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Structure and function study of paramyxovirus fusion protein heptad repeat peptides

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Abstract

Paramyxovirus might adopt a molecular mechanism of membrane fusion similar to that of other class I viruses in which the heptad repeat (HR) regions of fusion protein (F) HR1 and HR2 form a six-helix bundle structure inducing membrane fusion. In this study, we examined the structure and function of HR1 and HR2 from the avian paramyxovirus-2 (APMV-2) F protein. The study showed that APMV-2 HR1 and HR2 formed a stable six-helix bundle. Only a soluble APMV-2 HR2 peptide showed potent and specific virus-cell fusion inhibition activity. Cross-inhibiting activity with APMV-1 (Newcastle disease virus, NDV) was not found. A possible mechanism of membrane fusion inhibition by the paramyxovirus HR2 peptide is discussed.

Keywords: Paramyxovirus; Heptad repeat; Six-helix bundle; Cross-inhibiting activity

Membrane fusion between the virus envelope and host cells is the first step of the enveloped virus's entry into the host cells. This process involves the interaction of viral envelope glycoproteins and their cellular receptors (proteins or sialic aids), which leads to the conformational changes of the envelope glycoproteins [1]. The viral envelope contains two important glycoproteins, including attachment glycoprotein and fusion glycoprotein, which have two highly conserved heptad repeat (HR)[1] HR1 and HR2 regions in class I viral envelope proteins. When membrane fusion occurs, HR1 and HR2 can form a six-helix bundle structure. Synthetic peptides corresponding to HR1 or HR2 regions exhibit the ability to inhibit virus infection [2]. These are shown in many enveloped viruses, including human immunodeficiency virus (HIV) and Avian sarcoma and leucosis virus (ASLV) of *Retroviridae* [3–5], possibly human cytomegalovirus (CMV) and Bovine herpesvirus-1 (BoHv-1) of *Herpesviridae* [6,7], severe acute respiratory syndrome cononavirus (SARS) of *Coronaviridae* [8,9], Ebola virus of *Filoviridae* [10], and paramyxoviruses.

Paramyxoviridae family viruses are important human and animal respiratory tract pathogens [11]. Paramyxovirus contains two envelope glycoproteins, hemagglutinin-neuraminidase (HN) and fusion protein (F). HN is involved in the virus attachment to the sialic acid-containing host-cell surface receptor. The current favored mechanism [12–14] involves binding sialates to HN. This alters its binding conformation so that it can activate the F protein to undergo three conformation

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[1] *Abbreviations used:* HR, heptad repeat; APMV-2, avian paramyxovirus-2; HIV, human immunodeficiency virus; ASLV, Avian sarcoma and leucosis virus; CMV, human cytomegalovirus; BoHv-1, Bovine herpesvirus-1; SARS, severe acute respiratory syndrome cononavirus; HN, hemagglutinin-neuraminidase; SeV, Sendai virus; MuV, Mumps virus; SV5, Simian virus 5; RSV, Respiratory syncytial virus.

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states including native state, pre-hairpin intermediate state, and post-fusion hairpin state. In the process, the F protein HR1 and HR2 tend to interact with each other to form a stable six-helix bundle, and then to pull the cellular and viral membranes closer to mediate the membrane fusion. The added synthetic HR1 or HR2, or both, of paramyxoviruses would prevent the formation of a six-helix bundle by binding to the respective partner in the F protein intermediate conformational state. Consensus fusion mechanism has been proposed for some paramyxoviruses, such as Avian paramyxovirus-1 (APMV-1) (popular nomenclature is Newcastle disease virus, NDV) [15], Sendai virus (SeV) [16,17], Mumps virus (MuV) [18], Simian virus 5 (SV5) [19], Measles virus (MeV) [20], and Respiratory syncytial virus (RSV) [21].

Avian paramyxovirus-2 (APMV-2) infects a wide variety of avian species, thus posing a potential threat to the animal industry. APMV-2 contains HN and F glycoproteins, the inactive precursor of APMV-2, F_0 (55.75 kDa), which is cleaved by the enzyme isolating F_1 and F_2 subunits [22,23]. APMV-1 (NDV) is a model virus for the study of viral fusion mechanism, which together with APMV-2 is a member of the genus *Rubulavirus* in the family *Paramyxoviridae*. No correlative report exists, however, regarding APMV-2 membrane fusion. In this paper, the HR1 and HR2 regions of APMV-2 F glycoproteins were predicted by the computer programs LearnCoil-VMF and ExPASy-Coils, in which HR1 and HR2 were synthesized and studied to determine a series of structure and functions. To examine the ability of the HR1 and HR2 peptides to form a stable six-helix bundle, we performed circular dichroism (CD) and gel-filtration experiments. A cell fusion inhibition assay and a cross-inhibition assay were designed to examine the specificity and susceptibility of APMV-1 and APMV-2 Env-mediated fusion to inhibition by homologous or heterologous HR peptides. Certain conflicts exist concerning the inhibition mechanism of viral–cell membrane fusion, and this paper discusses the novel possible mechanism based on the structure and function of paramyxovirus HR peptides.

