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Biophysical characterization of HRC peptide analogs interaction with heptad repeat regions of the SARS-coronavirus Spike fusion protein core

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Abstract

The Spike (S) protein of SARS-coronavirus (SARS-CoV) mediates viral entry into host cells. It contains two heptad repeat regions, denoted HRN and HRC. We have identified the location of the two interacting HR regions that form the six-helix bundle (B. Tripet, et al, J. Biol. Chem., 279: 20836–20849, 2004). In this study, HRC peptide (1150–1185) was chosen as the region to make structure-based substitutions to design a series of HRC analogs with increased hydrophobicity, helical propensity and electrostatic interactions, or with a covalent constraint (lactam bridge) to stabilize the α-helical conformation. Effects of the substitutions on α-helical structure of HRC peptides and their abilities to interact with HRN or HRC have been examined by biophysical techniques. Our results show that the binding of the HRC analogs to HRN does not correlate with the coiled-coil stability of the HRC analogs, but their interactions with HRC does correlate with their stability, except for HRC7. This study also suggested three types of potential peptide inhibitors against viral entry can be designed, those that simultaneously inhibit interaction with HRC and HRN and those that are either HRC-specific or HRN-specific. For example, our study shows the important role of α-helical structure in the formation of the six-helix bundle where the lactam bridge constrained analog (HRC5) provided the best interaction with HRN. The importance of α-helical structure in the interaction with native HRC was demonstrated with analog HRC4 which binds best to HRC.

Keywords: Severe acute respiratory syndrome (SARS); Coronavirus (CoV); SARS-CoV; Spike protein; Heptad repeat regions (HRN and HRC); Coiled-coil; Six-helix bundle state

1. Introduction

Severe acute respiratory syndrome (SARS) is an acute respiratory illness caused by infection with a novel coronavirus (SARS-CoV). The severity and mortality of this illness was witnessed during the global pandemic of SARS-CoV in 2003. The virus has not re-emerged since July 2003, except for several cases of laboratory-acquired infections and one natural outbreak resulting in four infected people (Normile, 2004a; Normile, 2004b). However, since SARS-CoV-related viruses have been detected in some animals, it still remains a threat due to its highly transmittable nature to human populations and the mysterious origin of SARS-CoV (Guan et al., 2003; Hartley and Smith, 2003). Currently, since there is no effective agent for the anti-viral therapy of SARS-CoV infection, it is imperative to learn as much as possible about this virus to accelerate the development of therapeutics and vaccines for its re-emergence.

Infection by SARS coronavirus requires fusion of the viral and cellular membranes, which is mediated by the viral envelope Spike (S) glycoprotein and receptors on the target cell (Holmes, 2005). The S protein is a type I viral fusion protein which contains two highly conserved heptad repeat (HR) domains that have been shown to form coiled-coil structures (Bosch et al., 2003; Hakansson-McReynolds et al., 2006; Spaan et al., 1988; Tripet et al., 2004). The HR

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region located closest to the N-terminus in the sequence is denoted HRN and the HR region located closest to the C-terminus is denoted HRC. Although the exact mechanism by which the SARS-CoV enters the host cell has not been elucidated, it is most likely similar to other coronaviruses. Upon binding to the receptor at the cell membrane, the fusion protein will be induced into the fusogenic intermediate state with a dramatic conformation change. Collapse of this fusogenic intermediate leads to a six-helix bundle (trimer of dimers) formation between HRN and HRC, which ultimately promotes membrane fusion (Eckert and Kim, 2001b). Our laboratory and other researchers recently reported (Bosch et al., 2004; Ingallinella et al., 2004; Tripet et al., 2004; Xu et al., 2004b) that HR regions of SARS-CoV S protein can associate to form a very stable α-helical six-stranded structure and the orientation of the HR regions is anti-parallel. Residues 902–950 in the HRN region and 1151–1185 in the HRC region were identified to be crucial for their interaction. The structure of the ectodomain of SARS Spike protein in its fusogenic/post-fusogenic state and HR interaction regions have recently been confirmed by crystallography (Duquerroy et al., 2005; Supekar et al., 2004; Xu et al., 2004a). Most recently, Li et al. (2005) reported the crystal structure of the SARS-CoV S receptor binding domain (residues 306–575) complexed with receptor ACE2 (angiotensin-converting enzyme 2), which further confirms the prediction that SARS-CoV fusion process is similar to other viruses with type I fusion proteins. Hakansson-McReynolds et al. (2006) also reported the solution structure of the SARS-Coronavirus HRC domain (1143–1193) in the prefusion state, which forms a coiled-coil symmetric trimer.

