Virology 432 (2012) 485–494

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Coronavirus E protein forms ion channels with functionally and structurally-involved membrane lipids

Carmina Verdiá-Báguena [a,1], Jose L. Nieto-Torres [b,1], Antonio Alcaraz [a], Marta L. DeDiego [b], Jaume Torres [c], Vicente M. Aguilella [a,*], Luis Enjuanes [b,*]

[a] Department of Physics, Laboratory of Molecular Biophysics, Universitat Jaume I, 12071 Castellón, Spain
[b] Department of Molecular and Cell Biology, Centro Nacional de Biotecnología (CNB-CSIC), Campus Universidad Autónoma de Madrid, Darwin 3, 28049 Madrid, Spain
[c] School of Biological Sciences, Division of Structural and Computational Biology, Nanyang Technological University, Singapore 637551, Singapore

ARTICLE INFO

Article history:
Received 11 May 2012
Returned to author for revisions
6 June 2012
Accepted 6 July 2012
Available online 24 July 2012

Keywords:
Coronavirus
SARS
Envelope protein
Ion channel
HCoV-229E
Lipid membranes

ABSTRACT

Coronavirus (CoV) envelope (E) protein ion channel activity was determined in channels formed in planar lipid bilayers by peptides representing either the transmembrane domain of severe acute respiratory syndrome CoV (SARS-CoV) E protein, or the full-length E protein. Both of them formed a voltage independent ion conductive pore with symmetric ion transport properties. Mutations N15A and V25F located in the transmembrane domain prevented the ion conductivity. E protein derived channels showed no cation preference in non-charged lipid membranes, whereas they behaved as pores with mild cation selectivity in negatively-charged lipid membranes. The ion conductance was also controlled by the lipid composition of the membrane. Lipid charge also regulated the selectivity of a HCoV-229E E protein derived peptide. These results suggested that the lipids are functionally involved in E protein ion channel activity, forming a protein–lipid pore, a novel concept for CoV E protein ion channel entity.

Introduction

Coronaviruses (CoV) are vertebrate pathogens that cause common colds, bronchiolitis and acute respiratory distress syndrome that may lead to death in humans, and lethal diseases of high economic importance in animals (Perlman and Netland, 2009). In fact, their relevance increased when the causative agent of the severe acute respiratory syndrome (SARS) was identified as a CoV. SARS-CoV emerged at the end of 2002 in Guangdong province, China, causing an epidemic with 8000 infected people and a death rate close to 10% (Drosten et al., 2003; Rota et al., 2003). SARS-CoV like viruses have been identified in bat reservoirs all over the world (Chu et al., 2008; Drexler et al., 2010; Muller et al., 2007; Quan et al., 2010), making SARS-CoV reemergence a realistic possibility.

Members of the *Coronaviridae* family (de Groot et al., 2012) have a plus-strand RNA genome of around 30 kb in length (Enjuanes et al., 2008). CoV viral genome is packed by the nucleocapsid (N) protein, to form a helicoidal nucleocapsid that is protected by a lipid envelope. Several viral proteins, including the spike (S), envelope (E), and membrane (M) proteins are embedded within this lipid envelope. In addition, a variable set of proteins is also present within the membrane, depending on the CoV species. In the case of SARS-CoV, proteins 3a, 6, 7a and 7b have also been identified in the viral membrane (Huang et al., 2006, 2007; Schaecher et al., 2007; Shen et al., 2005).

CoV E protein is a small transmembrane protein of between 76 and 109 amino acids in length (Arbely et al., 2004; Raamsman et al., 2000). E protein amino acid sequence is quite divergent among different CoVs, nevertheless its predicted structure is highly conserved and includes a short N-terminal amino acids stretch, an alpha helical transmembrane domain and a carboxy terminal region (Torres et al., 2007).

