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Linearity, saturation and blocking in a large multiionic channel: Divalent cation modulation of the OmpF porin conductance

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ABSTRACT

Measurement of unitary conductance is a fundamental step in the characterization of a protein ion channel permeabilizing a membrane. We study here the effect of salts of divalent cations on the OmpF channel conductance with a particular emphasis in dissecting the role of the electrolyte itself, the role of the counterion accumulation induced by the protein channel charges and other effects not found in salts of monovalent cations. We show that current saturation and blocking are not exclusive properties of narrow (single-file) ion channels but may be observed in large, multiionic channels like bacterial porins. Single-channel conductance measurements performed over a wide range of salt concentrations (up to 3 M) combined with continuum electrodiffusion calculations demonstrate that current saturation cannot be simply ascribed to ion interaction with protein channel residues.

1. Introduction

The elucidation of the mechanisms involved in the transport of small ions inside large channels such as bacterial porins, toxins or voltage gated channels of the outer mitochondria membranes is crucial for biotechnological, analytical and medical applications [1–3]. In this paper we study the outer membrane porin F (OmpF) from *Escherichia coli*, a protein channel that forms homotrimeric wide channels when inserted in planar lipid bilayers. OmpF porin can be considered as a mesoscopic channel: the size is too large to be specific whereas the channel walls do have an influence on the permeability. The transport of small inorganic ions (K^+, Na^+, Cl^-, etc.) across the channel is mainly regulated by the electrostatic interactions between the permeating ions and the channel ionizable residues, as revealed by experiments [4–7] and theoretical approaches including molecular dynamics and Brownian dynamics simulations and continuum electrodiffusion models [8,9].

The close relationship between channel charge and selectivity found in experiments with salts of monovalent cations over a wide range of pH [7] is lost when another type of short-range interaction between permeant ions and protein groups prevails over the Coulombic attraction or repulsion. Thus, we have recently reported that the moderate cationic selectivity of OmpF in 1:1 salts [4,7,8,10] is inversed in solutions with multivalent cations. Two major factors contribute to this channel feature: the binding of permeant ions to the channel, which compensates, or slightly overcompensates, the negative charge of the OmpF molecule, and the anionic selectivity coming from the differences in ion mobilities [11,12].

In this paper we take a further step investigating the effect of salts of divalent cations on the OmpF channel conductance. Measuring single-channel conductance is an indispensable task in the characterization of a protein ion channel permeabilizing a membrane. Thus, in order to assess the channel permeability properties one needs to discriminate the role of the electrolyte itself, the role of the counterion accumulation induced by the protein channel charges and any other effect that may be specific for a particular ionic species [13,14]. Commonly, the interpretation of ionic currents in channels relies on the principle of independent movement of ions [15]. This may be a good assumption when ion concentrations are low enough, but at salt concentrations well above the physiological values, deviations from ideality in solutions play an important role and current saturation becomes common in narrow channels. In order to see all the details of the complex conductance–concentration relation of multi-ion channels, the concentration needs to be varied over several orders of magnitude. To this end, single-channel conductance measurements are performed over a wide range of salt concentrations (up to 3 M).

We find that the change of channel conductance with salt activity in bulk solution exhibits different features in salts of monovalent cations and in salts of divalent cations. In order to separate channel and electrolyte effects we analyze the correlation between channel conductance and bulk solution conductivity. While one scales with the other in solutions of NaCl and KCl over the whole concentration range studied, the conductance for $CaCl_2$ and $MgCl_2$ exhibits a linear regime, saturation and a somewhat surprising decrease of channel conductance as bulk conductivity increases. Once

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one account for the large variation of activity coefficients of divalent salts at high concentrations, the experimental results agree quantitatively with Poisson–Nernst–Planck (PNP) calculations revealing that at low and moderate concentrations the pore conductance is controlled mainly by the electrolyte properties. Finally, we discuss the possible existence of sub-states of lower conductance as one of the causes of the conductance decrease in the high concentration regime in $CaCl_2$ and $MgCl_2$ solutions.

