Comments

On Molecular Protrusion as the Source of Hydration Forces

Israelachvili and Wennerstrom (IW)[1] have proposed an unusual idea for the origin of hydration forces, asserted to apply specifically only to what they call "amphiphilic surfaces" or actually lipid bilayers. The argument can have implications for similar forces observed between DNA double helices and linear polysaccharides. However, the argument misrepresents much of the data on lipids and neglects other systems where hydration forces have been carefully measured.

According to the IW picture, the forces "originate from the entropic (osmotic) repulsion of molecular groups that are thermally excited to protrude from these fluid-like surfaces". It is asserted that there is significant likelihood of individual molecules moving out of the plane of the bilayer and that the work of this molecular motion is a linear function of the displacement. They speak of an unfavorable energy of a fingerlike protrusion given by the excess area of the hydrocarbon exposed to water. One thus imagines a dilute cloud of errant molecules, of exponentially decreasing density, whose assumed rejection by a facing layer incurs entropic work seen as an exponential repulsive force.

The source of the interaction, in uncorrelated out-of-plane motion, is quite different from the order parameter mechanism for the extended perturbation of solvent proposed by Marcelja and Radic.[2] While the idea of protruding molecules seems implausible, such is the importance and generality of hydration forces that one should take seriously any rational, testable model for their explication. We would like to sketch here what we think should be considered to develop properly and to test critically this hypothesis about the origin of hydration forces.

(1) The Model Must Be Solved Completely Rather Than by Considering Only One of Its Possible Elements.

The work of displacing one molecule, without correlated neighbor displacement, is so large that one should include the more probable lower-energy laterally correlated fluctuations as well. Eventually one must include all modes of molecular displacement rather than the selectively few allowed in the IW formulation. It has been painfully clear in some past work that when using only a partial solution to this type of problem one can even obtain the wrong sign for an interaction. For example, a comparison of the results of the Jonsson and Wennerstrom[3] "first approximation" treatment of an image charge model[4] with Kjellander's full analysis of the same problem shows J & W's fit to the osmotic stress data was "fortuitous" due to the assumption of "very special, unrealistic conditions", and in one limit the J & W repulsion force could even be attractive.[5]

In the present case, how does one know that such a full summation-with-correlation applied to the protruding molecule hypothesis will not lead to a generalization of the undulatory behavior pointed out by Helfrich,[6] behavior which on dimensional grounds alone led to power-law rather than exponential variation?

(2) The Model Must Account for Known Experimental Data.

(a) If molecules protrude with the linear energy of protrusion required by IW, the 1–3 Å exponential decay constant would lead to impossibly high phospholipid solubilities. In the IW model, there is an assumed cost of kT for the molecule to move out by a decay distance λ (1–3 Å).

Consider dilaurylphosphatidylcholine (DLPC), which has a fully stretched 12-carbon acyl chain of length about 15 Å. The measured decay distance for the DLPC hydration force is about 2 Å.[7] In the molecular protrusion picture, there is therefore a Boltzmann factor of

$$e^{-15/2} = e^{-7.5} \simeq 0.5 \times 10^{-3} \qquad (1)$$

for this chain to come out of the bilayer into the polar medium. In the extreme limit where one imagined one of the two chains to emerge first and the other to follow this factor is 0.25×10^{-6} for the whole DLPC molecule to move out of the bilayer and into solution. From multilayer structural data,[7] the effective molar concentration of lipids in a multilayer is on the order of 1 M. Molecular protrusion then will lead to a predicted 10^{-7} to 10^{-6} M concentration of DLPC in the excess water phase that coexists with multilayers. This is at least FOUR orders of magnitude too high a predicted solubility.[8]

(b) It is difficult to see how phospholipid molecules could move as easily in and out of their gel-phase lattices, yet bilayers with frozen, gel state hydrocarbon chains repel at separations less than 15 Å with a force similar to those measured between bilayers with melted chains (though with shorter decay distance and greater coefficient.[7] One sees similar decay lengths for hydration forces between bilayers of frozen, interdigitated hydrocarbon chains as for melted chain phosphatidylcholines; the area per head group is almost double that of gel-phase bilayers.[9]

(c) Available information does not support the likelihood of a stronger hydration force with single chain molecules whose solubility and likely probability of protrusion is higher than that of double chain molecules. For single chain molecules with their lower cross section, one would expect a near doubling of the force coefficient and near halving of the exponential decay rate according to IW's reasoning. But lyso- and diacyl egg phosphatidylcholine phases exhibit only small differences in hydration, whether their chains are frozen or disordered by cholesterol.[10] More recently, it has been found that the repeat spacing of dioleylPE in its lamellar form is some 52 Å with a volume-average water layer thickness of 15 Å;[11] that of the combined lyso-mono- oleylPE with oleic acid is 55 Å

(1) Israelachvili, J. W.; Wennerstrom, H. *Langmuir*, 1990, 6, 873–876.
(2) Marcelja, S.; Radic, N. *Chem. Phys. Lett.* 1976, 42, 129–130.
(3) Jonsson, B.; Wennerstrom, H. *Chem. Scr.* 1983, 22, 221–225.
(4) Jonsson, B.; Wennerstrom, H. *J. Chem. Soc., Trans. