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MERS coronavirus envelope protein has a single transmembrane domain that forms pentameric ion channels

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ABSTRACT

The Middle East respiratory syndrome coronavirus (MERS-CoV) is a newly identified pathogen able of human transmission that causes a mortality of almost 40%. As in the case of SARS-CoV, MERS virus lacking E protein represents a potential vaccine. In both cases, abolishment of channel activity may be a contributor to the attenuation observed in E-deleted viruses. Herein, we report that purified MERS-CoV E protein, like SARS-CoV E protein, is almost fully α-helical, has a single α-helical transmembrane domain, and forms pentameric ion channels in lipid bilayers. Based on these similarities, and the proposed involvement of channel activity as virulence factor in SARS-CoV E protein, MERS-CoV E protein may constitute a potential drug target.

1. Introduction

In September 2012 the first case of a novel coronavirus (CoV) infection was reported (Zaki et al., 2012) and the etiological agent was named Middle East respiratory syndrome coronavirus (MERS-CoV) (de Groot et al., 2013). As of 23 May 2014, 635 cases and 193 deaths have been confirmed (http://www.who.int/csr/don/2014_05_23_mers/en/). An investigation on 47 cases between September 2012 and June 2013 revealed that MERS patients display similar clinical features to severe acute respiratory syndrome (SARS) virus infection, but progress more rapidly towards respiratory failure (Assiri et al., 2013). In addition, a majority of MERS patients (estimated at 75%) has at least one co-morbid condition, in contrast to only 10–30% in SARS patients (Assiri et al., 2013; Drosten, 2013; Raj et al., 2014). Mortality rate in MERS-CoV-infected individuals is about 40%, compared to 10% during the SARS outbreak (http://www.who.int/csr/sarsarchive/2003_05_07a/en/). Human-to-human transmission has been reported, although it is still limited and inefficient (Raj et al., 2014). Although there are still no effective treatments, a mouse model suitable for studying MERS-CoV infection has been engineered (Zhao et al., 2014). The genomic sequence of MERS-CoV is closely related to bat CoVs HKU4 and HKU5, which belong to clade c of β-coronaviruses (Annan et al., 2013; Drexler et al., 2014). Like SARS-CoV, MERS-CoV is thought to have crossed over from bats to humans, although MERS-CoV used camels as an intermediate species (Haagmans et al., 2014).

The structural proteins in coronaviruses are N (nucleocapsid) and integral membrane proteins S (spike), M (membrane) and E (envelope). The S protein is involved in host recognition and entry into the cell. Recently, the crystal structure of the fusion core (Gao et al., 2013) and the receptor binding domain (Chen et al., 2013) of MERS S protein have been solved. The M protein is the most abundant protein in the lipid envelope of virions and defines their shape (Barcena et al., 2009; Neuman et al., 2011), although high resolution structural data is still not available. The E protein is scarcely present in the virion, but abundantly expressed in the infected cell, mainly distributed in intracellular membranes between ER and

Abbreviations: AQPZ, *E. coli* aquaporin Z; DDM, n-dodecyl β-D-maltoside; DHPC, 1,2-dihexanoyl-sn-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DPhPC, 1,2-diphytanoyl-sn-glycero-3-phosphocholine; DOPS, 1,2-dioleoyl-sn-glycero-3-phospho-L-serine; DPC, dodecyl phosphocholine; E, envelope; ER, endoplasmic reticulum; ERGIC, ER Golgi intermediate compartment; FTIR, Fourier-transform infrared spectroscopy; IBV, infectious bronchitis virus; LMPG, 1-myristoyl-2-hydroxy-sn-glycero-3-phosphoglycerol; M, membrane; MHV, murine hepatitis virus; MERS-CoV, Middle East respiratory syndrome coronavirus; N, nucleocapsid; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; SARS, severe acute respiratory syndrome; S, spike; SDS, sodium dodecyl sulphate; TM, transmembrane; TGEV, transmissible gastroenteritis virus.

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Golgi compartments (Lim and Liu, 2001; Nal et al., 2005; Raamsman et al., 2000). In those sites, it participates in virus assembly, budding and intracellular trafficking. In the case of SARS-CoV, the E protein accumulates at the ERGIC (Nieto-Torres et al., 2011).

CoV E proteins are 76–109 amino acids long and predicted to have at least one α-helical transmembrane (TM) domain based on prediction algorithms (Surya et al., 2013). The extramembrane C-terminal domain has some hydrophobic character, and could represent in some cases a second TM domain, e.g., in infectious bronchitis virus (IBV) and in murine hepatitis virus (MHV) E proteins, although this has not been confirmed experimentally. SARS-CoV E protein has been shown unequivocally to have just one TM domain (Li et al., 2014), and is the only E protein for which structural data is available (Li et al., 2014; Pervushin et al., 2009). E proteins have been shown to form ion channels in synthetic membranes (Torres et al., 2007; Wilson et al., 2006), with reported conductance of 20–30 pS. However, characterization with properly purified full length and transmembrane peptides of SARS-CoV E protein has produced much higher (>10 times) conductances (Baguena et al., 2012; Verdia-Baguena et al., 2012, 2013a,b). In SARS-CoV, this channel activity has been linked to activation of caspase-1 and maturation of pro-inflammatory IL-1β during infection (Nieto-Torres et al., 2014), although a detailed mechanism to explain this functional link is still not available.

