Selectivity of Protein Ion Channels and the Role of Buried Charges. Analytical Solutions, Numerical Calculations, and MD Simulations

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Supporting Information

ABSTRACT: The preference of large protein ion channels for cations or anions is mainly determined by the electrostatic interactions of mobile ions with charged residues of the protein. Here we discuss the widely spread paradigm that the charges determining the channel selectivity are only those that can be considered solvent-accessible because of their location near the permeation pathways of ions and water molecules. Theoretical predictions for the electric potential and average ion densities inside the pore are presented using several approaches of increasing resolution: from analytical and numerical solutions of electrostatic equations in a model channel up to all-atom molecular dynamics simulations and continuum electrostatic calculations performed in a particular biological channel, the bacterial porin OmpF. The results highlight the role of protein dielectric properties and the importance of the initial choice of the residue ionization states in the understanding of the molecular basis of large channel selectivity irrespective of the level of resolution of the computational approach used.

INTRODUCTION

Advances in methods of tertiary structure determination down to the atomic level have revealed the importance of electrostatic interactions in the structural conformation and the physiological function of proteins.1,2 This has been particularly evident in ion channels, a kind of transmembrane proteins crowding biological membranes and performing a variety of physiological functions. Many of these functions are based on the channel selectivity, that is, the ability to discriminate between differently charged solutes.3,4 In large channels like bacterial porins, toxins, or peptide channels, the selective transport is largely regulated by the net charge of the protein ionizable residues.5 Thus, the partial exclusion (accumulation) of charged solutes with the same (opposite) charge as that of the protein channel determines the channel selectivity. The ion selectivity of protein channels has been conventionally explored using the concept of “selectivity filter.”6 This term assumes that the channel discrimination is controlled by the narrower region of the pore, where the permeating ions and the protein residues interact more intensely. Furthermore, the distribution of ionizable residues in that “selectivity filter” is typically probed via site-directed mutagenesis. If the measured selectivity is not affected by the mutation this is regarded as a proof that such residue is not exposed to the ion stream. The overall approach is based on the idea, stated explicitly or not, that residues outside that “selectivity filter” (especially those buried in the protein) do not contribute significantly to the channel selectivity.7–9 Here we challenge this established understanding showing that the contribution of buried residues could become not only important but actually crucial in some cases. We take advantage of the extensive characterization of three weakly selective channels (OmpF porin of E. coli,10–12 alpha-hemolysin (aHL) secreted by Staphylococcus aureus13–16 and meningococcal Class 1 porin (PorA/C1) of Neisseria meningitidis17,18) where experimental evidence convey some important messages19 that can be summarized as follows:

1. Ion selectivity of wide channels is not dictated just by the charged amino acids located at the selectivity filter, but it is the result of the concerted action of many charged residues of the protein channel.

2. There is no unambiguous connection between changes in selectivity and changes in the charge state of the ionizable residues exposed to mobile ions. Therefore, the use of the standard method of site-directed mutagenesis to identify ion accessible protein residues through changes in selectivity of the corresponding mutants may not be generally valid for any kind of channels.

3. The charged residues that are not lining the pore wall (i.e., not solvent accessible) may also take part in the selective function of the channel, and, in some cases, the channel selectivity cannot be properly understood unless the net buried charge outweighs the net “pore wall” charge.

Although their contribution could be theoretically imaginable, buried residues have been traditionally ignored because the high energy penalties requested to titrate residues located in hydrophobic environments make these processes to seem relatively improbable. However, recent studies performed on globular proteins like staphylococcal nuclease confirm that some proteins can host a large number of ionizable residues at buried positions although at the expense of large pKₐ shifts.20–22

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We tackle this issue considering the outcomes of electrostatic calculations performed in a protein channel at three different levels of complexity. First, we present the analytical solutions for a model channel involving an ideal charge distribution. Second, the continuum electrostatic equations for a channel of finite length are solved numerically using a finite volume algorithm and realistic parameters that we obtain from the known structure of the bacterial porin OmpF. Finally, all-atom molecular dynamics (MD) simulations are performed in the same protein system, the OmpF channel. In each approach, we analyze how the dielectric environment of buried residues influences the channel selectivity. This is relevant not only to continuum electrostatics analyses in which the charge state of every residue and the value of the protein dielectric constant are input parameters, but also to MD simulations because the requirement of charge neutrality in the simulation box demands knowing beforehand the charge state of every single ionizable residue.

**METHODS**

Poisson–Boltzmann equations are numerically solved using the finite volume algorithm implemented in Fipy based on Python. The MD system used in this section comprises the full trimeric OmpF channel embedded in a POPC membrane (ca. 125 000 atoms). The protein/membrane system was solvated and enough K⁺ and Cl⁻ ions were added to simulate a ~1 M KCl solution. To calculate the 3D ion charge distribution the Volmap module in VMD was used as input. Then we divided the overall ion charge box in slices 1 Å wide along the axial coordinate direction (that is, slices parallel to the membrane) and averaged over them. A very similar procedure was used in ref 26.

