Does Arginine Remain Protonated in the Lipid Membrane? Insights from Microscopic pK_a Calculations

Jejoong Yoo* and Qiang Cui*†
*Graduate Program of Biophysics, and †Department of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706

ABSTRACT Free energy perturbation calculations are carried out to estimate the effective pK_a of an arginine (Arg) sidechain as a function of its location in the dipalmitoylphosphatidylcholine bilayer. Similar to previous all-atom simulations of the voltage sensor domain of a potassium channel in the membrane with charged Arg residues, the membrane and water structures deform to stabilize the charge of the Arg analog. As a result, the computed pK_a is >7 throughout the membrane although the value is very close to 7 near the center of the bilayer. With additional stabilizations from negatively charged amino acids or lipid molecules, it is reasonable to expect that Arg in membrane proteins (once in the membrane) can adopt the protonated state despite the low dielectric nature of the bulk lipid membrane.

Received for publication 27 September 2007 and in final form 17 December 2007.

Address reprint requests and inquiries to Qiang Cui, E-mail: cui@chem.wisc.edu.

There has been great interest in recent years concerning the protonation state of arginine (Arg) residues in a lipid bilayer environment. This interest was in part triggered by the observation that several Arg residues on the S4 helix may come in contact with the hydrophobic region of the lipid membrane in a crystal structure of the potassium channel (1). Conventional wisdom based on continuum electrostatic models strongly disfavors the transfer of charges from solution to the low-dielectric region of lipid. The new ''biological hydrophobicity'' scale established by White and co-workers (2) based on a translocon assay, however, indicates that the transfer free energy of charged residues into the lipid is much smaller than traditionally thought, although the location of the residue in the membrane is unclear. Molecular dynamics (MD) simulations of the voltage sensor domain of a potassium channel (3) showed that the lipid membrane can deform locally such that the polar headgroups of the lipid and water molecules can stabilize the charged Arg residues in the membrane. Coarse-grained molecular dynamics simulations (4) also showed that insertion of the Kv channel voltage sensor domain into the lipid bilayer can proceed spontaneously with all the Arg residues charged, although the energetics of the coarse-grained models need to be better calibrated. One might argue, however, that the deformation of the lipid and water structure observed in these simulations was due to the fact that Arg was assumed to be charged in the first place. It is possible that the pK_a of Arg is significantly shifted in the membrane environment such that at a certain depth of the bilayer, the sidechain is predominantly charged neutral. In a somewhat related context, poly-Arg and related cationic peptides are known to penetrate through lipid bilayers efficiently. (5) The mechanism of such translocation is poorly understood, and it is possible that deprotonation of Arg is involved in the process.

To better understand the protonation state of Arg in the membrane environment, we have carried out free energy perturbation calculations to estimate the pK_a of an Arg analog (Fig. 1) as a function of its location in the dipalmitoylphosphatidylcholine (DPPC) lipid bilayer. The results support previous all-atom simulation of the voltage sensor domain (3) that the structure of the lipid bilayer and water molecules can locally deform to stabilize the charged Arg sidechain. As a result, the effective pK_a of the Arg sidechain remains substantially >7 except for a very narrow region in the center of the bilayer. With additional stabilization from negatively charged amino acids or lipid molecules, it is reasonable to expect that Arg in membrane proteins can very well adopt the protonated state despite the low dielectric nature of the bulk lipid membrane. While our work was in progress, MacCallum, Bennet, and Tieleman (6) reported results from potential of mean force calculations for the transfer of the Arg sidechain analog in the neutral and charged states, from which the effective pK_a at different locations in the membrane also can be estimated (see below). However, since different force fields and simulation protocols are used in the two studies, our study complements and reinforces the results of MacCallum et al. (6). In this context, we note that our study is not meant to address the issue of transferring Arg from solution to membrane but to probe the favorable protonation state of Arg once it is stabilized somewhere in the membrane.

