Modeling Charged Protein Side Chains in Lipid Membranes

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The recent perspectives on membrane protein insertion, protein–bilayer interactions, and amino acid side hydrophobicity (*J. Gen. Physiol.* 129:351–377) have provided a great opportunity to explore an important problem that has challenged our basic understanding of protein–lipid interactions and membrane protein function. Biological membranes consist primarily of lipid bilayers that exhibit hydrophobic cores which present significant barriers to all polar and charged species. This essential character of membranes was brought into question after the proposal of the "paddle" model of voltage-gated ion channel gating (Jiang et al., 2003) in which voltage-sensing domains, containing multiple charged arginine (Arg) side chains, were supposed to move across the core of a lipid membrane, despite theoretical energy cost predictions of 100s of kcal/mol (Grabe et al., 2004). This discrepancy underscores the importance of critically evaluating all models in terms of the fundamental thermodynamics of charged side chain–membrane interactions.

What information can be derived experimentally to answer such questions? Partitioning experiments between water and various bulk solvents, though greatly simplified representations of real membranes, have long provided the best guide to the underlying thermodynamics of membrane insertion. While hydrocarbons, such as cyclohexane for example, may provide good bulk solvent models for the center of a membrane, these measurements tell us only about the neutral states of titratable side chains in this nonpolar phase (Wolfenden, 2007; despite correcting for protonation in the aqueous phase). Consequently, n-octanol has been used to measure the energetics associated with moving charged side chains from water into a "nonpolar" membrane mimetic. Yet, octanol possesses the ability to hydrogen bond and may contain >20 mol% water, which would explain the small partitioning free energies (of the order of 2 kcal/mol for charged side chains) emerging from those studies. The close correlation between octanol measurements and partitioning experiments to the interface of membranes (Wimley and White, 1996) tells us that octanol is a mimic for the membrane–solution interface, not the nonpolar core of a bilayer.

It is therefore not clear whether any bulk solvent can adequately mimic the complex solvation environment provided by a lipid bilayer membrane. White, von Heijne, and colleagues have designed a biological tool, using the translocon/oligosaccharyl transferase complex in the endoplasmic reticulum, to extract sequence-specific apparent free energies associated with membrane insertion (Hessa et al., 2005). These studies found surprisingly low free energies to insert charged side chains at the central residue of a transmembrane helix (also of the order of 2 kcal/mol). Given that the hydration free energy of protonated Arg is around −60 kcal/mol (see Li et al., 2007 and references therein), these results tell us that the glycosylation of the nascent peptide occurs with the side chain in an environment that is nearly as hydrophilic as water (as also suggested by the good correlation between the translocon and octanol scales; Hessa et al., 2005). The exact nature of the partitioning environment is not known and could involve interactions with the translocon protein (Hessa et al., 2005) or may even indicate that the hydrophobic segment remains within the translocon pore during glycosylation, as suggested experimentally (Plath et al., 1998). If this is the case, these experimental results would represent only the energetics associated with the onset of membrane incorporation. While it maybe true that the translocon reduces the activation barrier to form a transmembrane inserted protein (von Heijne, 2007), the path independence of the free energy dictates that what is being measured here is an equilibrium between two fairly hydrophilic environments, not likely between water and the center of an isolated membrane.

Recently, we used molecular dynamics computations to study the engineered hydrophobic segment used in the translocon experiments, and found that this segment by itself is incapable of sufficiently constraining a transmembrane configuration to map out free energies of charged side chains as a function of position relative to the membrane (Dorairaj and Allen, 2007). The large forces imparted on the charged side chain by the membrane cause the hydrophobic segment to slide such that side chain ends up residing at the interface. The energetic penalties for sliding would be all that is needed to account for the small variations in apparent free energies as a function of position in the sequence. This result is consistent with previous sliding observations with

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Abbreviation used in this paper: Arg, arginine.

