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Structural characterization of Mumps virus fusion protein core

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Abstract

The fusion proteins of enveloped viruses mediating the fusion between the viral and cellular membranes comprise two discontinuous heptad repeat (HR) domains located at the ectodomain of the enveloped glycoproteins. The crystal structure of the fusion protein core of Mumps virus (MuV) was determined at 2.2 Å resolution. The complex is a six-helix bundle in which three HR1 peptides form a central highly hydrophobic coiled-coil and three HR2 peptides pack against the hydrophobic grooves on the surface of central coiled-coil in an oblique antiparallel manner. Fusion core of MuV, like those of simian virus 5 and human respiratory syncytium virus, forms typical 3-4-4-4-3 spacing. The similar charecterization in HR1 regions, as well as the existence of O–X–O motif in extended regions of HR2 helix, suggests a basic rule for the formation of the fusion core of viral fusion proteins.

Keywords: Mumps virus; Fusion protein; Heptad repeat domains; Fusion core; Crystal structure

Mumps virus (MuV) belongs to genus *Rubulavirus*, family *Paramyxoviridae*, and can infect the glands, the central nervous system, the respiratory tract, and possibly also muscle and connective tissue [1]. Both surface glycoproteins, the hemagglutinin neuraminidase protein (HN) and the fusion (F) protein, are needed to effectuate an efficient viral fusion process mediated by Mumps virus [2]. MuV fusion protein shares many common features with those of other viral enveloped glycoproteins, including the hemagglutinin (HA) protein of influenza virus, gp160 of human immunodeficiency virus (HIV-1) and simian immunodeficiency virus (SIV), glycoprotein of Ebola virus, and fusion protein of paramyxovirus [3,4]. The fusion protein of MuV is synthesized as a precursor protein molecule (F0) which is cleaved in vivo by a host cell protease to yield the active F protein [5]. The mature F protein is composed of two disulfide-linked polypeptides, the distal subunit F2 and the transmembrane subunit F1, of unequal size (Fig. 1A). A highly hydrophobic conserved domain in the membrane-anchored subunit (F1), termed the fusion peptide, is considered to participate in the membrane fusion event as it would insert into the targeted cellular membrane.

The regions following the fusion peptide have a 4-3 heptad repeat (HR) of conserved hydrophobic residues for class I viral fusion proteins. The first heptad repeat, termed the HR1, is followed by a long spacer domain (in some viruses it is short) and a second heptad repeat, termed the HR2, followed by another short spacer and the transmembrane (TM) anchor (Fig. 1A). Previous studies of HA2 [6], HIV-1/SIV gp41 [7–13], Ebola virus GP2 [14,15], Simian virus 5 (SV5) F1 [16], and MHV fusion core [17] indicate that these heptad repeat regions form six-helix bundles which are considered as fusion core of these glycoproteins in a post-fusion form. Three HR1 peptides form hydrophobic central coiled-coil and three HR2 helices pack against the central core in an oblique antiparallel manner [3].

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Previous biochemical studies indicate that the viral fusion proteins undergo a series of conformational changes when activated [4,18]. Earlier studies on HA of influenza and gp41 of HIV-1 led to a model for membrane fusion mediated by envelope glycoproteins [3]. In this model, the N (HR1) and C (HR2) peptides of HIV gp41 come together and form a highly stable coiled-coil aligning the vial and cellular membranes juxtaposition, facilitating membrane fusion, and viral entry [19].

Our recent studies of HR1 and HR2 regions in MuV fusion protein have shown that its HR1 and HR2 also form a stable six-helix bundle, suggesting a common core architecture similar to those of other viral fusion protein [20]. These methods have been successfully used in the biochemical and structural analysis of several other viral fusion protein core, including SARS-CoV [21,22], MHV [17], Newcastle disease virus [23,24], Nipah virus, and Hendra virus [25]. Here, we report the determination of crystal structure of MuV fusion core to 2.2 Å resolution by X-ray crystallography. The structure confirms the similarity between the MuV and other viral fusion proteins, indicating a similar mechanism for viral fusion mediated by Mumps virus. The C terminus of HR1 peptide shows a 3-4-4-4-3 spacing, which is conserved in known SV5 F and HRSV F structure, suggesting the conserved fusion function and mode in family *Paramyxoviridae*. This structure also adds the repertoire of fusion cores of viral fusion proteins, providing more structural information for understanding of formation of post-fusion state of viral fusion proteins.