Removal of E protein is in general deleterious to coronaviruses, although the severity of the damage is strongly virus-dependent. For example, deletion of E gene in transmissible gastroenteritis virus (TGEV) results in blockage of virus trafficking in the cellular secretory pathway and prevention of virus maturation (Ortego et al., 2002). Mutations at the C-terminal region of MHV E protein results in impaired virion assembly and maturation (Fischer et al., 1998). SARS-CoV lacking the E gene is attenuated (DeDiego et al., 2007), and MERS-CoV lacking the E gene cannot propagate, despite showing similar viral RNA level to wild-type virus (Almazán et al., 2013). Based on the attenuating effect of E protein removal, a SARS-CoV lacking E gene is currently being studied as potential vaccine (Enjuanes et al., 2011; Fett et al., 2013; Netland et al., 2010), and a similar E-deletion approach has been suggested for controlling the MERS outbreak to obtain a virus that is replication-competent, but propagation-defective (Almazán et al., 2013).

The only coronavirus E protein structurally characterized in some detail is the one in SARS-CoV, as a transmembrane domain (Pervushin et al., 2009; Torres et al., 2006) and latter as a truncated form (residues 8–65), obtained using a 20 residue N-terminal tag that included a poly-His tail (Li et al., 2014). Herein, we report for the first time the expression, purification, and preliminary structural study on full-length 82 residue MERS-CoV E protein without any tag. This purified protein allowed its biophysical characterization to conclude that it has striking similarities to SARS-CoV E protein.

2. Materials and methods

2.1. Protein expression and purification

The DNA sequence encoding E protein from MERS-CoV-EMC12 (GenBank accession number JX869059) was fused N-terminally to a 6-His-MBP tag, followed by a TEV protease cleavage site, to form the construct 6-His-MBP-TEV-E. This was subcloned into pTB-MalE for expression. To investigate the effect of the four native cysteines (Fig. 1A), in addition to wild type MERS-CoV E, three additional Cys-to-Ala mutants were generated, at positions 23 and 30 (E_{AACC}), 40 and 43 (E_{CCAA}), and 23, 30, 40 and 43 (E_{AAAA}). Expression and purification of non labeled and ^{15}N-labeled E_{AAAA} was performed following the protocol described previously for MBP-tagged full-length SARS-COV E protein (Li et al., 2014), except that the elution buffer contained 1.6 mM n-dodecyl β-D-maltoside (DDM, Calbiochem).

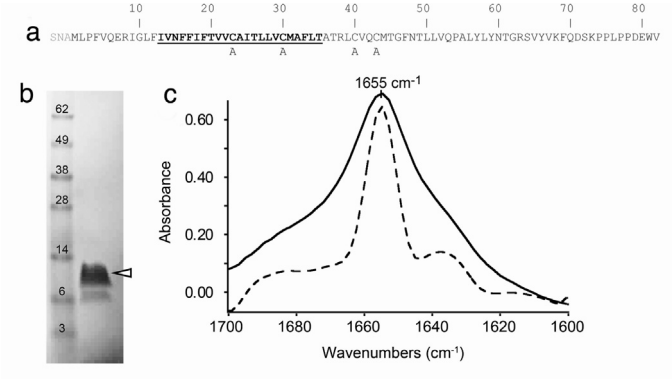

Fig. 1. Purification and secondary structure of MERS-CoV E protein. (A) Amino acid sequence of full-length E protein used in this study with predicted TM region in bold and underlined. The three N-terminal amino acids, SNA (gray), result from MBP tag removal by TEV protease cleavage. Positions of Cys residues, which were mutated into alanine, are indicated by 'A'; (B) SDS-NuPAGE of purified Cys-less mutant, E_{AAAA}. The protein band migrated approximately at its calculated molecular weight of 9.6 kDa; (C) infrared amide I region of MERS-CoV E in hydrated POPC lipid bilayers (solid line) and its corresponding Fourier self-deconvolved spectrum (dashed line).

2.2. Gel electrophoresis

For SDS-NuPAGE, samples were run in 4–12% NuPAGE® Bis–Tris gels (Invitrogen) with NuPAGE® MES SDS running buffer and stained with SimplyBlue™ SafeStain (Invitrogen) according to the manufacturer's protocol. Blue-native PAGE (BN-PAGE) was performed as described previously (Gan et al., 2011). Lyophilized peptide was solubilized to 0.1 mM in a sample buffer containing 50 mM DHPC (Avanti Polar Lipids), 5, 25 and 50 mM DPC (Avanti Polar Lipids), or 21 mM LMPG (Anatrace). *Escherichia coli* aquaporin Z (AqpZ) in 20 mM SDS was included as additional molecular weight marker.

2.3. Fourier-transform infrared spectroscopy

Sample preparation, ATR data collection and H/D exchange were performed as described previously (Torres et al., 2006) on a Nicolet Nexus spectrometer (Madison, USA). Briefly, the peptide was incorporated in multilamellar liposomes by dissolving a dry mixture of peptide and POPC (Avanti Polar Lipids) or DMPC (Avanti Polar Lipids) at a 1:50 molar ratio. Fourier self-deconvolution was performed on the amide I region using FWHH of 20 cm^{-1} and a narrowing factor k of 1.5 (Kauppinen et al., 1981).

2.4. Analytical ultracentrifugation

Sedimentation equilibrium data were collected at 20 °C in a Beckman XL-I analytical ultracentrifuge monitoring the absorbance at 280 nm. Lyophilized peptide was dissolved to OD_{280} of 0.3, 0.5, and 0.8 (12 mm pathlength) in 50 mM Tris buffer (pH 7.3), 100 mM NaCl, 5 mM C14-betaine and 32.3% D_2O. The samples were centrifuged in six-channel charcoal-filled Epon centerpieces with quartz windows. Radial distribution profiles were acquired at 18,500, 22,700, and 27,800 rpm after reaching equilibrium. The latter was tested by HeteroAnalysis. Data were processed and fitted to several monomer-N-mer models in SEDFIT and SEDPHAT (Schuck, 2003).