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The SARS-CoV heptad repeat 2 exhibits pH-induced helix formation

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ABSTRACT

The heptad repeats 1 and 2 of SARS-CoV spike, termed HR1 and HR2, play critical roles in viral entry. Moreover, HR1 and HR2 derived free peptides are inhibitors of SARS-CoV entry. In this work we used circular dichroism to show that HR2 helix formation is induced at pH 5, the pH of the endosome. In addition, we demonstrate that the HR2 helix is further stabilized at physiological ionic strengths. Together, these observations provide new insight into the mechanism of SARS-CoV entry and suggest that HR2 may be an attractive target for therapeutic intervention.

1. Introduction

Severe Acute Respiratory Syndrome (SARS) is caused by the coronavirus SARS-CoV [1,2]. The entry of SARS-CoV, as well as other enveloped viruses including Ebola, HIV and Influenza, is mediated by envelope proteins [3,4]. The SARS-CoV envelope protein consists of S1, which binds to receptor, and S2, which mediates fusion of the viral and target membranes. In the first step of entry, S1 attaches to a target cell receptor, human angiotensin-converting enzyme 2 (hACE2) and the virus enters the cell via receptor-mediated endocytosis [3,5]. In the endosome, proteolysis by proteases of the cathespin family trigger conformational changes within S2 that are necessary for membrane fusion [6].

The S2 protein of SARS-CoV contains 2 heptad repeats, termed HR1 and HR2, which play a critical role in membrane fusion, similar to that of other enveloped viruses [7,8]. Interestingly, peptide versions of the HIV HR1 and HR2 are potent inhibitors of HIV entry [4]. In contrast, peptide versions of SARS-CoV HR1 and HR2 are relatively modest inhibitors of SARS-CoV entry [8–10].

The SARS-CoV HR2 have been extensively studied by X-ray crystallography and NMR. In the presence of HR1, HR1 and HR2 form a trimer of antiparallel helices, which is thought to represent a low energy final conformation that brings the virus and endosomal membranes into close contact and subsequent fusion [11–13]. In the presence of the co-solvent TFE, isolated HR2 forms a trimer of parallel helices [14]. On the other hand, under aqueous conditions, isolated HR2 has been shown to be in an equilibrium between extended coil monomer and helical trimer [15,16]. In this work, we further characterize the structural properties of SARS-CoV HR2 under different conditions of pH and ionic strength by circular dichroism.

2. Materials and methods

2.1. Protein preparation

SARS-CoV HR2 was prepared as a HIS-PG-HR2 fusion protein as previously described [14,15]. Briefly, protein expression was achieved by growing *Escherichia coli* strain SG13009 in the presence of the appropriate plasmid in 1 L of LB media supplemented with 100 μg/mL ampicillin and 50 μg/mL of kanamycin at 37 °C until they reached an OD_{600} of 0.6. Expression was induced by addition of 0.8 mM IPTG and the culture was grown for an additional 4–5 h at 37 °C. The HIS-PG-HR2 fusion protein was purified from the soluble fraction using a Ni^{2+} fast-flow Sepharose column (Qiagen, Valencia, CA). The protein was then cleaved using TEV protease and run once more over the Ni^{2+} column to remove His-PG and TEV protease, which also contains a polyhistidine tag, as well as uncut HIS-PG-HR2. The flow-through fraction containing HR2 was then dialyzed extensively against 10 mM $NaPO_4$, pH 7.0 and concentrated by ultrafiltration (YM3; Amicon, Billerica, MA). The purity and identity of HR2 was confirmed using SDS–PAGE and MALDI–TOF mass spectrometry.

2.2. Circular dichroism

Circular dichroism (CD) spectra were measured on a Jasco-710 spectropolarimeter. Wavelength spectra were recorded from 190–260 nm at peptide concentrations of 110 μM (0.7 mg/mL) in cells of 0.2 mm path length. For all experiments the spectra were corrected with the subtraction of a blank corresponding to the

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buffer of that experiment. Buffer conditions were 10 mM $NaPO_4$, pH 7 or 5, and 0–300 mM NaCl at 20 °C. The percentage of alpha-helical residues was calculated by the observed molar ellipticity at 222 nm divided by the theoretical molar ellipticity (Θ_t), where $(\Theta_t) = 40{,}000 \times (1-4.6/N)$ and $N = 55$ (the total number of residues).

3. Results and discussion

As noted in the introduction, SARS-CoV enters cells via receptor-mediated endocytosis. Accordingly, we first tested the effect of pH on SARS-CoV HR2 structure at pH 7 and 5, the pH of the extracellular and endosomal spaces, respectively. As shown in Fig. 1A, the circular dichroism spectrum at pH 7 reveals the presence of helical structure, which is estimated to be ~32% (~18 of 55 residues). At pH 5, the helical content of HR2 increases substantially to ~90% (~50 of 55 residues).

