Lonely Arginine Seeks Friendly Environment

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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) provide an excellent opportunity to return to the fundamental issue of the solvation of amino acids and their thermodynamic stability in different environments (Andersen, 2007). Though seemingly "solved" long ago, in recent years this issue has resurfaced in discussions of the voltage-gating mechanism of K^+ channels, and the likely configuration of charged arginine side chain of the voltage sensor with respect to the membrane lipids (Mackinnon, 2005). The issues have, at some level, been wrapped in controversy. The perspectives point to ways to dissipate some of the controversies, upon which I wish to elaborate further in this letter.

First, let us recall the obvious. Water is a good "high dielectric" solvent for polar and charged species and a poor solvent for nonpolar substances (Friedman and Krishnan, 1973; Wolfenden, 2007), which gives rise to the hydrophobic effect. Indeed, electrostatics and hydrophobicity are two fundamentally opposing solvation forces. Charged molecules interact strongly with water molecules and are difficult to dehydrate, whereas nonpolar molecules are difficult to dissolve in water. To compare the relative magnitude of those two forces, it is useful to consider first a simple situation corresponding to the partition of molecules between aqueous and hydrocarbon phases. Such basic two-phase partitioning experiments can be converted into a thermodynamic scale of transfer free energy. The transfer free energy of a charged side chain from water to oil is unfavorable and on the order of tens of kcal/mol. On the other hand, the transfer of a nonpolar side chain from water to oil is favorable and on the order of a few kcal/mol. Electrostatic solvation forces are much larger than the hydrophobic effect. For example, electrostatic solvation free energy of a single arginine side chain is ∼10–20 larger than the hydrophobic solvation for a single leucine side chain (Deng and Roux, 2004; Shirts and Pande, 2005; MacCallum et al., 2007). The guanidinium group of the arginine side chain is by no means a hydrophobic cation.

Let us now move on to a more complex (and ambiguous) situation, the partition of amino acids between water and octanol (Wimley et al., 1996). The observed trend in the water/octanol scale is somewhat similar to that in the water/cyclohexane scale, namely, the transfer of nonpolar species from water to octanol is favorable whereas that of charged and polar species is unfavorable. Importantly, transfer free energy for the charged species is much smaller than for the water/cyclohexane scale (by one order of magnitude) (Wolfenden, 2007). The reason can be traced back to the experimental conditions used for measuring the equilibrium partition of amino acids between water and octanol, which implies that there is free exchange of water between the two phases. Therefore, the octanol is saturated with water (up to 5–10%). The real "hydrophobic" phase in this case is therefore wet-octanol, a complex environment in which there are droplets of water surrounded by hydrocarbon chains (see Fig. 1). The transfer of a nonpolar compound from water to octanol is favorable because it is surrounded by hydrocarbon chains. But the transfer of a charged side chain such as arginine, though slightly unfavorable, is not prohibitive because the guanidinium group can be solvated by a small droplet of water molecules in octanol (Fig. 1). As a result, the free energy cost of transfer for arginine from water to octanol is much smaller than the free energy transfer to a true bulk alkane phase.

Let us now consider the transfer of amino acids from water to a bilayer membrane. According to the calculations of MacCallum et al. (2007), transfer from bulk water to the membrane interior correlates well with the water/cyclohexane scale. In contrast, the transfer of free energy from bulk water to the membrane interface correlates very well with the water/octanol scale. This is the result one would expect a priori. Indeed, it was noted previously that the partitioning of short peptide, designed to occupy the membrane–solution interface, correlates well with the water/octanol scale (Wimley and White, 1996; Wimley et al., 1996). In summary, the water/octanol thermodynamic scale does not reflect the partitioning of amino acids in the interior of membranes but to the membrane–solution interface. Finally, let us consider an even more complex situation, the

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Abbreviations used in this paper: MD, molecular dynamics; TM, transmembrane.

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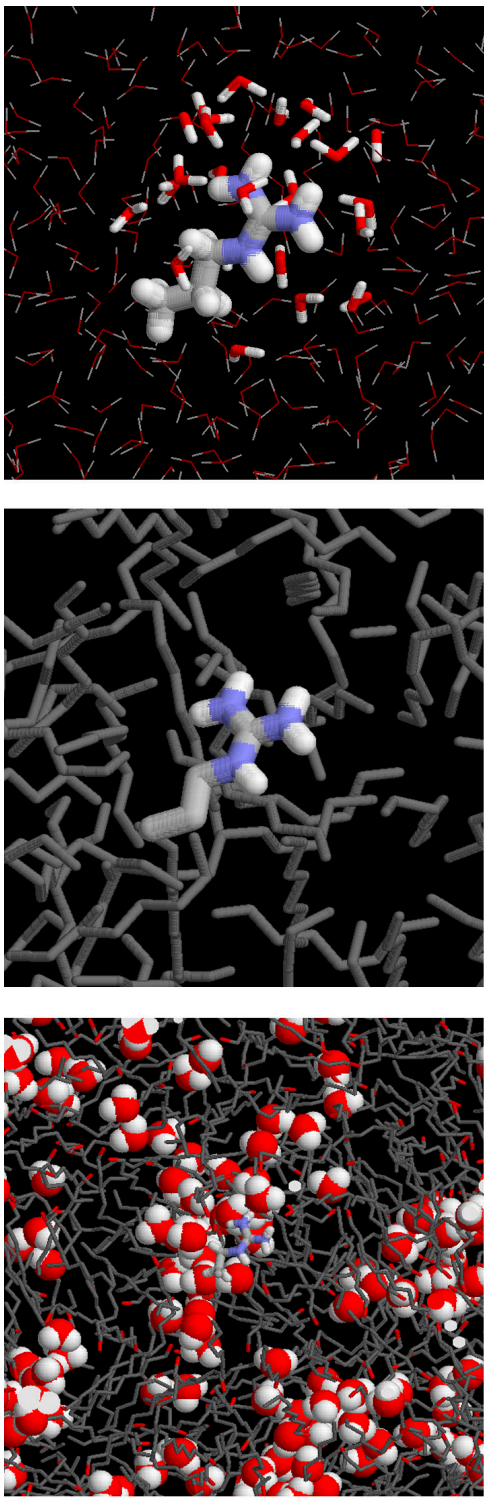

Figure 1. Molecular representations of an arginine side chain (guanidinium cation) in different liquid phases. In liquid water (top), in liquid hydrocarbon (middle), and in a wet-octanol with 10% water (bottom). The top two figures (water and hydrocarbon) show the arginine side chain at close range, while the last figure (wet-octanol) displays a larger fraction of the system (~40 Å across) to show the clustering of water molecules around the arginine side chain. The figure in bulk water shows the water molecules in sticks (thicker for the first hydration shell) while the figure in wet-octanol shows the water molecules in space-filling (CPK) representation.

insertion of transmembrane helices using the translocon expression system. The fascinating measurements of Hessa et al. (2005a) are suggestive of a thermodynamic partitioning between two microscopic phases, one being somewhat more polar or "aqueous," and the other being somewhat more nonpolar or "greasy." The identity of those microphases at the molecular level is not known (see below). Only the end result is known; a given helix is synthesized and ends up in bulk solution or inserted in the membrane. When the nascent peptide reaches the "crossroads" where this decision is being made, it presumably experiences a complex environment when it makes a thermodynamic choice between two microscopic phases. The experimental "readout" for the outcome of this decision is the number of glycosylation events on the nascent helix; glycosylated only once is interpreted to mean that the helix was membrane inserted; glycosylated twice is interpreted to mean that the helix was not. However, in interpreting the glycosylation patterns, one should bear in mind that oligosaccharyl transferase (OT), the enzyme responsible for glycosylation, is actually part of the translocon protein complex (Goder and Spiess, 2001; Chavan and Lennarz, 2006). This means that the helix is still interacting with (maybe even inside?) the translocon complex as it gets glycosylated. Consequently, the peptide is neither in water nor in the lipid bilayer membrane when it is at the crossroads. Furthermore, glycosylation rates and efficacy are quite sensitive to local details about the nascent peptide (Killian and von Heijne, 2000). A legitimate question is, thus, to ask whether the observed glycosylation patterns always faithfully reflect the ultimate fate of the peptide. After all, it is a negative event—the "failure" to glycosylate a nascent peptide twice by OT—that carries the information underlying the interpretation of those experiments. For instance, it seems conceivable that some amphipathic peptide could settle into a position within the translocon relative to OT that permits only a single glycosylation event, and end up adopting a helical configuration parallel to the membrane surface while being misinterpreted as a transmembrane (TM) helix.

Interestingly, the water/octanol scale matches very well the data obtained with the translocon. This is the reason why the water/octanol scale has good predictive value to detect the existence of transmembrane helices in amino acid sequences of proteins (Jayasinghe et al., 2001). Noting the correlation between the water/octanol and translocon scales, Hessa et al. (2005a) concluded that "direct protein–lipid interactions are critical during translocon-mediated membrane insertion." Contributing significantly to confusion, the correlation with the octanol scale was then translated by the authors into a simple view, hereafter taken as "proven fact," whereby the translocon experiments directly report the free energy of an helix in "the" membrane-inserted state

(i.e., a TM helix completely surrounded by lipids) relative to a more hydrophilic environment (e.g., inside the translocon) (Freites et al., 2005; Hessa et al., 2005b; White, 2007). This view is not supported by the facts because the water/octanol scale does not reflect the partitioning of amino acids from water to the interior of membranes, but rather to the membrane–solution interface (Wimley and White, 1996; MacCallum et al., 2007). To take the predictive value of the water/octanol scale to detect TM helices in amino acid sequences as evidence that this scale must reflect the insertion of isolated helices into a membrane thus becomes a circular argument (Hessa et al., 2005a). In retrospect, the most likely reason why the water/octanol scale has some predictive value in detecting TM helices is because of its similarity to the translocon scale. Systematic comparisons show that the predictive value of the water/octanol scale is rather moderate (Chen et al., 2002), and that it may not necessarily reflect the true propensity of lipid-exposed residues in fully formed and folded membrane proteins. While there is no doubt that the translocon experiments of Hessa et al. (2005a) reflect a thermodynamic equilibrium between two microphases, the reality is that neither of them is known. Furthermore, there are good reasons to believe that these two microphases do not correspond to a helix in pure aqueous solution, nor to a helix inserted into the membrane (Shental-Bechor et al., 2006). For instance, the high correlation between the translocon and water/octanol scales suggests that the nascent peptide is experiencing a complex environment in which there are lots of aqueous microregions, as in wet-octanol (Fig. 1).

