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Analysis of SARS-CoV E protein ion channel activity by tuning the protein and lipid charge

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ABSTRACT

A partial characterization of the ion channels formed by the SARS coronavirus (CoV) envelope (E) protein was previously reported (C. Verdiá-Báguena et al., 2012 [12]). Here, we provide new significant insights on the involvement of lipids in the structure and function of the CoV E protein channel on the basis of three series of experiments. First, reversal potential measurements over a wide range of pH allow the dissection of the contributions to channel selectivity coming from ionizable residues of the protein transmembrane domain and also from the negatively charged groups of diphytanoyl phosphatidylserine (DPhPS) lipid. The corresponding effective pKas are consistent with the model pKas of the acidic residue candidates for titration. Second, the change of channel conductance with salt concentration reveals two distinct regimes (Donnan-controlled electrodiffusion and bulk-like electrodiffusion) fully compatible with the outcomes of selectivity experiments. Third, by measuring channel conductance in mixtures of neutral diphytanoyl phosphatidylcholine (DPhPC) lipids and negatively charged DPhPS lipids in low and high salt concentrations we conclude that the protein–lipid conformation in the channel is likely the same in charged and neutral lipids. Overall, the whole set of experiments supports the proteolipidic structure of SARS-CoV E channels and explains the large difference in channel conductance observed between neutral and charged membranes.

1. Introduction

Coronaviruses are enveloped viruses that cause common colds in humans and other serious diseases in birds and mammals [1]. One of these coronaviruses is responsible for the severe acute respiratory syndrome (SARS-CoV), which, between 2002 and 2003, affected 8000 people worldwide and resulted lethal in the 10% of the cases. All coronaviruses express the envelope (E) protein, a multifunctional short polypeptide involved in virus morphogenesis and virulence [2–5]. SARS-CoV E protein is 76 amino acid long and contains an alpha-helical transmembrane (TM) domain that spans the lipid membrane with ~28 residues [6] (Fig. 1) SARS-CoV E protein oligomerizes forming a pentameric structure that displays ion channel activity [7–12] a remarkable function for this protein that may affect virus host interaction.

In a recent paper [12] we reported that SARS-CoV E protein channels (as well as a synthetic peptide representing just the protein TM) exhibit different functional properties when reconstituted in neutral or charged planar lipid membranes. This data suggested that lipid molecules likely assemble with E protein oligomers to form a combined proteolipidic structure. In this structure the lipids could be only located at the channel entrances, or alternatively, within the lipid polar heads, stabilized by peptides, lining totally or partially the pore wall. The ion channel activity of a number of transmembrane proteins, as well as of small peptides and antimicrobial peptides, is strongly dependent on the lipid environment [13,14]. Actually, evidence on the lipid involvement in the channel structure is often obtained from the sensitivity of the pore-forming activity to the curvature of the lipid bilayer membranes [15]. The reason for that lies in the high energy cost of assembling lipidic structures in membranes with intrinsic curvature that usually inhibits the channel activity [16]. In this sense, we observed a lower probability of pore formation by SARS-CoV E protein in membranes containing phosphatidylethanolamine (DOPE), a lipid with negative intrinsic curvature [12], which suggests a significant involvement of lipid molecules in channel structure.

However, the correlation between the pore forming potency of peptides and the spontaneous curvature of the lipid is not a definitive argument to elucidate the actual structure of the SARS-CoV E channels. Similar correlations have been reported in well-known proteinaceous pores like alamethicin. In this case, the sensitivity of the channel to the lipid charge comes from a peptide-induced membrane thinning [13,17]. Therefore, in the case of SARS-CoV E protein, it seems important to obtain additional lines of evidence on the mechanism of pore formation and the functional properties of the resulting ion channels [12].

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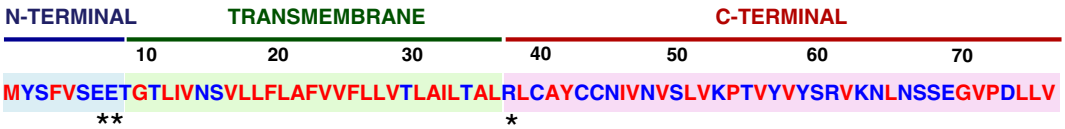

Fig. 1. SARS-CoV E protein sequence. E protein is divided into three domains: the amino terminal (N-terminal), the transmembrane and the carboxy terminal (C-terminal). Red letters represent hydrophobic amino acids, and blue letters indicate hydrophilic amino acids. Asterisks highlight polar charged amino acids located at the beginning (two glutamic acid residues) and at the end (an arginine) of the transmembrane domain, respectively.

To address these issues a different strategy has been adopted in this manuscript, which is complementary to other structural studies [7]. We generated useful information on the CoV E channel structure by focusing on the effect of lipid charge on channel conductance and ionic selectivity under a variety of conditions. Several series of experiments are reported that have in common the modulation of the effective protein and lipid charge presented to the small ions crossing the aqueous pore [18]. First, reversal potential measurements under different pH conditions enabled us to identify the contributions of ionizable residues of the protein TM and also of the negatively charged groups of DPhPS lipid to channel selectivity. Secondly, the change of channel conductance in membranes containing varying ratios of neutral DPhPC lipids and charged DPhPS lipids in low and high salt concentrations was studied. Finally, protein and lipid charges were modified by changing the salt concentration of the solution both in neutral and charged membranes and the corresponding channel conductance and the solution conductivity were determined.