The simulation system contains 72 DPPC molecules and 2511 water molecules in a periodic rectangular box of 50 Å $\times$ 50 Å $\times$ 72 Å, and Particle-Mesh-Ewald (7) is used for treating the long-range electrostatics; no sodium or chloride ions have

Editor: Anthony Watts.

doi: 10.1529/biophysj.107.122945

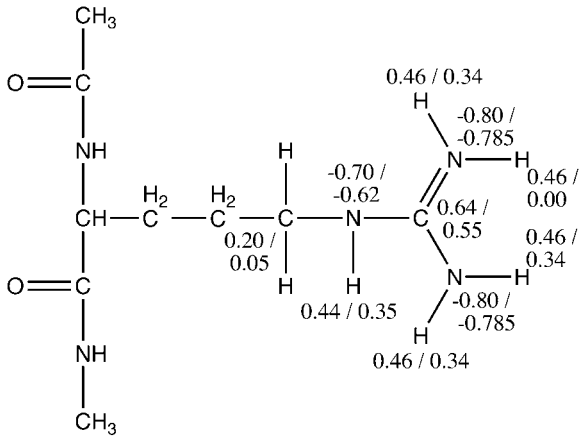

FIGURE 1 The Arg analog used in the study; partial charges for the charged/neutral states are shown.

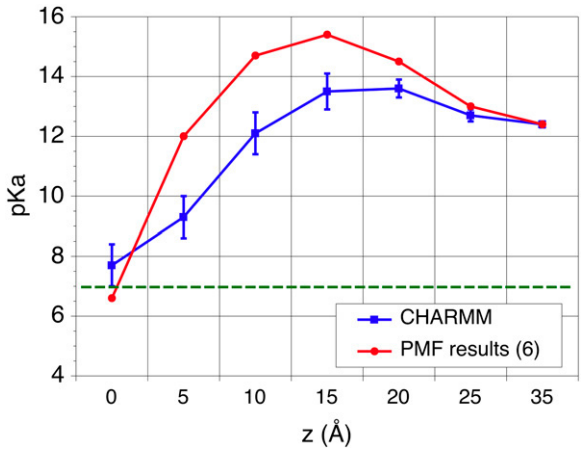

FIGURE 2 Computed pK_a value for an Arg analog in different locations in a DPPC bilayer from our (CHARMM) work and a recent potential of mean force study (6); $z = 0.0$ corresponds to the mid-plane of the bilayer. The solution pK_a is 12.48. (8).

been included. A sidechain analog of Arg (Fig. 1) is placed in the box and the center-of-mass z-coordinate of the guanidinium group is constrained to be at specific values during different simulations using a rather stiff harmonic restraint with a force constant of 100 kJ/mol·Å^2. The pK_a shift of the Arg analog relative to the bulk solution (pK_a = 12.5 (8)) is computed using the free energy perturbation (FEP) approach (9) in which the reverse work associated with converting the Arg analog from the protonated to the neutral form is calculated. Since the overall charge of the system changes from +1 to 0 during the process, care is exercised to correct for effects that might arise from the artificial periodicity, following the protocol in the literature based on continuum electrostatics at the Possion-Boltzmann level (10). As discussed in the Supplementary Material. However, the magnitude of the correction is small for the our setup. The potential energy function employs the CHARMM22 force field (11) for the lipid/water (12) and the Arg analog. The system is equilibrated under constant surface tension of 17 dyne/cm in a tetragonal box using the CHARMM package (13), which is then subjected to production runs under constant volume at 323 K using Gromacs 3.3.1 (14).

Since the local structure of the membrane and water can be significantly perturbed by the presence of the charged Arg analog, care is exercised to monitor the statistical convergence of the FEP simulations using a protocol similar to that proposed by Schiferl and Wallace (15). As shown in the Supplementary Material, the free energy derivatives converge rather quickly (in <10 ns) with a standard deviation <0.3 kcal/mol when the Arg analog is in solution whereasmuch longer sampling is necessary when the Arg analog is deep inside the membrane. The most challenging windows involve the neutral form of the Arg analog at z = 0, 5, or 10 Å, for which the statistical error is ~1 kcal/mol after more than 30 ns (Fig. S1).