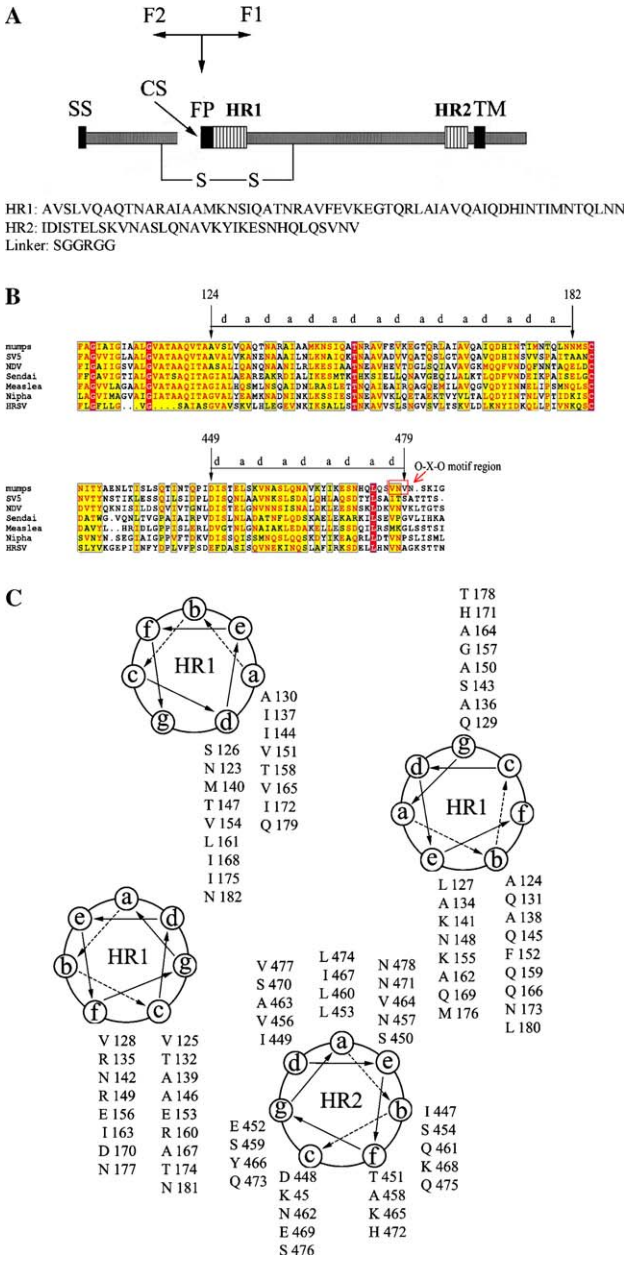

Fig. 1. Construction and sequence characterization of the MuV F protein 2-helix. (A) Schematic diagram of MuV F protein 2-helix constructs. F1 and F2 are formed after proteolytic cleavage (vertical arrow) and covalently linked by a disulfide bond. The fusion protein has an N-terminal signal sequence (SS) and a transmembrane domain (TM) adjacent to the C terminus. F1 contains two HR (heptad repeat) regions (hatched bars), termed HR1 and HR2, as indicated. FP (hatched bars) is an N-terminal fusion peptide followed by HR1 region. MuV 2-helix was constructed as a single polypeptide by linking two HR regions with a six-residue linker (SGGRGG). The amino-acid sequences for HR1 and HR2 used in this study were shown under schematic diagram. (B) Sequence alignment of paramyxovirus spike protein HR1 and HR2 regions. The identical and similar amino acids were indicated in red and yellow background, respectively. Letters above the sequence indicate the predicted hydrophobic heptad repeat a and d residues, which are highly conserved. O–X–O motif of Mumps virus HR2 region is shown in red box. (C) Helical wheel representation of HR1 and HR2. Three HR1 helices and one HR2 helix are represented as helical wheel projections in a top view form. The three central HR1 helices form a central trimic coiled-coil with the hydrophobic interaction of residues in the a and d positions. The three HR2 helices pack against grooves on the surface of the central coiled-coil, aligning residues at the a and d positions in HR2 and the residues at the e and g positions in HR1. These residues, mediating the interactions between HR1 and HR2, are always hydrophobic and highly conserved (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Materials and methods

Expression, purification, and crystallization. The construction, expression, purification, and biochemical studies of MuV fusion core protein was described previously [20]. The purified MuV fusion core protein were dialyzed against 10 mM Tris, pH8.0, 10 mM NaCl, and concentrated to 20 mg ml^{-1}. Crystallization was performed by the hanging-drop vapor-diffusion method at 291 K. One microliter protein solution was mixed with 1 μl reservoir solution and the mixture was equilibrated against 200 μl reservoir solution at 291 K. Crystallization conditions were screened using Crystal Screen reagent kits (Hampton Research). Crystals with good quality can be obtained in 15% PEG800, 0.5 M lithium sulfate in 2 weeks.

Data collection and processing. Data collection of MuV fusion core was performed in-house on a Rigaku RU2000 rotating-copper-anode X-ray generator operated at 48 kV and 98 mA (CuKα; $\lambda = 1.5418$ Å) with a MAR 345 image-plate detector. The crystal was mounted on nylon loops and flash-cooled in cold nitrogen-gas stream at 100 K using an Oxford Cryosystems with reservoir solution as the cryoprotectant. Data were indexed, integrated, and scaled using DENZO and SCALEPACK programs [26].