E protein is incorporated at a low copy number in the viral envelope (Maeda et al., 2001; Raamsman et al., 2000). Nevertheless, high amounts of E protein are accumulated within cells during viral infection, suggesting an important role of this protein during virus cycle. CoV E protein mainly distributes between ER and Golgi apparatus membranes where it actively participates in virus budding, morphogenesis and trafficking (Corse and Machamer, 2000; Lim and Liu, 2001; Nal et al., 2005; Nguyen and Hogue, 1997; Raamsman et al., 2000; Ruch and Machamer, 2012a). Particularly, SARS-CoV E protein mainly localizes in the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) when expressed alone or during virus infection (Nieto-Torres

* Corresponding authors.
E-mail addresses: aguilell@uji.es (V.M. Aguilella), L.Enjuanes@cnb.csic.es (L. Enjuanes).
[1] Both authors equally contributed to this work.

0042-6822/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2012.07.005

et al., 2011). It has been proposed that SARS-CoV E protein sequesters protein associated with lin seven 1 (PALS1), a member of the tight junctions complex, to the ERGIC, disrupting the epithelia and possibly contributing to the lung damage observed in SARS patients (Teoh et al., 2010). The presence of SARS-CoV E protein at the cell plasma membrane has been previously suggested (Pervushin et al., 2009), however, these results have not been confirmed in recent studies (Nieto-Torres et al., 2011).

CoV E protein self-associates forming an oligomeric structure that delimits an ion conductive pore in planar lipid bilayers and micelles (Pervushin et al., 2009; Torres et al., 2006; Wilson et al., 2006, 2004). Synthetic CoV E proteins of human coronavirus 229E (HCoV-229E), mouse hepatitis virus (MHV), SARS-CoV and avian infectious bronchitis virus (IBV), behaved as cation-selective channels when they were reconstituted into planar lipid bilayers. Furthermore, it has been reported that HCoV-229E (genus α CoV) E protein was more selective to K^+ than to Na^+, whereas MHV and SARS-CoV (genus β CoVs) and IBV (genus γ CoV) E proteins were more selective to Na^+ than K^+ ions (Wilson et al., 2006, 2004). Studies using SARS-CoV E protein derived peptides showed that the transmembrane domain had ion conductive properties (Torres et al., 2007; Wilson et al., 2004), and some crucial residues involved in this activity have been identified (Torres et al., 2007).

Generation of MHV recombinant viruses, in which E protein was interchanged by CoV genus α, β and γ E proteins indicated that only E proteins corresponding to genus β and γ could partially replace the function of MHV E protein (Kuo et al., 2006). It was speculated that the selectivity of the channel could be important for the functional replacement. However, the possible biological relevance of ion selectivity in different CoV E proteins requires additional studies.

Other RNA viruses also encode small transmembrane proteins with ion channel properties. Influenza virus M2, hepatitis C virus p7, human immunodeficiency virus (HIV) vpu, and poliovirus 2B proteins are some examples of a growing list of these virally encoded ion channels called viroporins (de Jong et al., 2006; Ewart et al., 1996; Pinto et al., 1992; Wozniak et al., 2010). It has been described that these proteins are involved in diverse processes such as virus entry, trafficking and maturation, inflammation, and apoptosis (Campanella et al., 2004; Ichinohe et al., 2010; Wozniak et al., 2010).

Deletion of E gene in different CoVs may result in a complete abrogation of virus maturation and release, as shown for transmissible gastroenteritis virus (TGEV) (Ortego et al., 2007, 2002) or in a reduction of virus growth, as described for MHV (Kuo and Masters, 2003) and SARS-CoV (DeDiego et al., 2007). In addition, a SARS-CoV lacking E gene (SARS-CoV-ΔE) was attenuated in three animal models (DeDiego et al., 2007, 2008; Netland et al., 2010). Experimental data comparing SARS-CoV-ΔE and the parental virus revealed that E protein reduced cellular stress and virus-induced apoptosis (DeDiego et al., 2011).

