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Experimental demonstration of charge inversion in a protein channel in presence of monovalent cations.

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Abstract

Charge inversion takes place when interfacial charges attract counterions in excess of their own nominal charge. Up to date, this phenomenon has been reported in a variety of membrane channel systems in presence of multivalent cations. Here, we investigate the charge inversion phenomenon in a protein channel in solutions of monovalent cations. To this end, we perform selectivity experiments in salts of different cations, changing both the electrolyte concentration and the solution acidity. We show that charge inversion can be produced by two different mechanisms, namely specific adsorption and competitive binding.

Keywords: bioelectrochemistry, electrophysiology, electrostatics, membrane protein, ion channel

1. Introduction

The electrostatic interaction with the charge on the pore surface produces the exclusion of coions and the accumulation of counterions. An extreme case occurs when interfacial charges attract counterions in excess of their own nominal charge, thus leading to an effective charge inversion of the system [1, 2]. This phenomenon has been reported in diverse systems that are in contact with an aqueous solution containing multivalent ions: lipid vesicles, colloids, polyelectrolytes, Langmuir monolayers, membranes, nanopores and ion channels among others [1-5]. Here, we focus on the bacterial channel OmpF of E. coli [6] to report experimental evidence of charge inversion in presence of monovalent cations. We perform selectivity experiments in different conditions of pH
and salt concentration to analyze both the effect of cation type and size in the selectivity changes. In order to assess the channel preference for anions or cations, we measure the reversal potential $E_{\text{rev}}$, defined as the electrical potential difference that should be applied across the channel to obtain a null electric current when there is a concentration difference between the external solutions [3]. The fact that the channel residues are titratable leads to the well-known result that the overall charge of the channel is pH dependent [6]. Thus, decreasing pH can turn a channel preference for cations into a preference for anions [6]. By “charge inversion” we are not referring to this situation that is only consequence of the protonation of acidic residues. Quite the opposite, we are referring to the situation where pH is kept constant and we investigate the channel selectivity as a function of the solution concentration. In principle, concentrated solutions screen the channel fixed charges more effectively, and, therefore, the channel ionic selectivity should decrease as concentration increases [6]. However, we show that in certain specific conditions this is not the case. Increasing salt concentration could reverse channel selectivity. In absence of other effects, we ascribe this change to the overcharging of the channel charges by the ions from the bathing solution.

2. Materials and methods

Wild-type OmpF, kindly provided by Dr. S. Bezrukov (NIH, Bethesda, USA), was isolated and purified from an E. Coli culture. Planar membranes were formed by the apposition of monolayers across orifices with diameters of 70-100 µm on a 15-µm-thick Teflon partition using diphytanoyl phosphatidylcholine. The orifices were pre-treated with a 1% solution of hexadecane in pentane. An electric potential was applied using Ag/AgCl electrodes in 2 M KCl, 1.5% agarose bridges assembled within standard 250 ml pipette tips. The potential was defined as positive when it was higher on the side of the protein addition (the cis side of the membrane chamber), 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 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 pH was adjusted by adding HCl or KOH and controlled during the experiments with a GLP22 pH meter (Crison). Measurements were obtained at $T = (23.0 \pm 1.5)^{\circ}C$. The reversal potential was measured as the potential needed to achieve zero current when one or several channels
were inserted into the bilayer. It was corrected with the liquid junction potential calculated from Henderson’s equation, as described in detail elsewhere [6].

