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Directional ion selectivity in a biological nanopore with bipolar structure

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ABSTRACT

Ion transport features of a biological nanopore, the bacterial porin OmpF from *Escherichia coli*, have been investigated by patch-clamp experiments performed at the single channel level. Membrane potential measurements done under asymmetric conditions of pH and electrolyte concentration provide important evidences about the charge regulation exerted by the channel that cannot be extracted from the rectification displayed in current–voltage curves. The pH gradient imposed across the pore induces an asymmetric fixed-charge distribution that resembles the structure of synthetic bipolar membranes. This particular arrangement demonstrates that the ionic selectivity of a non-uniformly charged structure is not an intrinsic quality of the system but depends crucially on several external factors. Amazingly, changing the direction of the salt concentration gradient can turn a cation selective channel into an anion selective one.

1. Introduction

The promising connections between biological molecular structures and engineered nano-scale sensors have attracted the interest and imagination of researchers. Over the last years, exhaustive attention has been paid to synthetic pores that mimic several relevant physiological mechanisms carried out by ion channels. Selective permeation of inorganic ions, metabolites or other small solutes that are crucial in electrical signaling and other biological functions has been also found in abiotic nanopores [1–15]. Conversely, the manipulation of biological membranes and ion channels to take advantage of their "sensing" properties has already been achieved in a variety of biotechnological and analytical applications [16–18]. In a recent study, we showed that the OmpF channel, a biological pore found in the outer membrane of *Escherichia coli*, may function as a pH-regulated, biological, nanofluidic diode [19]. Continuum electrostatic calculations suggested as well that the origin of the current rectification found might be the asymmetric distribution of fixed-charges, similar to that of a bipolar ion exchange membrane.

Bipolar membranes (BMs), a composite of an anion-permeable membrane and a cation-permeable membrane, have been known for more than half a century [20]. Since the development of the first commercial BMs for industrial purposes [21], a great progress has been made, and intensive research is currently being done both in fabrication [22] and in characterization of BM structure and physico-chemical properties [23]. Today, BM technology is well established [24], regarded as a clean technology, and numerous separation processes involve the use of BM in the fields of chemical engineering and biotechnology [25–30].

There are very few historical records of biological systems analogue to BMs. To our knowledge, only Mauro [31] and Coster [32] reported biological systems with properties similar to those of BM. Under asymmetric pH conditions the OmpF channel has been reported to display current rectification [19]. Despite the fact that electrostatic calculations pointed to a bipolar-like charge structure, that study was not totally conclusive about the origin of the diode-like rectifying behavior since an asymmetric conduction has been also reported in other ion exchange systems like conical nanopores [4]. Here we attempt to clarify whether the rectification properties arise from a merely asymmetric fixed-charge distribution or from a bipolar distribution, i.e., with two regions of opposite fixed charge. The exploration of OmpF channel selectivity is appealing as it tests our understanding of physical principles underlying transport through BMs. To this end, we present here a thorough characterization of the pore under an applied pH gradient: current–voltage (I–V) curves and membrane potential. We show that under certain conditions the fixed-charge distribution is unambiguously bipolar. Furthermore, our results raise the question of the definition of selectivity in non-homogenous systems, since in such membranes or nanopores the selectivity is no longer an exclusive feature of them but depends largely on the pH and ion concentrations of the surrounding solutions. These results suggest that the pH-regulated selectivity of ion channels and synthetic nanopores is somewhat similar to the permselectivity of synthetic membranes with grafted ionizable groups and amphoteric membranes [33–40].

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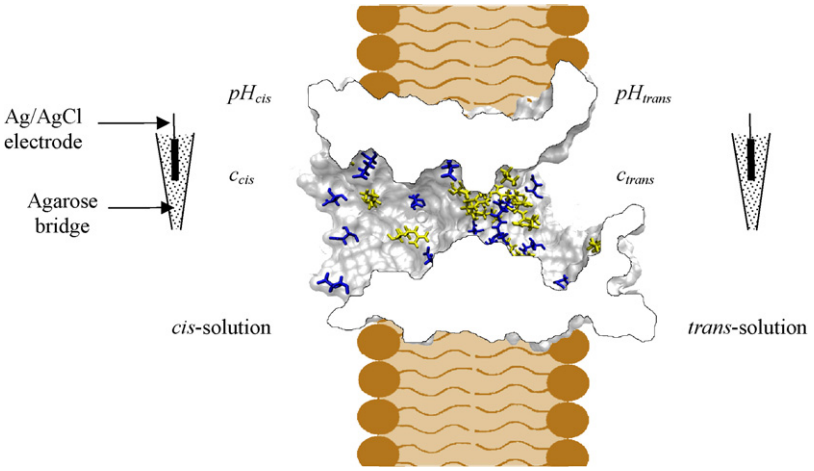

Fig. 1. Longitudinal cross-section of the OmpF porin where acidic residues (blue in web version) and basic residues (yellow in web version) have been highlighted. The channel visualization (using Visual Molecular Dynamics (VMD) software) has been made from the atomic coordinates of OmpF crystal structure taken from the Protein Data Bank repository. Only one of the three monomers is depicted and superimposed over a cartoon that represents the lipid bilayer membrane. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

All these systems have in common that electrostatic interactions between titratable fixed charges and permeating ions are the main determinants of ion selectivity.