These three ways of modifying the effective fixed charge in CoV E channels strongly supported a proteolipidic structure of the channel that is likely to be the same in charged and neutral membranes. In other words, if this assumption proves correct, the large difference (two-fold change) between channel conductance in 1 M KCl in DPhPS and DPhPC host membranes would be simply an effect of the partial Donnan exclusion of anions in the aqueous pore [19].

Overall, exploring the lipid charge effects on channel conductance and selectivity over a wide range of lipid compositions, salt concentration and solution pH provided a unitary message for the protein–lipid composition of the channel. The results supported a proteolipidic structure without the need of additional sophisticated structural techniques.

2. Materials and methods

2.1. Protein synthesis

Full-length SARS-CoV E protein was kindly provided by Dr. Jaume Torres and synthesized and purified as previously described [12].

2.2. Ion channel reconstitution and ionic current recording

Planar bilayers were formed by apposition of two monolayers prepared from a solution of 1% pure diphytanoyl phosphatidylcholine (DPhPC), pure diphytanoyl phosphatidylserine (DPhPS), or a mixture of both lipids (Avanti polar lipids, Inc., Alabaster, AL) in pentane. Lipids were added on 70–90 μm diameter orifices in the 15 μm-thick Teflon partition that separated two identical chambers [20,21]. The orifices were pretreated with a 1% solution of hexadecane in pentane. Aqueous solutions of KCl were buffered with 5 mM HEPES at pH 6. All measurements were performed at room temperature (23 ± 1 °C). Ion channel insertion was achieved by adding 0.5–1 μl of a 300 μg/ml solution of synthetic protein in the buffer containing acetonitrile:isopropanol (40:60) on one side of the chamber (hereafter referred to as *cis* side).

An electric potential was applied using Ag/AgCl electrodes in 2 M KCl, 1.5% agarose bridges assembled within standard 250 μl pipette tips. The potential was defined as positive when it was higher on the side of the peptide addition (*cis* side), whereas the *trans* side was set to ground. An Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) in the voltage-clamp mode was used to measure the current and the applied potential. The chamber and the head stage were isolated from external noise sources with a double metal screen (Amuneal Manufacturing Corp., Philadelphia, PA). The channel conductance was obtained from current measurements under an applied potential of +100 mV in symmetrical salt solutions of variable KCl concentration. The conductance values were evaluated using the Gaussian fit tool of Sigma Plot 10.0 (Systat Software, Inc.).

The reversal potential, E_{rev}, was obtained as follows. First, a lipid membrane was formed at a given salt concentration gradient. Second, one or several channels were inserted into the bilayer and a net ionic current appeared due to the concentration gradient. Third, the ionic current through the channel was manually set to zero by adjusting the applied potential. The potential needed to achieve zero current was then corrected by the liquid junction potentials of the electrode salt bridges [22] to obtain the E_{rev}.

3. Results and discussion

3.1. Ion channel activity in planar lipid bilayers

The spontaneous formation of full-length SARS-CoV E protein ion channels in DPhPC and DPhPS membranes as well as their current recording did not show significant differences to the analogous process done previously for synthetic peptides derived from the SARS-CoV E protein TM domain [12]. The reversal potential was measured in multichannel experiments as the voltage required to null the ionic current. To estimate the most probable value of channel conductance, we recorded more than 40 long duration (~200 s each) current traces and made a statistical analysis of all the current jump events, including positive (increase) and negative (decrease) bursts.

Fig. 2 shows typical current traces recorded in DPhPC membranes (panel A) and DPhPS membranes (panel C) of SARS-CoV E channel. As already observed in electrophysiological measurements with synthetic peptides [12], CoV E channels formed in DPhPS membranes showed a better defined conductance (190 ± 60 pS), compared with channels formed in DPhPC membranes, which exhibited a higher dispersion in the magnitude of the current bursts (370 ± 160 pS). This trend can be clearly observed in the histograms included in Fig. 2. The origin of this different conductance variability depending on the host lipid remains unexplained.

3.2. Channel selectivity change with pH

The SARS-CoV E channel was reported to be slightly cationic selective in negatively charged membranes and almost non-selective in neutral membranes at pH 6 [8,12]. From those results it became apparent the large influence of the lipid charge on the channel preference for cations. To further analyze this effect, channel selectivity in solutions of varying acidity was measured using the same concentration gradient used in previous studies (500 mM *cis*|50 mM *trans*). Two series of reversal potential measurements were performed over a wide range of pH (1.5–7): first in neutral membranes (DPhPC) and then, using negatively charged membranes (DPhPS). The channel selectivity was strongly dependent on the net charge of the host lipid (Fig. 3). When reconstituted in DPhPC, the channel displayed a very