Some reports have revealed the importance of CoV E protein transmembrane domain in virus production and maturation. When alanine residues were introduced in the MHV E protein transmembrane domain, disrupting the alpha helix structure and repositioning polar residues, virus growth was compromised, suggesting an important function for E protein transmembrane domain in virus biogenesis (Ye and Hogue, 2007). Replacement of IBV E transmembrane domain by a heterologous one that lacked ion conductance activity resulted in a virus that was poorly secreted to the extracellular media (Ruch and Machamer, 2011).

In the present work we have characterized the ion conductive properties of the transmembrane domain of the SARS-CoV E protein, with or without amino acid substitutions, and the full-length E protein, aiming to determine the functions affected by SARS-CoV E protein ion channel activity. Residues N15 and V25 were essential for SARS-CoV E protein ion channel activity. In all cases where the bilayer membrane was permeabilized, the channel showed symmetric ion transport properties either for positive or negative applied voltages, and its conductance was not regulated by voltage. Interestingly, when E protein was reconstituted in negatively charged lipid bilayers, the ion channel became slightly more selective to cations than to anions. The transmembrane domain of HCoV-229E E protein was also analyzed as a putative K^+ selective channel. A very weak selectivity for K^+ over Na^+ was observed for this peptide and its small preference for cations was also regulated by the charge of the lipid membranes, as was the case of SARS-CoV E protein. We propose that lipids are an integral component of the CoV E protein derived ion channels, a novel finding for these structures.

Results and discussion

E protein conductance on neutral planar lipid bilayers

Previous studies attributed the ion channel activity of SARS-CoV E protein to the transmembrane domain of the protein (Torres et al., 2007; Wilson et al., 2004). In order to identify residues involved in ion channel activity, the spatial distribution of SARS-CoV Urbani strain E protein transmembrane domain amino acids was predicted by helical wheel modeling using Protean (DNASTAR Software, Lasergene) and information from nuclear magnetic resonance (NMR) studies (Pervushin et al., 2009) (Fig. 1). In addition, conserved amino acids located within the E protein transmembrane domain of different CoV species were identified by sequence alignment (Fig. S1, supplementary data). Using information from these sources, conserved polar amino acids located at the beginning and the end of the transmembrane domain, and conserved amino acids central to the CoV E protein transmembrane domain, which in many cases were tentatively located towards the lumen of the pore formed by SARS-CoV E protein, were mutated (Table 1 and Fig. 1). A total of nine peptides derived from SARS-CoV E protein were synthesized, one of them (*wt*) representing the wild type protein transmembrane domain and its flanking polar amino acids, and eight mutant peptides including different residue substitutions. One (M2 to M7), two (M1) or four (M8) amino acids were replaced in each peptide at different positions along the transmembrane domain, or at both the amino- or carboxy-termini of this domain (Table 1 and Fig. 1).

The ion channel activity of these peptides was evaluated by electrophysiological measurements in artificial lipid bilayers. Single channel conductance was estimated from a statistical analysis of the current jump amplitudes, not from the total current measured. This procedure allows a reliable estimate of the most probable value of current change every time a new channel is inserted or disappears. Although several channels are being inserted, the magnitude of the current through a single channel could be discriminated. In order to exclude any peptide–lipid or peptide–peptide electrostatic interactions previous to peptide insertion (Sani et al., 2012), measurements were initially made in neutral lipid DPhPC bilayers. Six of the peptides investigated were able to permeabilize the phospholipid membrane. *Wt* as well as mutant peptides M1, M2, M5, M6 and M7 led to spontaneous current bursts 10–15 min after the peptide was added to the *cis* chamber (Fig. 2A). These results indicated that residues E7 and E8 (mutated in M1 peptide), T11 (mutated in M2 peptide), T30 (mutated in M5 peptide), T35 (mutated in M6 peptide) and R38 (mutated in M7 peptide) were not completely essential for the ion channel activity of SARS-CoV E protein derived peptides. M1, M5 and M7 peptides displayed conductance