coronavirus (SARS-CoV) poses a serious public health hazard. The S2 subunit of the S glycoprotein of SARS-CoV carries out fusion between the virus and the host cells. However, the exact mechanism of the cell fusion process is not well understood. Current model suggests that a conformational transition, upon receptor recognition, of the two heptad core regions of S2 may expose the hydrophobic fusogenic peptide or fusion peptide for membrane insertion. Three regions of the S2 subunit have been proposed to be involved in cell–cell fusion. The N-terminal fusion peptide (FP, residues 770–788), an internal fusion peptide (IFP, residues 873–888) and the pre-transmembrane region (PTM, residues 1185–1202) demonstrated interactions with model lipid membranes and potentially involved in the fusion process. Here, we have determined atomic resolution structures of these three peptides in DPC detergent micelles by solution NMR. FP assumes α-helical conformation with significant distortion at the central Gly residues; enabling a close packing among sidechains of aromatic residues including W, Y and F. The 3-D structure of PMT is characterized by a helix–loop–helix with extensive aromatic interactions within the helices. IFP adopts a rather straight α-helical conformation defined by packing among sidechains of aromatic and aliphatic residues. Paramagnetic spin labeled NMR has demonstrated surface localization of PMT whereas FP and IFP inserted into the micelles. Collectively, data presented in this study will aid in understanding fusion mechanism of SARS-CoV.

1. Introduction

Membrane fusion is an important step for a successful infection of the enveloped viruses causing the transfer of viral genetic materials into the host cell. The energy barrier of membrane fusion is overcome by a coordinated process mediated by specialized viral fusion proteins [1–4]. Most fusion proteins are produced as precursor proteins which are often cleaved by host cell proteases, yielding a metastable complex consisted of a receptor binding subunit and a membrane fusion subunit [1–4]. Upon binding to the cell surface receptor or being in the acidic environment of endosome, fusion proteins are activated following a dramatic conformational transition which essentially sets free a buried hydrophobic fusion peptide from the protein core [1,2,5–9]. The fusion peptide, once exposed, inserts into the outer-leaflet of the lipid bilayer, initiating the cell fusion process [5–9]. While, a part of the fusion protein undergoes an irreversible refolding to a stable conformation that releases free energy to overcome the membrane fusion barrier [5–9]. Consequently, a thorough understanding of the molecular basis of the fusion mechanism has been deemed vital not only for the virus life cycle but also for the rational design of potential therapeutics [10–14]. Therefore, atomic-resolution structures of fusion proteins are essential for obtaining mechanistic insights of membrane fusion process. Toward this, 3-D structures, using X-ray crystallography, of a number of fusion proteins, full length or fragment, are reported in their pre fusion or post fusion states [2,4,15–19]. As such, these atomic-resolution structures have provided important molecular insights for membrane fusion and drug discovery. However, atomic resolution structures of the full-length fusion proteins in complex with lipid membrane remain a challenging task. In order to infer membrane interactions of fusion processes, fusion peptides are investigated for structural and functional characterization in lipid or membrane mimetic environments [20–29].

During 2002–2003, SARS-CoV emerged as a global health risk which was speared over 29 countries infecting approximately 9000 people with 774 deaths worldwide [30–32]. More recently, a new coronavirus, designated hCoV-EMC or Middle East Respiratory Syndrome coronavirus (MERS-CoV), has been identified in humans in Middle Eastern countries and England causing death of several hundred of the infected people [33–36]. Coronaviruses, including SARS-CoV and MERS-CoV,

Abbreviations: SARS-CoV, severe acute respiratory syndrome-associated coronavirus AMPs; FP, fusion peptide; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy; DPC, dodecyl phosphocholine

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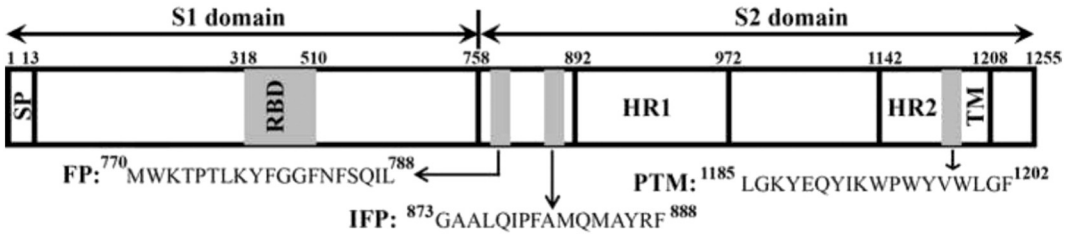

Fig. 1. Spike glycoprotein of SARS-CoV is composed of two domains S1 and S2 separated by a protease cleavage site. The N-terminal domain has a signal peptide (SP) and a transmembrane domain (TM) is present at the C-terminus. Other regions are receptor binding domain (RBD), two heptad repeat (HR1 and HR2) sequences and putative fusion peptides named as fusion peptide (FP), internal fusion peptide (IFP) and pre transmembrane domain (PTM).

display a broad host range and capable of causing chronic diseases related to the respiratory, hepatic and gastric systems. Due to its recurrent emergence, rapid and facile transmission, and high mortality, it is essential to gain a better understanding of the pathogenesis of this virus to develop antiviral drugs and vaccines for the cure and prevention of SARS and MERS [37–40].

