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Three-Dimensional Continuum Simulations of Ion Transport Through Biological Ion Channels: Effect of Charge Distribution in the Constriction Region of Porin

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Abstract. Drift-diffusion models are useful for studying ion transport in open protein channel systems over time scales that cannot be resolved practically by detailed molecular dynamics or quantum approaches. Water is treated as a uniform background medium with a specific dielectric constant and macroscopic current flow is resolved by assigning an appropriate mobility and diffusivity to each ionic species. The solution of Poisson's equation over the entire domain provides a simple way to include external boundary conditions and image force effects at dielectric discontinuities. Here we present a 3-D drift-diffusion model of ion (K^+ and Cl^-) permeation through the porin channel *ompF*, and its mutant *G119D*, implemented using the computational platform PROPHET.

Keywords: ion channels, Poisson-Nernst-Planck, transport simulation, *ompF* porin, nanotechnology

1. Introduction

Ion channels are a class of proteins that form nanoscopic aqueous tunnels to control the passage of ions through the otherwise almost impermeable membranes of all biological cells. Each channel consists of a chain of amino acids carrying a strong and rapidly varying localized charge. From a biological point of view, ion channels maintain the correct internal ion composition that is crucial to cell survival and function. They directly control electrical signaling in the nervous system, muscle contraction, and the delivery of many clinical drugs (Hille 1992). There are many types of ion channels, each with a specialized function. Some channels have the ability to selectively transmit or block a particular ion species and many exhibit switching properties similar to nanoscale electronic devices.

ompF is a trimeric porin channel that resides in the outer membrane of the *e-coli* bacterium. Its well-known and extremely stable molecular structure make it a good choice for experimental and computational studies. Each monomer appears to operate independently, making it a possible candidate for multilevel logic devices. *ompF* carries a net negative charge of approximately $-30|e|$, where e is the electron charge, and is moderately selective for cations. The narrow constriction region of each hourglass-shaped channel is highly charged due to the presence of three positively charged