We next tested the effects of ionic strength on SARS-CoV HR2 structure at pH 7. As shown in Fig. 1B, at pH 7 the helical content of HR2 increases from ~13% at low ionic strength to a maximum of 32% at physiological ionic strength. In a similar pattern, at pH 5 the helical content of HR2 increases from ~40% at low ionic strength to a maximum of ~90% at physiological ionic strength. At both pH, the helical content decreases at the highest ionic strength measured.

Taken together, the helical content of SARS-CoV HR2 is clearly stabilized at physiological ionic strength and at the pH of the endosome. Indeed, under these conditions the HR2 has a higher percentage of helix than the TFE-stabilized trimer of HR2 that was previously characterized [14]. Note that the helix stabilizing properties of NaCl at intermediate concentrations and the helix destabilizing properties of NaCl at relatively high concentrations have been previously observed in other peptides and are attributed to the interactions of Hofmeister ions [17,18].

To interpret the effects of pH and ionic strength, it is of interest to consider the structure of the SARS-CoV HR2 trimer [14]. As shown by the electrostatic profile of HR2 in Fig. 2A, there are numerous patches of like-charged sidechains in the TFE-stabilized helical trimer. For example, there are four regions of potential charge repulsion: (1) the negatively charged sidechains of D7, D9 and D12; (2) the positively charged sidechains of K25 and R29; (3) the negatively charged sidechains of E26, D28 and E32; (4) and the negatively charged sidechains of E39, D43 and E46. Increasing ionic strength would be expected to reduce the charge repulsion present in the helical form and thus stabilize the helical form with respect to the extended coil form. Furthermore, at pH 5 the acidic groups are expected to be partially protonated, thereby further reducing charge repulsion. Interestingly, in Influenza HA2, a region that connects two heptad repeat domains also exhibits pH-induced helix formation, presumably due to partial protonation of acidic

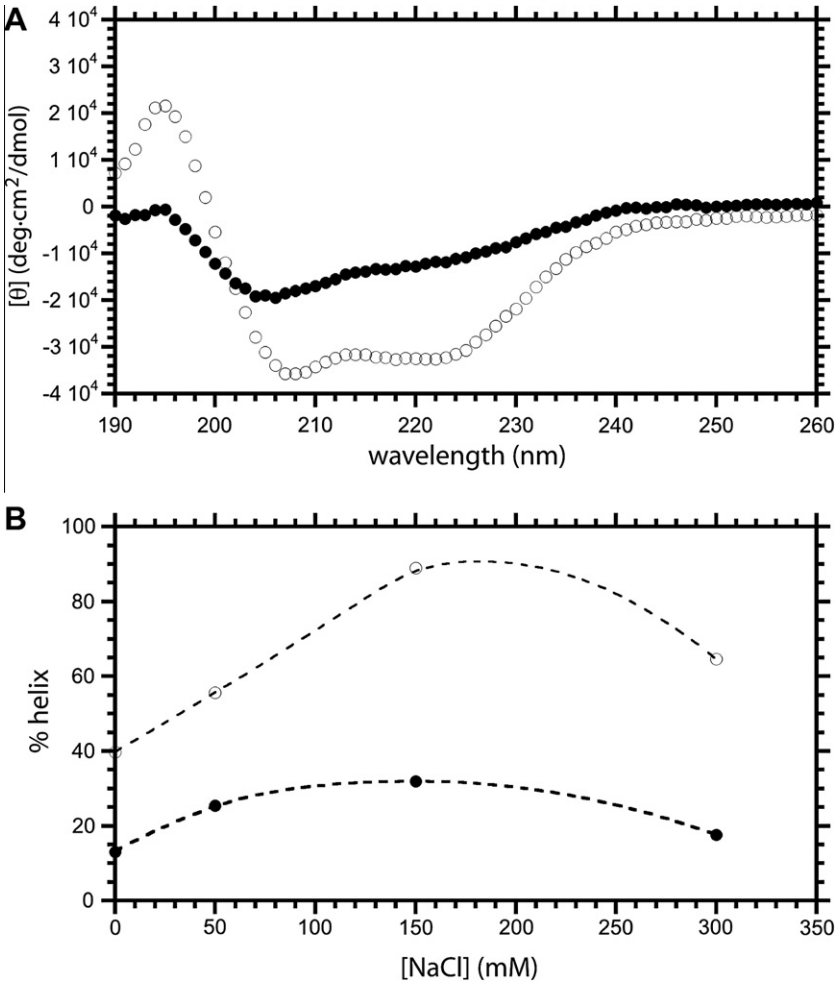

Fig. 1. (A) Circular dichroism studies of SARS-CoV HR2 at pH 7 (filled circles) and pH 5 (open circles). (B) SARS-CoV HR2 helix content as a function of ionic strength at pH 7 (filled circles) and pH 5 (open circles). The experimental conditions were 110 μM HR2 in 10 mM $NaHPO_4$ and 0–300 mM NaCl at 20 °C.