3. Results

To investigate the channel selectivity and hence the possibility of charge inversion, we measured the reversal potential of the OmpF channel as a function of the solution pH for a 0.02 M / 0.1 M concentration gradient in KCl (See Fig 1a). The results show that the selectivity of the channel is highly sensitive to the solution pH. The channel is selective to cations when the solution pH is higher than ~3.7 whereas it is anion selective below this value. When reversal potential experiments are carried out for the same concentration ratio (r = 5) but using different absolute concentration (0.5 M / 2.5 M) it is found that the inversion of selectivity occurs around pH ~ 2.5. Similar results (not shown here) are obtained in experiments performed with CsCl under the same conditions.
The fact that the selectivity of the OmpF channel is both proton and salt concentration dependent [7] is crucial for the charge inversion phenomenon. Figure 1b reveals the different roles of KCl solution as regards the interaction with the channel residues. The reversal potential measurements performed at a constant concentration ratio, \( r = 5 \), and pH = 6 reveals that the absolute value of the reversal potential decreases as salt concentration increases, meaning that concentrated solutions screen the channel fixed charges more effectively, and, therefore, the channel cationic preference gets weaker. However, when similar experiments are performed at pH = 3.3, increasing salt concentration reverses the channel selectivity, that changes from anionic to cationic. Because \( K^+ \) and \( Cl^- \) have almost equal bulk mobilities and, therefore hydrated sizes, diffusion potentials are negligible (~ 1 mV) and there should be an almost direct connection between the effective channel charge and the measured ionic selectivity [6]. Then, the channel selectivity inversion produced by increasing salt concentration could be an indication that interfacial charges attract counterions in excess of their own nominal charge, yielding charge inversion.

In previous theoretical studies, the possibility of finding charge inversion has been related to the relative size of cations and anions. In particular, it is expected to occur when coions are larger than counterions [8] as it is the case here (\( Cl^- \) is slightly larger than \( K^+ \)). As these theories can be applied to both naked and hydrated ions, we tackled this issue by comparing measurements done in KCl (\( K^+ \) is smaller than \( Cl^- \) either hydrated or dehydrated) with experiments performed under the same conditions in tetramethylammonium chloride (TMACl) (TMA\(^+\) is larger than \( Cl^- \) either hydrated or dehydrated) [9]. Results are shown in Figure 2a.
Figure 2. a) Dependence of the reversal potential on solution pH at Ccis = 0.02 M TMACl and Ctrans = 0.1 M TMACl (black circles) and at Ccis = 0.5 M TMACl and Ctrans = 2.5 M TMACl (green circles) b) Reversal potential measured at constant cis/trans TMACl concentration ratio (r = 5) but different absolute concentrations and solution. Inset: Reversal potential measured in the mutants D113C and D113R at constant cis/trans TMACl concentration ratio (r = 5) but different absolute concentrations and solution pH = 6. Note that in Figure 2 the transition from cationic to anionic selectivity is not at $E_{\text{rev}} = 0$, but at $E_{\text{rev}} = -11$ mV, corresponding to the diffusion potential for a concentration ratio $r = 5$.

Remarkably, the effects of solution acidity on channel selectivity in TMACl differ from those in KCl. In fact, around neutral pH (pH = 6) the channel is selective to cations for 0.02 M / 0.1 M gradient and selective to anions for 0.5 M / 2.5 M. In the case of TMACl, diffusional effects may play a role, since TMA$^+$ and Cl$^-$ have different mobilities. For the conditions studied here, where a concentration ratio of $r = 5$ is imposed, the diffusion potential (due to the difference in mobilities between TMA$^+$ and Cl$^-$) is about -11 mV and determines the change in selectivity. In Fig 2b, the reversal potential measurements performed at a constant concentration ratio, $r = 5$, and pH = 6 show clearly how increasing TMACl concentration turns channel selectivity from
cationic to anionic. In contrast, experiments at pH = 3 show that under these conditions, the channel selectivity is almost insensitive to TMACl absolute concentration. The inset of Fig 2b shows experiments performed at pH = 6 with mutant forms of the wild type protein OmpF that differed at the crucial residue D113 [3]. Interestingly, the substitution of the aspartic acid D113 with a neutral cysteine does not change the observed charge inversion. In contrast, when this acidic residue is replaced with a positively charged arginine the protein becomes insensitive to TMA concentration and the charge inversion is abolished.