2. Materials and methods

2.1. Single-channel conductance measurements

The experiments reported here have been carried out in single ion channels reconstituted on planar membranes. Lipid bilayers were formed by apposition of two monolayers made from 5 mg/ml solution of diphytanoylphosphatidylcholine (DPhPC) (Avanti polar lipids, Inc., Alabaster, AL) in pentane (Baker) on 70–100 μm diameter orifices in a 10 μm thick Teflon partition that separates two chambers. The orifices where pre-treated with 1% solution of hexadecane in pentane. The aqueous solutions were buffered by 5 mM HEPES and the pH was fixed at 6. Wild type OmpF, kindly provided by Dr. Mathias Winterhalter (Jacobs University, Germany), was isolated and purified from an *E. coli* culture. Channel insertion was achieved by adding 1 mg/ml solution of OmpF in the buffer that contained 0.1 M KCl and 1% (v/v) of OctylPOE (Alexis, Switzerland) to 2 ml of aqueous phase at the cis side of the membrane.

The measurements were taken using Ag/AgCl electrodes in 2 M KCl, 1.5% agarose bridges assembled within standard 250 ml pipette tips. An Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) in the voltage-clamp mode was used for measuring the current and applying potential. Electric potential is defined as positive when it is greater at the side of protein addition (the cis side of the membrane cell), while the other side (trans side) is set to ground. The membrane chamber and the head stage were inside a Faraday cage.

The single-channel conductance was obtained from the current measurement at applied potential of ±100 mV in symmetrical salt solutions. Each point was measured for at least three different experiments to assure reproducibility and to estimate the standard deviation. Solutions conductivity was measured with a CDM210 conductivity meter (Radiometer Analytical).

2.2. Channel conductance calculation using a macroscopic electrodiffusion model

The calculations of single-channel conductance were done by using the PNP electrodiffusion model [16], based on Nernst–Planck equations for ion fluxes and Poisson equation from electrostatics [15]. The PNP framework is a mean field theory that has been used for the interpretation of the transport properties of a large variety of membrane systems [17–21]. Properties of large channels including OmpF channel [9,16,22] and other porins [23,24] have also been studied by means of this formalism. We use here the one-dimensional version of PNP theory and the crystal structure of the OmpF channel. We start by using as input the atomic structure of the OmpF channel (obtained from X-ray diffraction with a resolution of 2.4 Å, Protein data Bank, code 2OMF) [25] to get the electric potential distribution created by the channel fixed charges following the procedure described by Antosiewicz and coworkers [26,27]. Then, we convert that 3D potential distribution into an effective fixed charge volume density averaged along the pore, which is introduced into PNP equations. The details of such conversion have been reported elsewhere [16].

The PNP equations are solved numerically using a grid of 10^3 equally spaced points. Given an applied potential and boundary conditions in terms of ion concentrations on both sides of the channel, a linear approximation is used in order to evaluate the initial potential profile along the channel. This solution is then iterated until convergence given a tolerance of 10^{-12}. The numerical procedure yields the ionic flux densities, from which the macroscopic current across the channel is computed. Conductance is then calculated as the current–voltage ratio when the applied potential is +100 mV.

3. Results and discussion

3.1. Channel conductance versus bulk solution conductivity

Channel conductance is usually measured in symmetrical solutions (the same salt concentration on *cis* and *trans* side) and the characterization of the channel permeability to a given salt involves a series of experiments varying the solution concentration. In order to separate channel and electrolyte effects, rather than looking to the change of conductance with concentration, it is much more illustrative to plot the measured channel conductance against the conductivity of the bulk solution. To this end, we measured OmpF single-channel conductance at pH 6 and bulk solution conductivity over a wide range of salt concentrations (0.1–3 M) for chloride salts of monovalent and divalent cations.