2. Experimental

Experiments have been performed on single ion channels reconstituted on lipid bilayer membranes (see Fig. 1). Wild type OmpF isolated and purified from an *Escherichia coli* culture was kindly provided by Dr. Mathias Winterhalter (Jacobs University, Germany). Bilayer lipidic membranes were formed from two monolayers prepared from 1% solution of diphytanoylphosphatidylcholine (DPhPC) (Avanti Polar Lipids, Inc.) in pentane (Baker) on 70–90 μm diameter orifices in the 12 μm-thick Teflon partition that separated two chambers [41,42]. The orifices were pretreated with 1% solution of hexadecane in pentane. The total capacitance depended on the actual location of the orifice in the film but it was always around 70–130 pF. In order to keep constant the pH value aqueous solutions of KCl were buffered by 5 mM MES (2-(N-morpholino)-ethanesulphonic acid) at pH values below pH 6, by 5 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) at pH values (6–8), by 5 mM CHES (2-(cyclohexylamino)-ethanesulfonic acid) at pH 9, and by 10 mM CAPS (3-(cyclohexylamino)-propanesulfonic acid) at pH values above 9. All measurements were performed on single OmpF channels at room temperature (23.0 ± 1.5) °C. Single-channel insertion was achieved by adding 0.1–0.3 μl of a 1 μg/ml solution of OmpF in the buffer that contained 1 M KCl and 1% (v/v) of Octyl POE (Alexis, Switzerland) to 2 ml aqueous phase at the *cis* side of the membrane only while stirring.

The *I–V* curves of OmpF were recorded under a variety of pH values and electrolyte concentration conditions of the surrounding solutions (see Fig. 1). For the sake of simplicity the pH configurations studied will be hereafter denoted as $pH_{cis}||pH_{trans}$. The voltage, *V*, was applied via Ag/AgCl electrodes in 2 M KCl, 1.5% agarose bridges assembled within standard 200 μl pipette tips [41]. Electric potential *V* is defined as positive when it is greater at the *trans* side of the membrane cell. An Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) in the voltage-clamp mode was used for measuring both *V* and the electric current, *I*, passing through the channel. *I* is defined as positive when it flows from solution *trans* to solution *cis* in Fig. 1. The membrane chamber and the headstage were isolated from external noise sources with a double μ-metal screen (Amuneal Manufacturing Corp., Philadelphia, PA).

The membrane potential, $\Delta\phi_M$, was obtained as follows. First, a lipid membrane was formed at a given salt concentration gradient. Second, a single OmpF channel was inserted at zero potential and the channel conductance was checked by applying +50 mV and then switching potential polarity. Third, the ionic current through the channel was manually set to zero by adjusting the applied potential. When measuring electric potential differences between two solutions of different concentration (as is the case of membrane potential), one cannot ignore that each bridge is in contact with a different solution. The potential difference generated across each bridge/solution interface, known as liquid junction potential (LJP), is different, so that the total contribution of both LJP to the total measured potential is different from zero. The LJP is commonly estimated by means of Henderson's equation, which is based upon two basic assumptions. First, the electrolyte solutions are treated as ideal (i.e., constant mobilities are assigned and activities are replaced by concentrations). Second, the junction may be represented by a continuous series of mixtures of the two end solutions (i.e., linear ion concentration profiles). The potential needed to achieve zero current was then corrected by the LJP calculated from Henderson's equation to obtain $\Delta\phi_M$ [43]. Each point was measured for at least three different channels in three different experiments to assure reproducibility and to estimate the standard deviation.

3. Results and discussion

Fig. 2 shows the measurements of $\Delta\phi_M$ under symmetric pH conditions ($pH_{cis} = pH_{trans} = pH$), in the case $c_{cis} = 1$ M and $c_{trans} = 0.1$ M KCl. We have represented in the right axis the transport number for cations calculated from [44]

$$t_+ = \frac{1}{2}\left(1 - \frac{F}{RT}\frac{\Delta\phi_M}{\ln(c_{trans}/c_{cis})}\right), \quad (1)$$

where *F*, *R* and *T* have their usual meaning. The results in Fig. 2 reveal some interesting features of the OmpF porin transport properties. In agreement with previous studies of this channel, we see that $\Delta\phi_M$, and consequently t_+, depend strongly on the pH of the external solutions [45,46]. Note that this is not only a quantitative change, but also a qualitative one: the cation selectivity found for the channel in neutral and basic medium turns into anionic selectivity in acidic solutions. This remarkable dependence on pH suggests that the mechanism responsible for the changes in selectivity is the titration of the porin acid and basic charged residues. Note how-