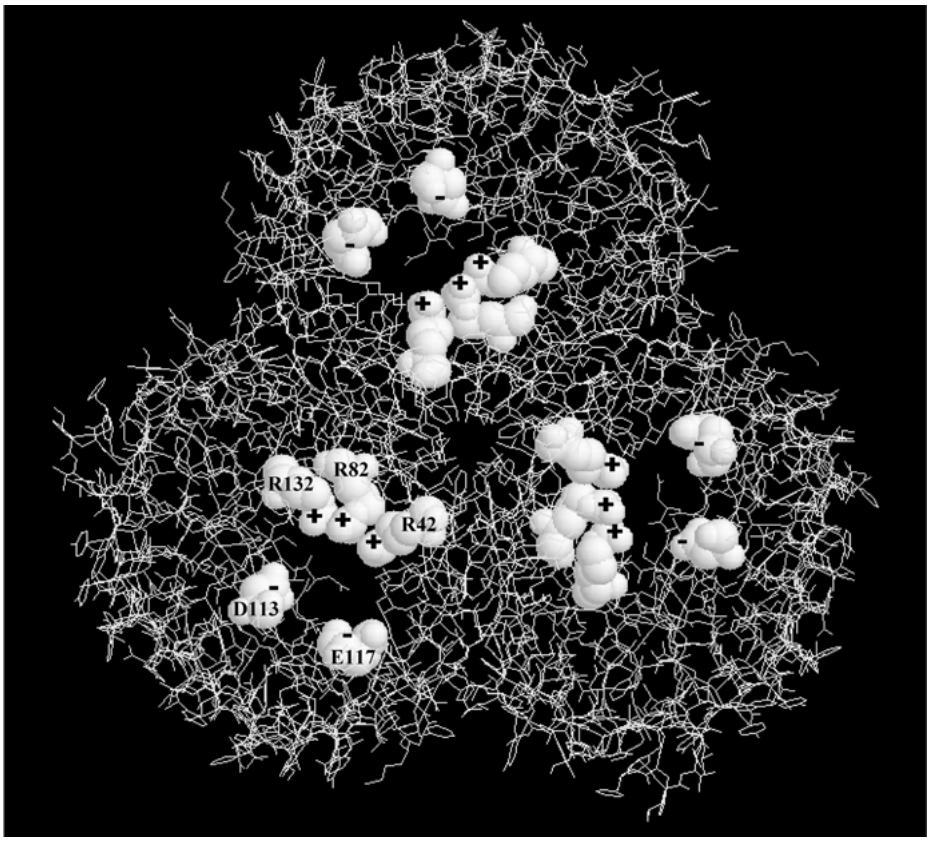

Figure 1. Molecular structure of *ompF*, projected along the length of the channel, showing the three-fold symmetry of the trimer. The five ionized amino acids in the constriction region of each pore are highlighted.

(R42, R82, R132) and two negatively charged (*D113*, *E117*) amino acids (Phale *et al.* 2001), as shown in Fig. 1. The arrangement of these charges gives rise to a very strong electric field parallel to the plane of the membrane, which is believed to govern the behavior of the open channel. *ompF* can be mutated by replacing or deleting one or more of the amino acids, thus altering the charge distribution along the channel. Engineering channels with specific conductances and selectivities is thus conceivable. The mutant *G119D*, formed by replacing an uncharged amino acid (glycine) with a negatively charged aspartate, has a structure almost unchanged from that of *ompF* yet its conductance is measured to be between 15% and 50% lower than *ompF*, depending on the concentration of the salt solution in which it is immmersed. Though the overall structure of the porin is not compromised by this mutation the constriction region becomes significantly narrower due to the bulk and orientation of the aspartate. The observed reduction in channel conductance is believed to be a result of changes in both the steric and electrostatic environment of the pore constriction.

Here we describe a three-dimensional (3-D) drift-diffusion model of ion (K^+ and Cl^-) permeation through *ompF* and its mutant *G119D*, implemented using the computational platform PROPHET (http://www-tcad.stanford.edu/). While continuum models sacrifice resolution of molecular detail they can be used to compute macroscopic current with a reasonable amount of computational effort and have been found to describe permeation through ion channels surprisingly well (Hollerbach *et al.* 2000). Using this approach we investigate the effect of the charge distribution in the pore constriction on the open channel conductance and selectivity. In Section 2 we describe our model; in Section 3 we present the results, in particular we compare the current-voltage characteristics and selectivities of *G119D* and *ompF*. Section 4 concludes with a brief discussion of this work and future plans.

2. Poisson-Drift-Diffusion Model

The channel system studied here is comprised of a porin trimer in situ in a cell membrane, immersed in an aqueous bath of KCl. Experimentally it is possible to maintain different salt concentrations on either side of the membrane (hereafter referred to as C_{left} and C_{right}). Electrodes are immersed in the baths to apply a fixed bias across the channel/membrane system. The electrostatic environment of the channel is determined by (i) mobile ions permeating through the open channel, (ii) fixed (permanent) charges that reside on the protein itself, and (iii) the charges in the aqueous baths and on the electrodes. The local electrostatic potential φ is related to all the charges in the system by Poisson's equation,

$$\nabla \cdot (\varepsilon \nabla \varphi) = -(\rho_{fixed} + \rho_+ + \rho_-) \tag{1}$$

where ε is the dielectric constant, and ρ_{fixed}, ρ_+ and ρ_- are the charge densities per unit volume of fixed charges residing on the protein, K^+ ions and Cl^- ions, respectively. Current flow $\vec{j}_\pm$ is described by the drift-diffusion equation

$$\vec{j}_\pm = -(\mu_\pm \rho_\pm \nabla \varphi \pm D_\pm \nabla \rho_\pm) \tag{2}$$

where $\mu_\pm$ and $D_\pm$ are, respectively, the mobilities and diffusivities of the ionic species. Conservation of charge dictates

$$\nabla \cdot \vec{j}_\pm + \frac{\partial \rho_\pm}{\partial t} = S_\pm \tag{3}$$

where $S_\pm$ can be any form describing the details of ion binding and other chemical phenomena that populate or deplete the ion densities. In the present model we do not consider such phenomena and set $S_\pm = 0$. We seek a steady-state solution for φ, ρ_+ and ρ_-that simultaneously satisfies Eqs. (1)–(3) subject to specific Dirichlet boundary conditions at the electrodes (C_{left}, C_{right} and V_{bias}).

The model described above was implemented using the PROPHET simulator, a computational platform originally developed at Lucent Technologies and currently being extended at Stanford University. The PROPHET simulator uses the "dial-an-operator" methodology to construct systems of partial differential equations by combining existing differential operators described using a scripting syntax. Equations (1)–(3) and the boundary conditions are constructed using existing PROPHET operators. After the channel geometry is defined on a customized mesh provided by the user, the equations are discretized and the linear system is solved using iterative methods. Physical parameters are assigned to the different regions of the domain at run-time. For the results presented here we use $\varepsilon = 80$, 20 and 2, for the aqueous, protein and membrane regions, respectively. Diffusivities were chosen to fit measured current-voltage (I-V) curves, assuming $D_+ = D_-$, and mobilities were assigned using the Einstein relations, reducing the number of adjustable parameters to one.

The distribution of charge on each amino acid is modelled by associating a fractional point charge (in units of $|e|$) to each atom. Values for these charges in neutral solutions are calculated using *ab initio* quantum chemistry codes. For this work we have used the tabulated OPLS partial charges, a force field used widely in molecular dynamics simulations of protein molecules (Jorgensen and Tirado-Rives 1988).

3. Results

As mentioned in the previous section, the distribution of permanent charge around each amino acid is obtained from quantum chemistry calculations that treat each amino acid as a free entity in solution. The dielectric environment and proximity of neighboring ionized amino acids in the folded protein is expected to alter the charge distribution. Electrostatic calculations by Karshikoff *et al.* (1994) suggest that at neutral pH the charges on amino acids R82, located inside the constriction region, and R167, located near the mouth of the channel, should be scaled down from +1 to zero (Dutzler private communication). More recent calculations (Schirmer and Phale 1999, Varma and Jakobsson private communication) however, indicate that both R82 and R167 should in fact be fully ionized at neutral pH. Since both R82 and R167 line the pore of the channel it is important that these charges be properly represented. Figure 2 shows I-V curves computed for symmetric bath concentrations of (a) 100 mM KCl and (b) 1 M KCl, with both representations of the charge distribution: R82, R167 uncharged—solid symbols; R82, R167 fully ionized—open symbols. Figure 2(c) shows the computed I-V curve for a system with 100 mM KCl on one side of the membrane and 1 M KCl on the other, assuming R82 and R167 are uncharged. A diffusivity in the range $(0.8\text{–}1.02) \times 10^{-5}$ cm^2 s^{-1} provides a satisfactory fit to the experimental data. Interestingly, the extra positive