**RESULTS AND DISCUSSION**

**Analytical Solution for an Ideal Model Channel.** We consider a cylindrical aqueous pore (dielectric constant εᵣ) enclosed in a protein hydrophobic region that is treated as a homogeneous structureless medium of low polarizability (dielectric constant εₛ). We calculate the electric potential ϕ created by protein fixed charges (ionized residues) at any point of the aqueous pore by solving the linearized Poisson–Boltzmann equation in the pore and the Laplace equation in the protein domain where there are no mobile ions. Assuming a high pore aspect ratio (length ≫ diameter) and radial symmetry for fixed charge distribution allows an analytical solution of the system of differential equations (see the Supporting Information). Then it follows that (a) the electric potential radial profile in the aqueous pore ϕ(r) is independent of the location of the buried charges, and only depends on the total electrical charge; (b) this electric potential ϕ(r) does not depend explicitly on the dielectric constant of the protein hydrophobic domain, εₛ and (c) in contrast to the conventional belief that only the solvent accessible charges influence the electrical potential in the aqueous pore, the model predictions suggest that all fixed charges are involved, being only important the overall value, rather than the actual charge distribution throughout the protein hydrophobic environment.

**Numerical Solution for a Finite Cylindrical Pore Model.** One could wonder whether the above results are just a consequence of the assumptions made to obtain the analytical result, and if they are valid and sound for a realistic protein channel. In principle, the radial symmetry of charge distribution should not be an oversimplification. It can be regarded as realistic in some multimeric channels like αHL, where the ionization properties and relative positions of the residues are virtually identical in each of the assembling monomers. This assumption is also correct for other channels like alamethicin or viroporins with an oligomeric structure of identical alpha helices either in a barrel stave configuration or any other toroidal pore assembly. Even in the case of channels with noncylindrical geometry but an hourglass shape like the bacterial porin OmpF, previous studies have shown that the pore transport properties can be qualitatively described using cylindrical symmetry and an averaging procedure to obtain the effective charge volume density along the pore. However, the assumption of high aspect ratio could be more problematic having in mind that most protein channels have lengths only about 3–5 times their diameter. To validate this assumption, we consider a model pore of radius a and length l with radial symmetry in the distribution of charges. Because no analytical solution exists for this case, Poisson–Boltzmann equations are numerically solved. To guarantee the use of realistic values of charge in the numerical solution we selected the bacterial porin OmpF as a case study. OmpF is a trimeric protein with a total of 102 titratable residues per monomer. Using a cutoff distance to account for the solvent penetration into the protein structure, these ionizable residues can be classified into two groups, one group of charges that are solvent accessible (Qₑ = 6e) and another comprising charges buried in the protein hydrophilic domain (Qₜ = −14e). We assumed the latter to be 0.5 nm deep from the pore wall and both of them (Qₑ and Qₜ) distributed with cylindrical symmetry.

Figure 1 shows the calculated electrostatic potential at the center of the pore ϕ(0) for different channel lengths while keeping constant the other parameters. For low (~2) aspect ratio channels the analytical and the numerical solutions for the electric potential agree by 80%, while for higher (~5) aspect ratio pores the coincidence is almost 100%. It is remarkable that the differences between analytical and numerical solutions are relatively small even in the worst case of a "wide" channel. This
means that the finite length of the pore is not a crucial issue in the analytical calculation of the pore electric potential and the numerical model could be considered consistent with the infinite pore approximation used in the analytical solution.

Determining the Charge Distribution in a Protein Channel from its 3D Atomic Structure. The numerical solution discussed in the last section confirms some general predictions of the analytical model: as long as charged residues are distributed with radial symmetry, the electrostatic potential at the center of the pore barely depends (<15%) on the pore length and almost imperceptibly on the protein dielectric constant, \( \epsilon_b \) (results not shown). Concerning the latter, note that \( \epsilon_b \) influences the numerical solution of \( \phi \) in two ways. It is involved in the solution of the equations for a channel of finite aspect ratio and for a particular channel it would be also implicit in the determination of the charge distribution used as input for the numerical solution. The protonation state (or apparent pK\(_a\)) of a particular residue could be significantly different from that in the standard free solution conditions due to both the interaction of the residue with the low dielectric protein environment and with other charged residues in their vicinity.\(^{10,11,33}\) Calculated pK\(_a\) values are very sensitive to the values of protein and solution dielectric constants\(^{10}\) and there is some consensus in the literature about the fact that values of \( \epsilon_b = 20 \) and \( \epsilon_p = 80 \) yield pore electric potential distributions in agreement with experiments.\(^{34,35}\) However, the meaning of \( \epsilon_b = 20 \) in a nonpolar medium like a lipid membrane or a protein hydrophobic domain is still unclear and smaller values closer to the canonical \( \epsilon_b = 2 \) for hydrocarbon material could probably be more realistic. Figure 2 shows the result of continuum electrostatics calculations of the protein charges present in the OmpF channel. It is seen that the protein dielectric constant determines the values of solvent accessible (\( Q_s \)) and buried charges (\( Q_b \)). Interestingly, the calculated net charge (\( Q_p + Q_s \)) of the protein could be reversed in the case that the protein electrode determines the values of solvent accessible (\( \sigma_p \)) whereas it determines decisively the buried charge (\( \sigma_s \) or \( Q_b \)). Our results stress the crucial role of the protein permittivity actuating in an indirect subtle way: its explicit contribution to the outcomes is negligible but it crucially determines implicitly the amount of buried charge in the system.

Molecular Dynamics Simulations in OmpF Channel. The role of buried charged residues and the dielectric properties of the protein hydrophobic domain can alternatively be studied using MD. Although these simulations do not make explicit use of the dielectric properties of the system, the force fields demand knowledge of the partial charges (protonation state) of all atoms in the system. To check how the initial charge assignments determine the outcome of MD simulations we compare the result of using two different values of the protein permittivity commonly used in studies of protein electrostatics: \( \epsilon_b = 4 \) (dry protein) and \( \epsilon_b = 20 \) (some water penetration into the protein structure). Figure 3A shows a snapshot of the MD system, comprising around 125 000 atoms.

Figure 3B shows the 45 protein residues (color spheres) whose charge state at neutral pH changes in sign depending on the value of \( \epsilon_b \) assumed. This yields a substantial shift in the global charge of 13e per monomer. As can be seen in the front (left panel) and lateral (right panel) view, they are evenly distributed over the entire monomer, with some of them lying near the permeation pathway and other buried inside the protein hydrophobic domain.

Concerning the influence of \( \epsilon_b \) on the concentration of mobile ions in the pore, Figure 4 shows two cross sections of the ion charge distribution for \( \epsilon_b = 20 \) (left) and \( \epsilon_b = 4 \) (right). Our results show clearly how the outcomes of MD are biased by initial choices of the residue ionization states. The left panel (\( \epsilon_b = 20 \)) captures accurately one of the essential physical features of the OmpF porin,\(^{36}\) cations (yellow to red) and anions (blue) follow well-separated permeation pathways along the channel due to the strong transversal electric field appearing in the central constriction. Such feature is not so well-defined in the right panel (\( \epsilon_b = 4 \)), where especially anions seem to accumulate near one of the pore vestibules.

In order to confront the results of MD simulations with the observed preference of OmpF channel for cations, we
calculated the average ion charge density along the aqueous pore of one OmpF monomer for the two values of \( \varepsilon_b \) (Figure 5). At each axial coordinate \((z)\), the average ion charge distribution over the ion accessible regions of the pore is computed (see Methods section). We find an overall shift toward negative charge density when \( \varepsilon_b \) is lowered from 20 to 4. However, the shift depends on the protein region, being more important near the constriction zone \((z = 0)\) and especially at \( z < 0 \). Only the positive average ion charge density calculated for \( \varepsilon_b = 20 \) is consistent with the channel cationic selectivity. Interestingly, we can also compare the outcomes of MD simulations with the corresponding electrostatic calculations performed with the Poisson–Boltzmann solver APBS under the same conditions. We observe how continuum models overestimate the ion charge densities, probably because of neglecting other effects (finite size of ions and shielding between them, etc.) that are actually accounted for simulations.

**CONCLUSIONS**

We have presented electrostatic calculations performed at different levels of complexity: analytical solutions for a model channel and an ideal charge distribution, numerical solutions for a realistic channel of finite length, and finally, all-atom molecular dynamics. The comparison between analytical and numerical calculations show that models invoking ideal conditions like cylindrical symmetry or infinite channel length can be satisfactorily applied to real channels and provide correct insights about them. When simplified models fail to describe observed features in experiments (the role of buried residues), it is not necessarily due to the simplifications made in the process to obtain an analytical solution. In fact, we show that, regardless of the level of complexity employed, the main problem could be that some parameters that in principle are considered to be independent could be actually reciprocally dependent. An example of this is the role of the protein dielectric properties: its explicit contribution to the outcomes of the calculations seems negligible, but it crucially determines indirectly the amount charge in the system. All-atom MD simulations performed in the same protein system reinforce this message. Our results show how the outcomes of MD are biased by initial choices of the residue ionization states.

**ASSOCIATED CONTENT**

* Supporting Information
  Derivation of analytical solution for the electric potential in a model cylindrical pore with buried charges. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.5b03547.

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**Notes**

The authors declare no competing financial interest.

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