Materials and methods

Gene construction

In this study, fusion protein (F) from the APMV-2 strain Yucaipa (GenBank Accession No. D13977) was used. We chose the LearnCoil-VMF(http://nightingale.lcs.mit.edu) and ExPASy-Coils (http://www.ch.embnet.org/software/COILS) programs, as they were designed specifically to identify viral heptad repeat coiled coils and have been used successfully for a number of virus fusion proteins. As shown in Fig. 1, the programs indeed predicted the F_1 protein HR1 and HR2 regions, which were quite similar to each other, but sequences predicted by LearnCoil-VMF were longer than those predicted by the ExPASy-Coils programs. The actual HR1 and HR2 sequences chosen in this paper were somewhat longer than those predicted by LearnCoil-VMF. For HR1 and HR2 gene construction, the HR1 region used was derived from amino acids 124 to 170, which gave

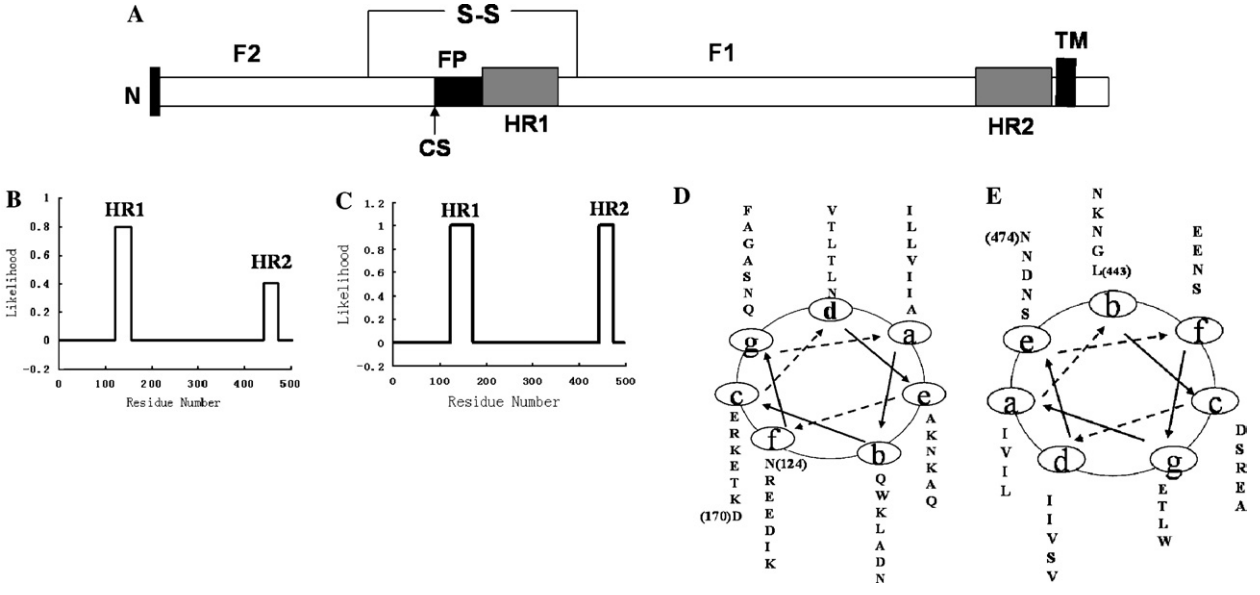

Fig. 1. Heptad repeat regions of the APMV-2 F protein. (A) Schematic diagram of the APMV-2 F protein with the location of structurally significant domains. "S-S" represents the disulfide bond linking the F_1 and F_2; CS, cleavage site; FP, fusion peptide; HR, heptad repeat; and TM, transmembrane region. (B) HR1 and HR2 sequences were predicted by the ExPASy-Coils program. (C) HR1 and HR2 sequences were predicted by the LearnCoil-VMF program. (D) Helical wheel of the HR1 (amino acids 124–170) is depicted. (E) Helical wheel of the HR2 (amino acids 443–474) is depicted.