The large positive-stranded RNA genome of SARS-CoV enters into the host through cell fusion mediated by the viral spike (S) glycoprotein [41].

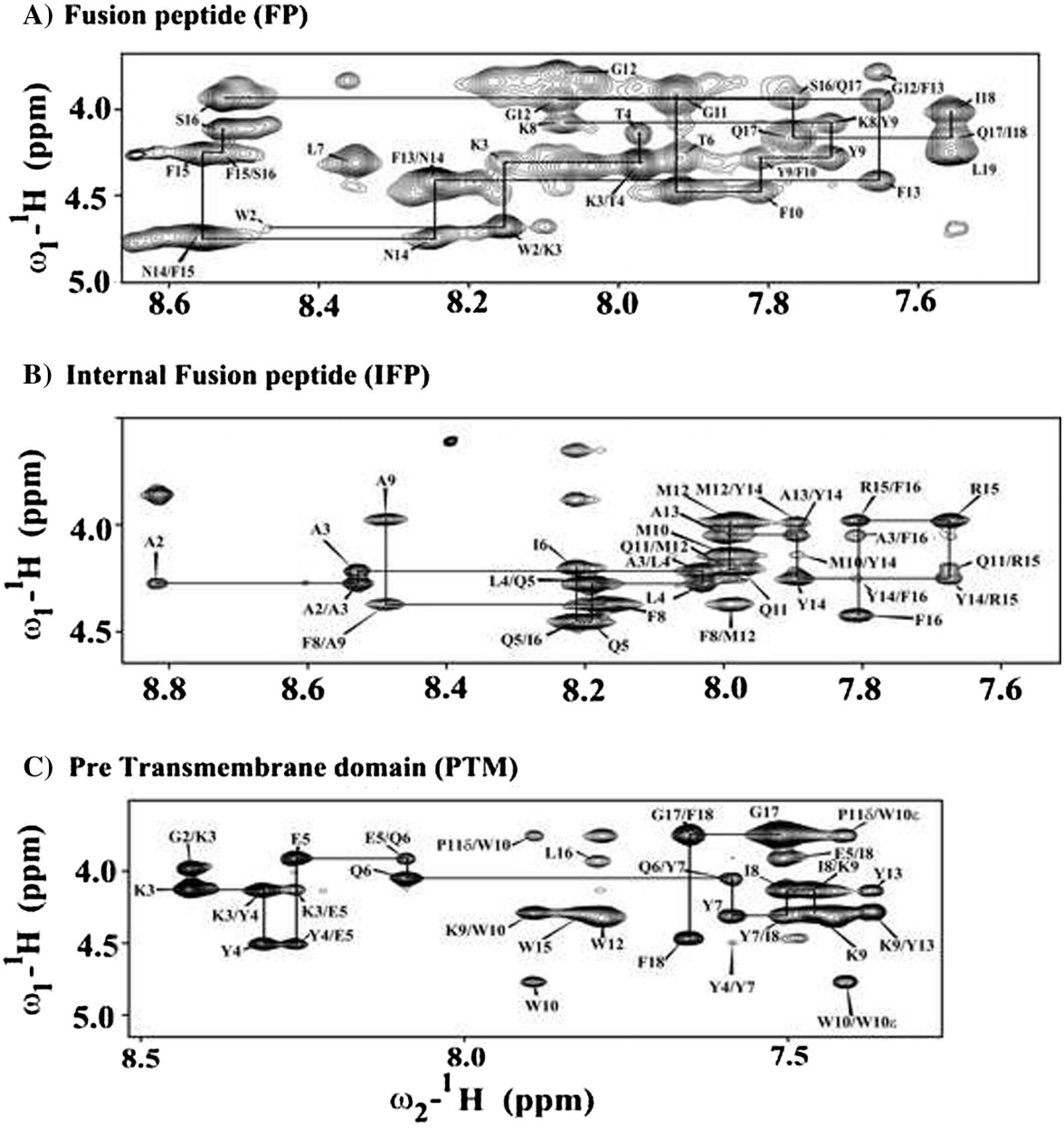

Fig. 2. Fingerprint regions of ^{1}H–^{1}H 2-D NOESY spectra of SARS-CoV fusion peptides showing sequence specific resonance assignments of FP (panel A), IFP (panel B) and PTM (panel C) in DPC micelles.