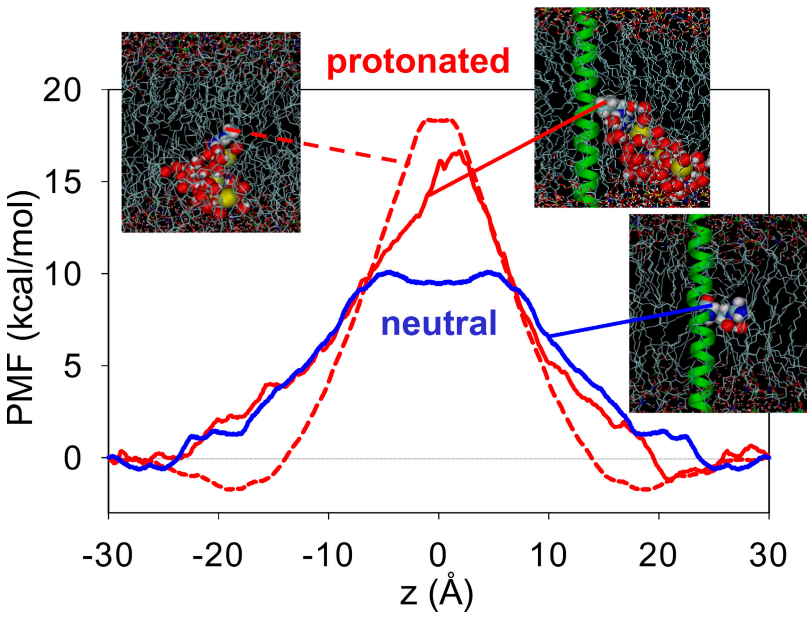

Figure 1. Free energy profiles for a protonated Arg side chain (solid red), protonated methyl-guanidinium (dashed red, symmetrized), and neutral Arg side chain (solid blue, symmetrized) with system images revealing membrane perturbations (lipid hydrocarbon, gray sticks; displaced lipid phosphorous atoms, yellow balls; displaced water, red/white balls; Arg, gray/blue balls; α-helix, green ribbons). Adapted from data in Li et al., 2007.

the glycosylation mapping technique and with the fact that glycosylation sites are far from the hydrophobic segment (see Li et al., 2007 and references therein). Therefore, currently there exists no experiment to tell us, unambiguously, the thermodynamics for charged side chains inside a membrane.

What can theory tell us about the thermodynamic stability of charged side chains in membranes? Continuum models, which assume the membrane is a rigid low dielectric slab, overestimate the dehydration energetics because they lack the ability to deform the interface to help stabilize charges inside the membrane. We therefore turned to fully atomistic, explicit solvent simulations to obtain accurate spatially resolved free energies and created an idealized model of an isolated transmembrane helix to extract the underlying free energies associated with moving an Arg side chain across a membrane (Dorairaj and Allen, 2007). Despite penetration of water and lipid head groups, with strong Arg–head group and Arg–water interaction energies of −100 kcal/mol and −50 kcal/mol, respectively, even at the center of the bilayer, the free energy barrier was 17 kcal/mol (Fig. 1, solid red curve). Free energy decompositions (that include all energetic and entropic contributions) have demonstrated that displaced head groups do not help stabilize the protein, and that while water penetration is the dominant stabilizing factor, it is unable to overcome the large dielectric barrier imposed by the membrane.

These simulations proved to be a challenge because of the complexities of sampling side chain structural isomers and interfacial connections inside a membrane. Could a simpler model have sufficed? Fig. 1 (dashed red curve) shows the free energy profile for a simple analogue molecule for the Arg side chain (methyl-guanidinium, with similar solvation free energies to propyl-guanidinium). The membrane perturbations are comparable to the actual side chain and lead to similar coordination at the membrane center (Li et al., 2007). However, due to increased orientational freedom and the absence of a nearby helix, the lipid head groups provide greater stabilization at the membrane interface (one to two more moieties and −50 kcal/mol interaction), which leads to interfacial minima in the free energy profile. Furthermore, the bulk water coordination is higher by two molecules, which lowers the bulk reference free energy, raising the barrier to cross the membrane (as seen in the free energy profile of Fig. 1). Importantly, despite the considerable differences in the free energy profiles, the overall barrier is high and fairly consistent among these models.