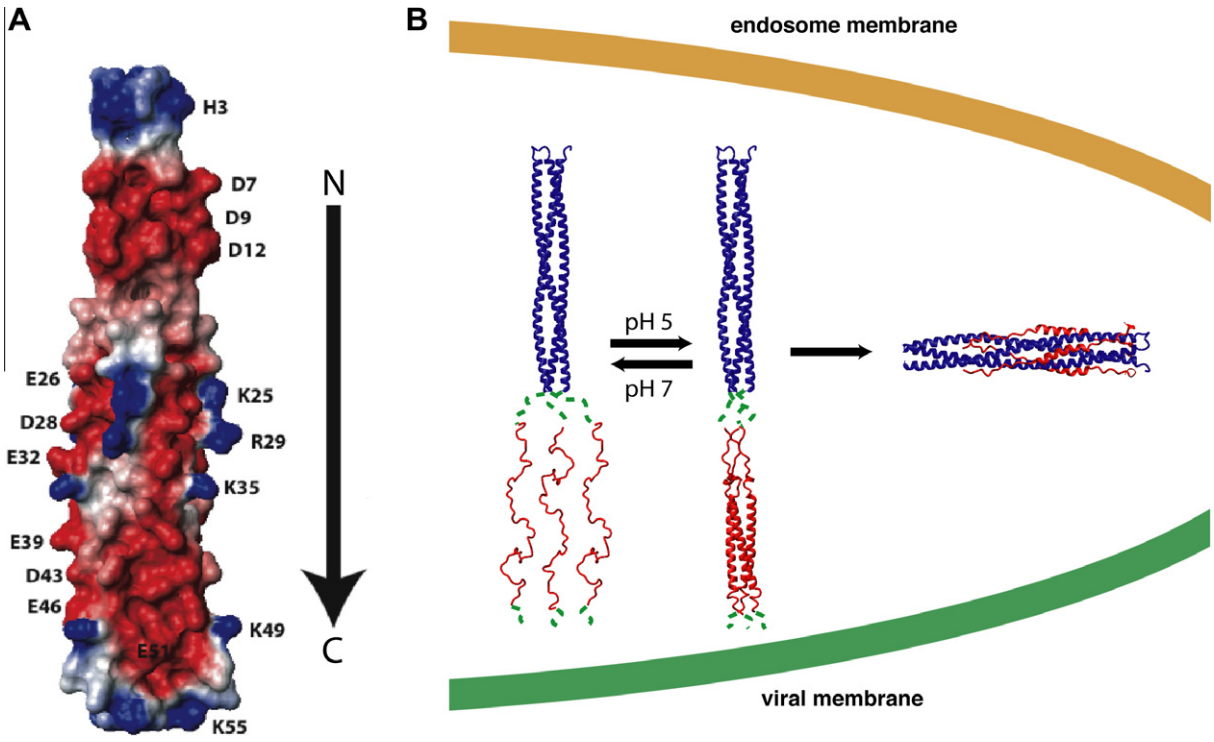

Fig. 2. (A) Electrostatic profile of SARS-CoV HR2. The structure is taken from Hakansson-McReynolds et al. [14]. (B) Model for HR2-mediated entry of SARS-CoV. HR1 and HR2 are depicted in blue and red, respectively. Undetermined structural domains are depicted as green dashes. Coordinates for isolated HR2 prefusion state are taken from [14]. Coordinates for the HR1 and HR1/HR2 are taken from [11]. (For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this article.)

sidechains and the reduction of electrostatic repulsion in the helical conformation [19]. In the case of the Influenza virus, the formation of helix in this region triggers a large conformational change in HA2 structure that is intrinsic to the viral entry mechanism in the endosome [19,20]. Thus, it is tempting to speculate that the SARS-CoV HR2 has evolved to favor the transition from coil to helix in the endosome.

We have previously presented a model for SARS-CoV entry in which the coil–helix equilibrium of HR2 played a role in allowing repositioning of the HR1 and HR2, based on biochemical and biophysical studies at pH 7 [15]. In light of the present results at pH 5, the model needs to be modified. In the modified model, at pH 7 HR2 is in equilibrium between extended coil and helix with the extended coil being favored (Fig. 2B). Upon entering the endosome (i.e. a transition to pH 5) the helix conformation of HR2 is greatly favored. Subsequently, the HR2 helix interacts with the HR1 helix to form the highly stable six helix bundle, which brings the viral and endosomal membranes into close proximity and allows membrane fusion to occur. As a consequence of this model, isolated HR2 helix is transiently present in the endosome, which therefore may present a novel target for therapeutic intervention. For example, small molecules or peptides that bind to the HR2 helix may be expected to disrupt the HR1–HR2 interaction that is critical for membrane fusion. Finally, we note that it will be interesting to consider whether pH-induced structural changes occur the HR2 of other viruses that enter via endocytosis such as Ebola and Influenza.

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