Fig. 1 shows marked differences between 1:1 and 2:1 salts. On the one hand, channel conductance measured in KCl and NaCl scales with bulk conductivity. Indeed, the conductance–conductivity linear correlation is almost identical in KCl and NaCl. This is consistent with a large number of previous studies [7,8,12,16,28–30] stressing that the OmpF channel does not display any chemical specificity, and any difference between K^+ and Na^+ permeation comes only from their slightly different intrinsic properties (mobilities, diffusivities, hydration radius, etc.). In other words, in salts of monovalent cations the OmpF porin is so weakly selective that the ions flowing through the channel do not differ significantly from a bulk electrolyte.

On the other hand, the channel permeability in $CaCl_2$ and $MgCl_2$ is strikingly different. The relationship between channel conductance and electrolyte conductivity is non-monotonic. For salt concentrations below ∼1 M, the channel conductance scales with the bulk conductivity of the solution in both salts. The slope differs from that of 1:1 salts because the counterion accumulation and coion depletion inside the channel is not the same. Above 1 M, the channel conductance in presence of divalent cations displays

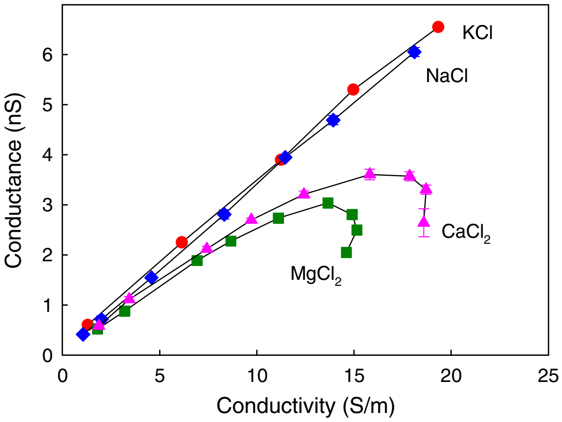

Fig. 1. OmpF single-channel conductance and bulk solution conductivity measured in solutions of different salts of concentrations ranging 0.1–3 M at pH 6.

an unexpected trend: both the conductance and the solution conductivity decrease with increasing concentration, breaking the linearity and creating the image of a spiral or clothoid. Given that both channel conductance and electrical conductivity depend on the electrolyte concentration, such representation does not mean that conductance is a bivaluated function of conductivity, but it is only a visual effect arising from the planar projection of a 3D surface. This peculiar dependence of the conductance on the conductivity at high salt concentrations suggests the channel could interact with the different divalent cations (Mg^{2+} and Ca^{2+}) in a particular way that we aim to investigate here.

3.2. Activity of the divalent solutions

For a right interpretation of the experiments over a wide range of salt concentrations, it is necessary to know accurately the "effective concentration" of each species, i.e. its activity. Fig. 2A shows the mean activity coefficient $\gamma_\pm$ of the salts used in experiments as a function of the salt concentration, c. The tabulated values [31] have been translated from molal to molar scale and later interpolated [31–33].

The difference in the $\gamma_\pm(c)$ dependence between 1:1 and 2:1 salts becomes apparent in Fig. 2A, especially above 1 M. While $\gamma_\pm$ in KCl and NaCl is almost insensitive to concentration, in $CaCl_2$ and $MgCl_2$ $\gamma_\pm$ slightly decreases in the low concentration range, then reaches a minimum and finally shows a steep increase.