We have so far discussed only the charged (protonated) Arg species, because that is the state that is pertinent to voltage-gated ion channel activation. However, recent experiments have shown large negative pKa shifts for basic amino acid residues in nonaqueous environments (Cymes et al., 2005). The complex environment surrounding the side chain as it enters the membrane (see Fig. 1) rules out protonation state determination with commonly used continuum-based calculations. Fig. 1 (solid blue curve) shows the free energy profile for the neutral side chain across the membrane from fully atomistic simulations (Li et al., 2007). This profile is different from that reported by MacCallum et al. for propyl-guanidine, with no interfacial binding and a barrier ∼4 kcal/mol higher, most likely reflecting the fact that as Arg enters on a poly-Leu helix, a Leu side chain is ejected into water, which costs ∼4 kcal/mol (MacCallum et al., 2007), with the presence of the helix having only a small additional effect on the solvation of this neutral species. Remarkably, the barrier for the

neutral Arg is not that low (~10 kcal/mol), showing that deprotonation is not the key to a "free" passage across a lipid membrane, a result that has implications for a wide range of charged peptide–mediated membrane translocating processes. We understand the high barrier for the neutral species because there are no bilayer perturbations to help stabilize the side chain; the energetics therefore are simply associated with dehydration per se. If we subtract the neutral from the charged free energy profile, we can deduce a pKa profile with a central value of between 7 and 8 (Li et al., 2007), indicating a slight preference for the protonated form (by maybe a factor of 10) and explaining nature's choice of the highly basic Arg as a voltage-sensing residue that can withstand deviations from an aqueous environment.

The CHARMM27 empirical force field used in these studies has been shown to reproduce correct hydration free energies, in agreement with high-level quantum mechanical and semi-empirical solvation models (Li et al., 2007). Moreover, interactions of the charged side chain with head groups and water inside the membrane match results from ab initio calculations to within a few percent. While this is expected to depend somewhat on the choice of force field (using a united atom lipid model, MacCallum et al. found an ~4 kcal/mol lower barrier for the charged analogue), it shows that we can trust the overall large free energy barrier that emerges from the MD simulations.

It is tempting, of course, to use simple models to reduce the computational cost of such simulations. For example, spontaneous membrane insertion of Arg-rich voltage sensor S4 helices has been observed in simulations based on a coarse-grained model (Bond and Sansom, 2007). But simple tests reveal that the governing energetic costs of dehydration (modeled artificially with Lennard-Jones potentials) are not properly accounted for in this model, with hydration free energies for charged species that are an order of magnitude too small for Arg (e.g., −6 kcal/mol; Marrink et al., 2007). Moreover, electrostatic shielding by a dielectric constant of 20 in that model leads to grossly underestimated interactions between the charged side chain and lipid head groups. This explains the absence of the membrane deformations that were essential for obtaining correct thermodynamics in the fully atomistic simulations. One must be very careful to parameterize the correct interaction and solvation energies before embarking on simulations of voltage-gated ion channels using coarse-grained models.

It has been suggested that strong interactions with head groups and water inside the bilayer would stabilize a transmembrane S4 segment containing multiple charged Arg residues (White, 2007), based on short unbiased simulations that do not allow for an equilibrium distribution of protein positions across the membrane (Freites et al., 2005). Yet, as argued above, the side chain experiences these strong and seemingly favorable interactions even at the free energy maximum (an unstable system), which shows that favorable interaction energies alone are not proof of thermodynamic stability. Furthermore, for an isolated S4 segment, though multiple nonpolar residues could offset the unfavorable free energy to insert Arg, we suggest that the true minimum in the free energy surface is likely to involve an interfacial rather than a transmembrane configuration, such that the Arg side chains are not exposed to the bilayer core.

The exclusion of charged side chains from the hydrocarbon core of membranes has implications for conformational changes in voltage-gated ion channels. Based on the slope of the free energy profile in Fig. 1, the transmembrane voltage that would be required to overcome this force will exceed 1 V near the membrane center, an order of magnitude greater than physiological membrane voltages. The barrier for multiple protonated Arg side chains, though not likely additive, should be even larger. Whereas membrane thinning, and associated focusing of electric fields, has been suggested by recent simulations (Treptow and Tarek, 2006; Jogini and Roux, 2007), this would not increase the driving force sufficiently for lipid-exposed movement across the membrane core. We therefore suggest that charged side chains that are protected by water, lipid head group, and protein interactions in a hydrophilic "gating pore" should be a key element of any model of membrane voltage sensing.

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