A critical step in viral entry is the conformational change of the fusion domain of the S protein. Peptides derived from the HRC region of the HIV-1 gp41 and other class I fusion proteins have been reported to show significant viral fusion inhibitory activity (Chan and Kim, 1998; Eckert and Kim, 2001a; Root et al., 2001). It has been proposed that HRC analogs bind strongly to the transiently exposed HRN coiled-coil trimer, and block the formation of the six-helical bundle necessary for the fusion. For HIV and murine CoV mouse hepatitis virus (MHV), HRC peptides have been shown to inhibit viral entry at nanomolar concentrations (Bosch et al., 2004; Chan and Kim, 1998). Recently, a HIV peptide inhibitor has been made by the fusion of gp41 HRN region and a trimeric forming coiled-coil peptide, where the three chains are covalently linked by disulfide bridges. This construct was a potent inhibitor of viral entry at pM concentration (Bianchi et al., 2005). One of the HRC peptides of HIV-1 gp41, Enfuvirtide (Fuzeon), was approved by the FDA for treatment of AIDS (LaBonte et al., 2003). In several recent studies (Bosch et al., 2004; Liu et al., 2004; Yuan et al., 2004; Zhu et al., 2004), SARS-CoV S-mediated fusion was shown to be inhibited by HRC-derived peptides; however, these peptides inhibited viral entry at concentrations in the micromolar range (Bosch et al., 2004; Liu et al., 2004). One possible reason for the higher inhibitory concentration is that the interaction between the inhibitory peptides and HRN or HRC is too weak to prevent the native intra-molecular association of HRN and HRC. As reported in studies of HIV peptide inhibitors, considerable genetic and biophysical evidence supports the concept that the ability of the Class I envelope glycoprotein to mediate membrane fusion is determined, in part, by the high stability of the six-helix bundle (Eckert and Kim, 2001b; Skehel and Wiley, 2000). Thus, peptides inhibiting the fusion process must be designed with the highest binding affinity/stability to be useful for preventing viral entry.

In the present study, HRC peptide (1150–1185) was chosen as the region to make structure-based substitutions to design a series of HRC analogs. These HRC analogs were designed with increased hydrophobicity, helical propensity and electrostatic interactions, or with a covalent constraint (lactam bridge) to stabilize the α-helical structure. Effects of the substitutions on the conformation of HRC peptides and their abilities to interact with HRN or HRC have been examined by biophysical techniques.

2. Materials and methods

2.1. Preparation of peptides

2.1.1. Peptide synthesis

The HRN and HRC peptides of SARS-CoVS glycoprotein were prepared by solid-phase synthesis methodology-using 4-benzylhydrylamine hydrochloride resin with conventional *fmoc* (fluorenylmethoxycarbonyl) chemistry as described by Tripet et al. (2000).

The synthesis of the covalent HRC trimer and HRN trimer for surface plasmon resonance analysis was carried out as described by Tripet et al. (2006).

The gene sequence Gene bank Accession No. for the SARS-CoV S protein is AY278741.

2.1.2. Lactam formation on peptide resin

The protecting groups for the side-chains of Lys1170 and Glu1166 in the HRC5 sequence which forms the *i*, *i* + 4 lactam bridge were the allyloxycarbonyl group (Aloc) for Lys and the allylester for Glu, Glu (OAl). These two protecting groups were removed by Tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$] as described by Kates et al. (1993). The reaction was carried our under argon. Formation of the lactam ring was achieved with benzotriazole-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (pyBOP) in the presence of *N*,*N*-diisopropylethylamine (DIEA).

2.1.3. Peptide purification and characterization

Peptides were N-terminally acetylated, cleaved from the-resin, and purified by reversed-phase high-performance liquid chromatography (RP-HPLC) on a Zorbax 300 SB-C8 preparative column (250 mm × 9.4 mm I.D., 6.5 μm particle size, 300-Å pore size; Agilent Technologies, Little Falls,