Having in mind the Fig. 2A, the reader could wonder now why ion activities were not mentioned in our previous studies characterizing the OmpF channel. Thus, the ion selectivity of the OmpF channel was investigated from measurements of reversal potential, defined as the applied transmembrane voltage that yields zero electric current when there is a concentration gradient across the channel [7,29,30,34]. But, in such studies performed up to moderate concentrations (∼1 M) of monovalent cations, there is no need to consider ion activities because the $\gamma_\pm$ is almost constant in this range (see Fig. 2A). Then, although concentrations and activity could differ considerably, the concentration gradient (c_{cis}/c_{trans}) is practically equal to the activity gradient (a_{cis}/a_{trans}). However, the use of activities seems mandatory here because this reasoning is no longer valid. First because the $\gamma_\pm$ of divalent cations changes dramatically at high concentrations and second, because the channel conductance is characterized in terms of an absolute value of concentration and not in terms of a concentration ratio.

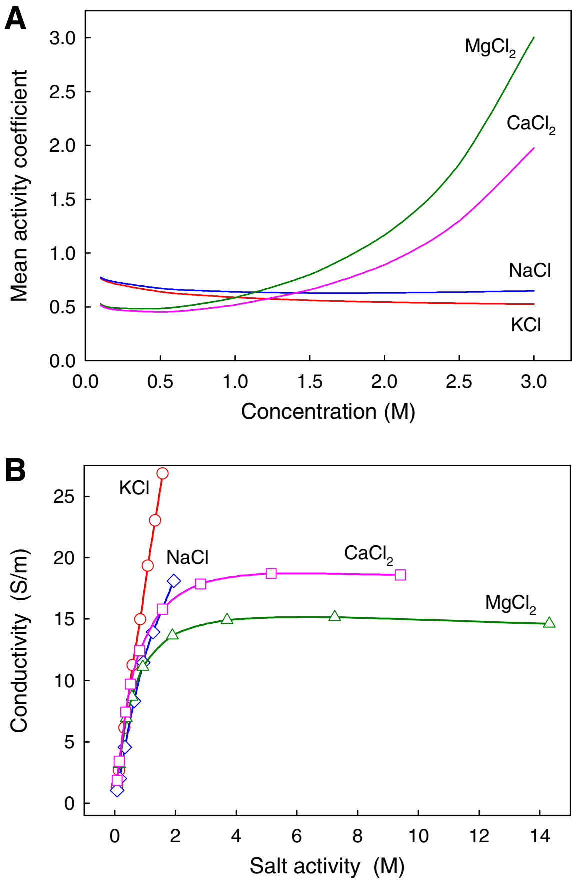

Fig. 2. (A) Mean activity coefficient in molar reference as a function of the solution concentration for the electrolytes used in the experiments. (B) Measured conductivity as a function of the electrolyte activity in solution.

Once it is clear that the electrochemical characterization of the channel conductance in concentrated solutions must be done in terms of activities, the next step is to separate the role of the channel from the intrinsic properties of the electrolyte. Measuring conductivity of electrolyte solutions of several activities, as shown in Fig. 2B, provides useful information.

In the range of activities studied, the conductivity of 1:1 salts scales with their activity, whereas in the case of 2:1 salts conductivity saturates at a certain value of the activity. We hypothesize that the reason behind this peculiarity of divalent cations is the well known fact that ion diffusion coefficients of some electrolytes are strongly dependent on ion activity [31]. Unfortunately, such information is only available for a reduced number of electrolytes (mainly salts of monovalent cations) and a limited range of ion activities [31]. To bypass this problem, we recall that there are no concentration gradients in bulk solution and the electrical current is directly proportional to the electric field. Then, the conductivity can be written as a function of the ion activities, the ion diffusion coefficients D_i and their valences z_i as follows:

$$\kappa = \frac{F^2}{RT}(D_+ z_+^2 a_+ + D_- z_-^2 a_-) \qquad (1)$$

Then, we can use the Eq. (1) to fit the conductivity measurements and obtain *effective* ion diffusion coefficients. To solve for the two unknowns, D_+ and D_-, we take advantage of the fact that although ion diffusion coefficients vary with salt activity, their ratio D_+/D_- remains practically independent of salt activity [31]. In the case of KCl and NaCl these *effective* diffusion coefficients are almost insensitive to ion activity and they compare rather well with the tabulated values. In contrast, the calculated ion diffusivities for divalent cations are a strong function of the ion activity. In the particular case of $CaCl_2$, there are measurements reported for D_{Ca} and D_{Cl} at different activities [35] that support our empirical approach.