Carefully conducted molecular dynamics (MD) simulations can shed some light on these issues. As shown in the perspective by MacCallum et al. (2007), when all-atom molecule dynamics simulations are used to compute the solvation free energy of amino acids, the results are in near-quantitative agreement with measured values. This also means that, though computations based on all-atom force field models could be improved, they currently are of sufficient accuracy to provide mechanistic insight into the solvation of amino acids in various environments. Yet, disappointingly, it may appear to many that MD simulations can be made to support any claim. For instance, the study by Freites et al. (2005) displayed an isolated S4 helix inserted in a bilayer membrane where the positively charged arginines formed strong interactions with the lipid phosphate groups (the helix surrounded completely by lipids was said to model the translocon experiments). Because no drift was observed during a single MD trajectory of ∼10 ns, the authors stated that the helix was "thermodynamically stable" in its inserted configuration. This statement is unwarranted; the results from a single MD trajectory such as that presented by Freites et al. (2005) do not allow one to draw any conclusion about thermodynamic stability. Though brute force all-atom MD trajectories are often limited in their ability to explore conformational space, thermodynamic stability of given systems such as a TM helix can be addressed rigorously with biased umbrella sampling techniques (Torrie, 1977). Such computations were done by Dorairaj and Allen (2007). Using the same all-atom force field as Freites et al. (2005), they carried extensive umbrella sampling all-atom MD simulations to compute the free energy profile to move a polyleucine α-helix with a single arginine across a lipid bilayer. The free energy of the helix with the arginine near the center of the membrane was >15 kcal/mol. The large unfavorable free energy was found despite a significant deformation of the membrane–solution interface and charge pairing of the arginine side chains with nearby phosphate groups of the lipids. Based on this result, there is the strong presumption that an isolated S4 helix with four arginines would not be thermodynamically stable in a TM configuration. Bond and Sansom (2007) also show MD simulations of S4 inserting into a membrane. Those simulations were based on an approximate "coarse-grained" model in which simple spherical particles carrying no electric dipole are used to represent effective "droplets" of approximately four water molecules. Water is not a "high dielectric" polar liquid in this model. Thus, whereas such coarse-grained models can be very useful in some applications, they have to be used with caution in complex systems where there are strong opposing electrostatic and hydrophobic forces. A direct comparison with the free energy results from Dorairaj and Allen (2007), which are based on realistic all-atom simulations, suggests that the simulations of Bond and Sansom (2007) are unlikely to resemble reality.

Lastly, what is the significance of these considerations for the voltage sensor of potassium channels? The x-ray structure of the Kv1.2 channel in an open state shows that a "proteinaceous gating pore" sequestering the S4 helix from the membrane does not exist (Long et al., 2005). Although such a feature has historically been a "staple" of simplified cartoons explaining voltage gating, its absence in the structure is not surprising. Even an early model of the *Shaker* channel showed that shielding S4 entirely from the lipids was neither possible nor necessary (Laine et al., 2003, 2004). About 75–80% of the total molecular surface area of S4 is covered by protein in Kv1.2 crystal structure, compared with ∼80–85% in the model of *Shaker* by Laine et al. (2003). In the Kv1.2 channel, the two outermost arginines along the S4 helix are near the membrane–solution interface (Long et al., 2005); MD simulations indicate that they are well hydrated (Treptow and Tarek, 2006; Jogini and Roux, 2007) and that they behave essentially according to the classical "snorkeling" picture (Mishra et al., 1994). Thus, the arginines of S4 do not project into the lipid hydrocarbon when the channel is in the open state.

No atomic resolution structure is available for the channel in the closed state. However, recent experiments constrain unambiguously the closed-state positions of the S4 segment with respect to the S1 and S2 segments (Campos et al., 2007). To satisfy these constraints, the S4 segment must undergo an axial rotation of ~180° and a vertical movement of ~6–8 Å at the level of the first charged residue in S4 (R362 in *Shaker*) in going from the open to the closed state of the channel. Essentially, the first arginine moves from the membrane–solution interface, as shown in the x-ray structure of the Kv1.2 channel (Long et al., 2005), to the middle of the outer leaflet of the bilayer (Campos et al., 2007). As a result of the rotation, the positively charged residues of S4 point toward the S1–S3 helices in the closed state and no charged residues is directly exposed to the lipid hydrocarbon region in the open state or in the closed state. Therefore, even though the S4 helix is not inside a gating pore as pictured traditionally, its charged residues may still move along an energetically favorable pathway, a "virtual" gating pore, during the voltage-gating transition. In conclusion, the concept of arginines being significantly exposed to the hydrocarbon region of the membrane does not appear to be relevant to the operation of the voltage sensor.

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