4. Discussion

As far as we know charge inversion has been reported in presence of salts of multivalent cations in systems of membrane channels [3-5], but only this is the first example concerning monovalent cations. Previous studies of charge inversion in systems in which the charge is pH-dependent mention specific adsorption of metal cations and correlation effects as potential candidates to explain charge inversion observations [10]. The simplest version of Bjerrum pairing theories predict no substantial correlations between monovalent cations and interfacial groups [11], as confirmed in recent all atom MD simulations performed in OmpF channel in KCl solutions [12]. In any case, these correlations should be less probable the larger the cation involved [11], what contradicts our findings involving TMA⁺ and K⁺. Interestingly, the distribution of the TMA⁺ cation in concentrated solutions (~ 4M) of TMACl was previously investigated by means of neutron diffraction with hydrogen/deuterium isotope substitution [13]. This approach discarded solute-solute correlations showing that the TMA ion behaves more like an apolar solute [13]. This suggests that the origin of selectivity inversion could be related to the adsorption of TMA⁺ cations onto the pore surface. The more concentrated the TMACl solution the more cations could be adsorbed onto the pore surface yielding the observed selectivity inversion. Overcharging could occur locally as adsorbed TMA⁺ cations directly overcompensate the charge of the acidic residues that are acting as their receptors. Alternatively, we can think in terms of the overall protein, considering that OmpF channel is an amphoteric structure that at pH = 6 has negative net charge. Thus, TMA⁺ cations could adsorb specifically to the surface just neutralizing some negative residues but inverting dramatically the global charge balance. In this context we use the term “specific” to indicate that ions of the same valence behave differently [2], as it is
the case of K$^+$ cations showing no adsorption under the same pH conditions. The reason for this specificity could lie on the fact that TMA$^+$ cations are kosmotropic (more hydrophilic) [14]. Wide channels like OmpF have evolved to configure a hydrophilic environment similar to that in bulk aqueous solution where ions are fully hydrated. Thus, kosmotropic cations could lose part of their hydration shell exchanging some solvation waters that are replaced by liganding groups within the channel. On the contrary, chaotropic cations like K$^+$ are less hydrophilic [14] and display a lower affinity towards the protein. Interestingly, hydrophobic/hydrophilic effects have been reported as the driving force behind charge inversion found in colloids in presence of monovalent ions [15].

At low pH the picture is different. Selectivity inversion is only found in the case of KCl, and differs from that found in the case of TMACl at neutral pH. There, increasing TMACl concentration turns cationic selectivity into anionic one. Here, it is just the opposite way, increasing KCl concentration turns anionic selectivity into cationic one. This discards the specific adsorption of K$^+$ cations as a potential source of selectivity change, because adsorbed cations would enhance the channel preference for anions. Indeed, available X-ray structures in presence of salt show that alkaline cations tend to locate in the vicinity of oppositely charged residues partially screening them but without forming any kind of complexation [16]. Previous studies show also that the pH sensing mechanism of OmpF channel in the case of alkaline cations operates via competitive binding: at a fixed pH, increasing salt concentration provokes the replacement of protons with cations in certain critical residues [7]. This peculiar salt-mediated titration means that the effective pK$_a$ of these residues actually depends on salt concentration [7] explaining our findings reported in Fig 1. The inversion of selectivity is then produced by the reversal of the net channel charge arising from the salt-induced deprotonation of acidic residues. This mechanism does not appear in presence of TMACl because the specific adsorption of TMA$^+$ is so strong that even at low pH the site is in practice unreachable for protons.

5. Conclusions

We have presented two examples of selectivity inversion in a protein channel, involving TMACl at neutral pH and KCl in acidic solutions. In both cases, the inversion of
selectivity is likely to be originated by a charge inversion phenomenon in the system, discarding that the relative size of cations and anions (TMA\(^+\) > Cl\(^-\) ≥ K\(^+\)) could play a decisive role. Interestingly, each electrolyte seems to display a particular mechanism, specific adsorption in TMACl and competitive binding in KCl. We have shown that both mechanisms are hardly compatible with the simplest versions of Bjerrum pairing theories suggesting no significant effects in the case of monovalent cations [11]. Our results emphasize also the importance of hydrophobic/hydrophilic effects in the interaction of monovalent cations with membrane proteins.

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