3.3. Channel conductance modulation at moderated salt concentration

Once the electrolyte properties are understood, we can try to establish a sound correlation between the available structural data and the channel conductance. By using the crystal 3D structure of the porin, the transport of ions across the OmpF channel in 1:1 salts has been modeled satisfactorily using mean field theories as the 1D PNP model described in subsection 2.2 [14,16].

We apply here such electrodiffusion model to calculate the channel conductance in 1:1 and 2:1 salts. In the case of monovalent cations, we use as diffusion coefficients the values tabulated for bulk solution whereas for the salts of divalent cations, we employ the values deduced from Fig. 2B and Eq. (1).

Measured channel conductance as a function of salt activity is shown in Fig. 3A. A double logarithmic plot is used to improve the visibility of the region of low activities (and concentrations). Fig. 3B shows the theoretical prediction of the conductance using the PNP formalism.

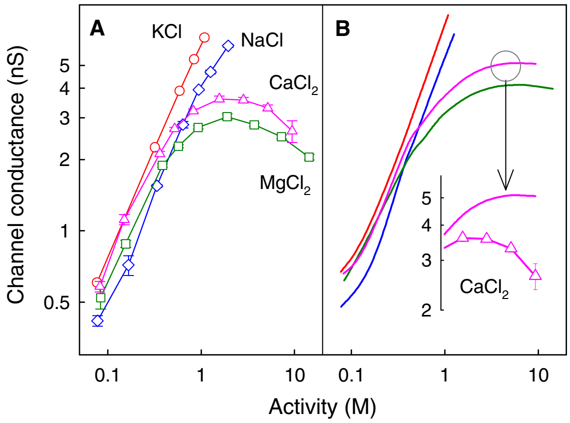

Fig. 3. OmpF channel conductance over a wide range of activities of several salt solutions. (A) Measured conductance. (B) Conductance calculated using the PNP electrodiffusion model and the crystal structure of the channel. (Inset of B) comparison of measured and calculated conductance in CaCl$_2$ solutions at high salt concentrations shows that the decrease observed at very high concentrations is not accounted for the model.

The comparison between Fig. 3A and B indicates that the model reproduces rather well, even quantitatively, the measured conductances in KCl and NaCl. The success of such a simple model based on averaged magnitudes (fixed charge concentration, pore diameter) and using infinite dilution diffusivities stresses an idea already mentioned: ion transport is similar within the pore and in bulk solution.

In the case of CaCl$_2$ and MgCl$_2$, two features are remarkable. On the one hand, the model satisfactorily predicts the saturation of conductance that appears around $a = 2$ M. This trait, inexplicable using infinite dilution diffusivities, can be explained by using the *effective* diffusion coefficients described above. Again, the ions flowing through the channel resemble to a bulk electrolyte but in contrast with the case of NaCl and KCl, unless the solution properties are properly accounted for, the saturation could be mistakenly attributed to the channel. On the other hand, the observed decrease of conductance found at high enough activities clashes with the plateau anticipated by the model (see the inset in Fig. 3B with the enlargement of measured and calculated CaCl$_2$ conductance at high concentrations). The channel conductance decreases despite the fact that the solution conductivity remains almost constant. This suggests that a close interaction between the channel residues and the permeating ions, not considered in the PNP framework, is taking place.

3.4. Channel conductance at high CaCl$_2$ and MgCl$_2$ concentrations

Recently, a new crystal structure of the OmpF channel was obtained in the presence of 1 M MgCl$_2$ aqueous solution (Protein data Bank, code 2ZFG) [36]. This structure is particularly relevant to our study, because it shows a Mg^{2+} cation bound in the selectivity filter between residues Asp113 and Glu117. That binding has been claimed to be responsible for the channel selectivity inversion: the fact that moderate cationic selectivity of the OmpF channel in KCl and NaCl solutions is reversed to anionic selectivity in salts of divalent and trivalent cations [11,12].