As shown in Fig. 2, the computed pK_a of the Arg analog varies nonmonotonically as it is transferred from bulk solution to the center of the bilayer. When the Arg analog is far above the membrane, the computed pK_a shift relative to an Arg analog solvated by pure water solution is essentially zero, which is a useful self-consistent validation for the membrane simulations. As the Arg analog approaches the membrane, the pK_a first increases slightly to ~13.6 for z = 15–20 Å, which is consistent with the dipole potential of the membrane at the membrane-solvent interface. (16) As the Arg analog goes deeper into the hydrophobic region of the bilayer, the effective pK_a value drops quickly, although it still remains >7 even at the center of the bilayer (z = 0.0 Å).

The qualitative trend in the FEP-computed pK_a values is in good agreement with that estimated from the potential of mean force results for the charged and neutral arginine by MacCallum, Bennet, and Tieleman (6) (the converted pK_a values are also shown in Fig. 2). For example, the potential

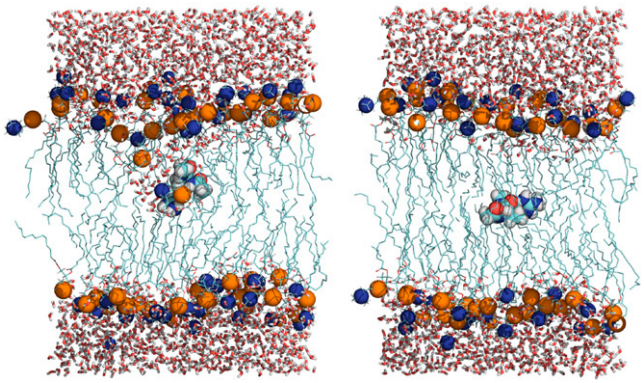

FIGURE 3 Snapshots during the production run at z = 0 Å with a charged state, λ = 0.0, (*left*) and a neutral state, λ = 1.0 (*right*). ARG analog and P and N atoms are shown as sphere. Phosphorus is in orange and nitrogen in blue.

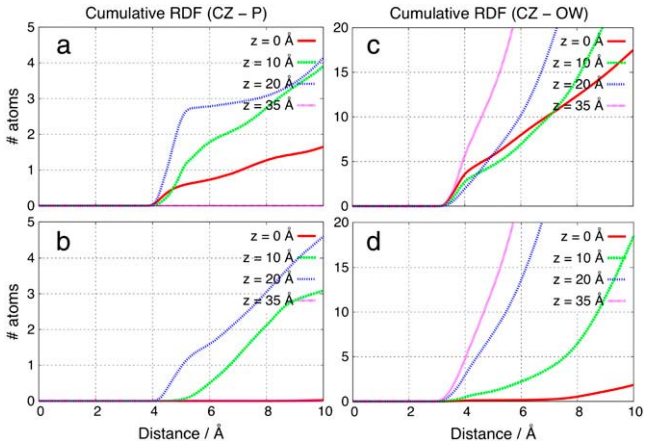

FIGURE 4 Cumulative distribution of phosphorus atom of lipid (*left*) and water (*right*) around a charged (*upper*)/neutral (*lower*) Arg sidechain (distance measured from its center of mass).

of mean force results indicated that the headgroup region favors the charged Arg but disfavors the neutral form, thus the largest effective pK_a is located at approximately $z = 15.0$ Å. At the center of the bilayer, the effective pK_a estimated based on results from MacCallum et al. (6) is 6.6, which is slightly lower than the value of 7.7 from our study. Both sets of values indicate that Arg likely has significant populations of the protonated and neutral forms near the center of the bilayer.

Further analysis of the free energy derivatives indicates that the drop in the pK_a in the hydrophobic region of the bilayer results mainly from the larger destabilization of the charged state ($\lambda = 0.0$, 0.2, 0.5, and 0.8) than the stabilization of the neutral form ($\lambda = 1.0$) of the Arg analog (see Supplementary Material, Table S1). As shown in Fig. 3, similar to previous simulation studies (6), the charged form of the Arg analog induces significant changes in the lipid and water structures when it approaches the hydrophobic region of the bilayer. At a more quantitative level, for $z = 0$, for example, there are about two P groups (Fig. 4 *a*) and more than 15 water molecules (Fig. 4 *c*) within 10 Å from the center of mass of the charged Arg analog. In fact, there are more water molecules within 6 Å of the Arg analog at $z = 0$ Å than $z = 10$ Å for the charged window (Fig. 4 *c*); the reverse is true, however, for the neutral window (Fig. 4 *d*). The new contribution of this work is to show explicitly that such deformations are not so energetically costly that Arg prefers to be deprotonated.