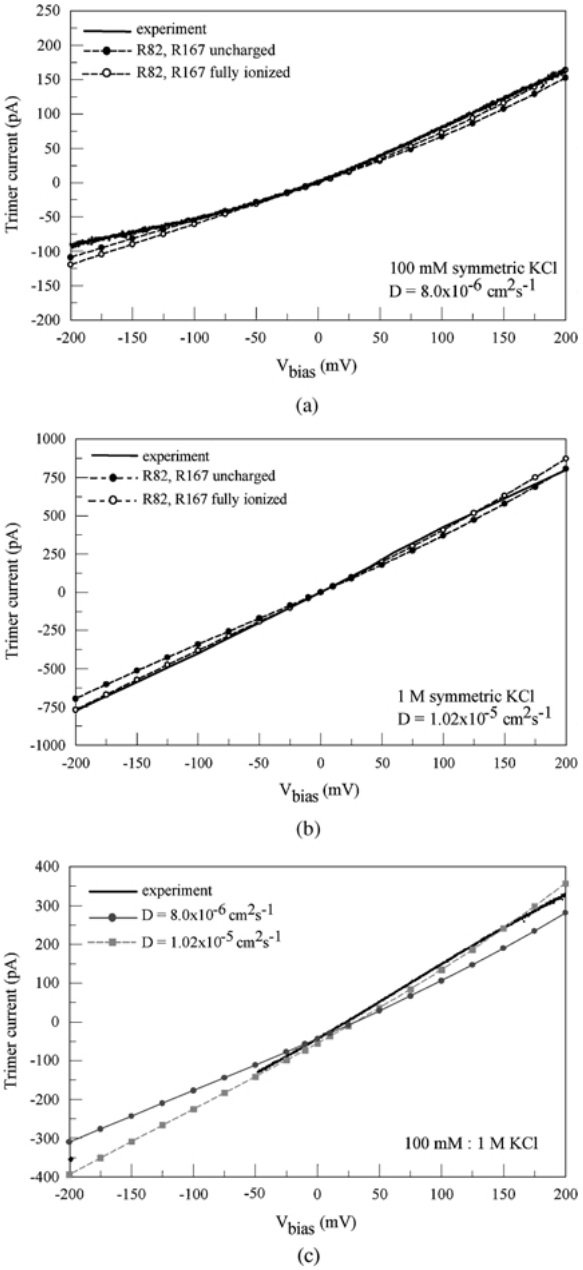

Figure 2. Comparison of drift-diffusion model with experimental *I-V* curves for *ompF* in (a) 100 mM (b) 1 M and (c) 100 mM : 1 M KCl. Simulations run with both R82 and R167 fully ionized are indicated with open circles.

charge on the protein does not make a significant difference to the open channel conductance. However the selectivity of the *ompF* for cations, defined here as the fraction of total current carried by cations, is reduced from 66% to 62% at 1 M KCl, and 88% to 84% at

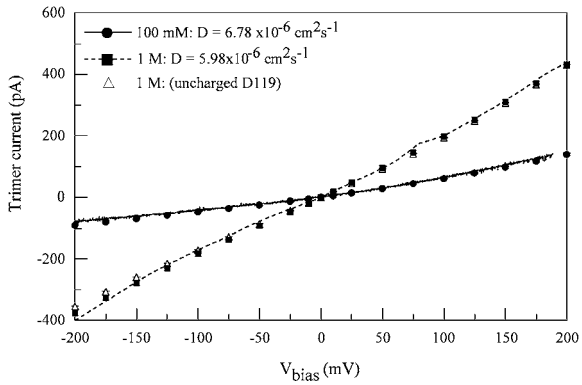

Figure 3. Comparison of drift-diffusion model with measured *I-V* curves for *G119D* in 100 mM and 1 M KCl. Measured data are indicated with solid (100 mM) and dashed (1 M) lines, the simulation data are indicated with symbols.

100 mM KCl, which consistent with the addition of $+6|e|$ making the channel less electrostatically accessible to cations. These values are commensurate with the value 77% measured for 100 mM – 1 M KCl (Schirmer and Phale 1999).

G119D is created by replacing the neutral glycine, *G119*, tucked between the aspartate *D113* and glutamate *E117* (see Fig. 1), both negatively charged, with an aspartate *D119*, also negatively charged. In addition to increasing the negative charge the bulkier aspartate also reduces the volume available for ions to pass through the constriction zone. Figure 3 shows the measured and computed *I-V* characteristics for *G119D*. At l00 mM KCl the conductance is about 15% lower than that of *ompF*, while at 1 M the conductance of *G119D* is about 40% lower. A diffusivity in the range (6.0–6.8) $\times$ 10^{-6} cm^2 s^{-1} is able to fit a fairly large range of experimental data. The simulations predict *G119D* to be more cation selective than *ompF* (69% at 1 M and 90% at 100 mM KCl), as would be expected from the addition of an extra charge in the constriction region.