Based on this evidence, the question arises whether such binding could be behind the conductance decrease observed at high salt concentration of CaCl$_2$ or MgCl$_2$. Indeed, the current traces recorded at several salt concentrations support this conjecture. Fig. 4 shows OmpF current recordings at several high CaCl$_2$ concentrations (1.5–3 M) for applied voltages of both polarities (±100 mV).

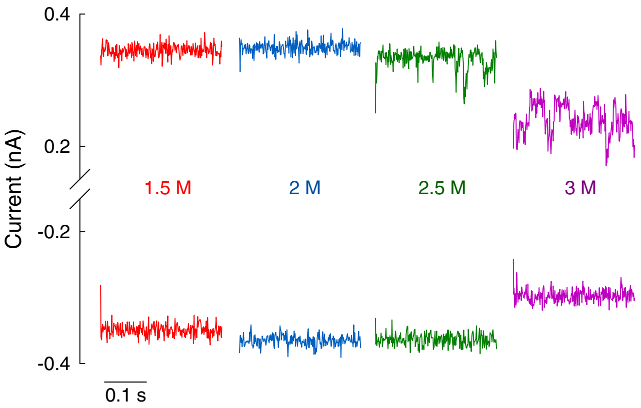

Fig. 4. OmpF single-channel current recordings in concentrated solutions of CaCl$_2$ for applied voltages of both polarities (+100 mV and −100 mV).

The traces in Fig. 4 indicate that the current changes with salt concentration in two different ways. First, we observe fluctuations in the current that depend on both the activity and the applied voltage. Second, both the average and the maximum values decrease with increasing activities. The visual inspection of current traces discards the applied voltage as the unique responsible for this channel closure. Thus, the decrease in conductance, in respect to the fully open channel does not follow the characteristic stepwise closure that the high applied voltage induces in the trimeric OmpF channel. (Reflecting the number of monomers that are in the open state).

One could hypothesize that the strong interaction between divalent cations and some channel residues promotes the existence of sub-states of lower conductance in the range of high concentrations that would be the cause of the decrease of conductance for salts of divalent cations. The existence of these sub-states opens the door to future studies on the influence of specific interactions between Ca^{2+} and Mg^{2+} ions and particular residues of the channel OmpF on its transport properties.

4. Conclusions

We have measured and analyzed the single-channel conductance of the bacterial porin OmpF in several salts of monovalent and divalent cations over a wide range of salt concentrations (up to 3 M). The main findings can be summarized as follows:

(1) At low and moderate salt concentrations (till ~1 M) there is a correlation between the channel conductance and the bulk solution conductivity (both in 1:1 and 2:1 salts). This suggests that, in this range, the ion transport through the channel is essentially regulated by the electrolyte properties. A close analysis of the measured solution conductivity shows that in the case of chlorides of divalent cations, the diffusion coefficients are a strong function of ion activity. This clearly indicates that a proper understanding of the electrolyte under study is mandatory before attempting the rationalization of the channel function.

(2) A PNP electrodiffusion approach has been used for the interpretation of the channel conductance measurements. Starting from the crystal structure of the porin, the transport of ion across the OmpF channel cations has been described with acceptable results at low and moderate salt concentrations.

(3) The decrease of channel conductance reported at high enough activities in CaCl2 and MgCl$_2$ contrasts with the plateau anticipated by the theoretical model. This suggests that

a close interaction between the channel residues and the permeating ions, not considered in the PNP framework might be involved. The current recordings in those 2:1 salts point to the existence of sub-states of lower conductance as one of the causes of the conductance decrease.

Acknowledgments

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