In short, carefully conducted FEP calculations indicate that it is energetically feasible for an Arg to adopt a protonated form in most regions of the bilayer (once it is transferred to a certain location) because the local lipid and water structures can deform fairly easily to accommodate the charge. With further stabilization from other residues or lipids, Arg in membrane proteins is most likely protonated.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

ACKNOWLEDGMENTS

Discussions with Professors N. Baker and R. Pastor are acknowledged. Computational resources from the National Center for Supercomputing Applications at the University of Illinois are greatly appreciated.

The study is supported by the National Institutes of Health (R01-GM071428-02). Q.C. acknowledges an A. P. Sloan Research Fellowship.

REFERENCES and FOOTNOTES

1. Jiang, Y., A. Lee, J. Chen, V. Ruta, M. Cadene, B. T. Chait, and R. MacKinnon. 2003. X-ray structure of a voltage-dependent K^+ channel. *Nature*. 423:33–41.
2. Hessa, T., H. Kim, K. Bihlmaier, C. Lundin, J. Boekel, H. Andersson, I. Nilsson, S. H. White, and G. von Heijne. 2005. Recognition of transmembrane helices by the endoplasmic reticulum translocon. *Nature*. 433:377–381.
3. Freites, J. A., D. J. Tobias, G. von Heijne, and S. H. White. 2005. Interface connections of a transmembrane voltage sensor. *Proc. Natl. Acad. Sci. USA*. 102:15059–15064.
4. Bond, P. J., and M. S. Sansom. 2007. Bilayer deformation by the Kv channel voltage sensor domain revealed by self-assembly simulations. *Proc. Natl. Acad. Sci. USA*. 104:2631–2636.
5. Fuchs, S. M., and R. T. Raines. 2006. Internalization of cationic peptides: the road less (or more?) traveled. *Cell. Mol. Life Sci*. 63:1819–1822.
6. MacCallum, J. L., W. F. D. Bennett, and D. P. Tieleman. 2007. Partitioning of amino acid side chains into lipid bilayers: results from computer simulations and comparison to experiment. *J. Gen. Physiol*. 129:371–377.
7. Darden, T., D. York, and L. Pedersen. 1993. Particle mesh Ewald: an N $\log_N$ method for Ewald sums in large systems. *J. Chem. Phys*. 98: 10089–10092.
8. Dawson, R. M. C., D. C. Elliot, W. H. Elliot, and K. M. Jones. 1986. Data for Biochemical Research. Clarendon Press, Oxford.
9. Simonson, T., J. Carlsson, and D. A. Case. 2004. Proton binding to proteins: pK_acalculations with explicit and implicit solvent models. *J. Am. Chem. Soc*. 126:4167–4180.
10. Kastenholz, M. A., and P. H. Hünenberger. 2006. Computation of methodology-independent ionic solvation free energies from molecular simulations. II. The hydration free energy of the sodium cation. *J. Chem. Phys*. 124:224501.
11. MacKerell, A. D., et al, and M. Karplus. 1998. All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins. *J. Phys. Chem. B*. 102:3586–3616.
12. Feller S. E., and A. D. MacKerell. 2000. An improved empirical potential energy function for molecular simulations of phospholipids. *J. Phys. Chem. B*. 104:7510–7515.
13. Brooks, B. R., R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus. 1983. CHARMM: a program for macromolecular energy minimization and dynamics calculations. *J. Comput. Chem*. 4:187–217.
14. Van Der Spoel, D., E. Lindahl, B. Hess, G. Groenhof, A. E. Mark, and H. J. Berendsen. 2005. GROMACS: fast, flexible, and free. *J. Comput. Chem*. 26:1701–1718.
15. Schiferl, S. K., and D. C. Wallace. 1985. Statistical errors in molecular dynamics averages. *J. Chem. Phys*. 83:5203–5209.
16. Flewelling, R. F., and W. L. Hubbell. 1986. The membrane dipole potential in a total membrane potential model. Applications to hydrophobic ion interactions with membranes. *Biophys. J*. 49:541–552.