The mutation introduces both steric and electrostatic changes in the constriction region of the channel. However, the relative contribution of each change to the reduced conductance observed in *G119D* is not known. Understanding the relationship between structure and function of ion channels is crucial if new channels are to be designed with specific properties and behaviors. Simulations can perhaps find their greatest use in providing insight that cannot be measured or inferred from experiment. As an example of this we have attempted to separate the steric and electrostatic effects in *G119D* by scaling down the charge distribution on the aspartate

to zero. This creates a channel (albeit, a virtual one) that should be electrostatically similar to *ompF* but structurally the same as *G119D*. Simulations at 1 M KCl reveal that the conductance (shown in Fig. 3) of such a channel, were it to exist, is almost the same as *G119D* despite the difference in the charge constellation in the constriction region. The selectivity, on the other hand, reduces to 56%. This raises the question of whether the lowered conductance of *G119D* is more the result of a narrowing of the pore constriction than any electrostatic changes. One issue that needs further probing is how the orientation of the key amino acids in constriction region depends on the local field. If it were possible to remove the charge on the aspartate would the charged amino acids reorient in such a way that the channel cross-section is appreciably altered? Detailed molecular dynamics simulations are required to answer these types of questions. An interesting mutation to consider experimentally would be *G119N* where the uncharged glycine is replaced with an asparagine, to emulate the bulk of the aspartate without the charge. If the above conjecture is correct then the conductance of *G119N* should be similar to *G119D* and its selectivity similar to *ompF*.

Ion occupancies, defined as the integral of the ion density over a specified volume, have been calculated for *ompF* and *G119D*, for a range of salt concentrations and applied bias. The latter has very little effect on the predicted ion occupancies, since the effective fixed charge lining the pore is so strong. Figure 4 compares the cation and anion occupancies for the four channel scenarios discussed above, computed for the conditions of zero applied potential and 1 M KCl. In all four cases, occupation of the constriction region is more favorable for cations, and the predicted changes in occupancy upon mutation are consistent with the changes in the net charge in the pore constriction.

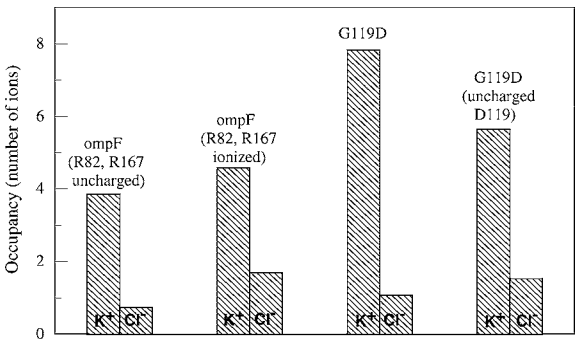

Figure 4. Effect of adding or deleting charges in the channel constriction on the cation and anion occupancies.

Conclusion

The success of the drift-diffusion theory in predicting measured *IV* curves of *ompF* and *G119D* is reasonably good considering that we have used only one adjustable parameter (diffusivity) to simulate ion permeation in a wide range of experimental conditions. The calibrated diffusivities agree to within a factor of 3 with the experimentally measured values in bulk salt solution. Theoretical values for the ion diffusivities inside the porin channel, as calculated from molecular dynamics simulations, also agree with the calibrated values to within 20%. Clearly a uniform diffusivity cannot capture all the physical effects affecting ion transport in an inherently three-dimensional restricted and highly charged volume. The development of a diffusivity model for each ionic species that includes a dependence on position (or local field) and/or ion density is the subject of ongoing work.

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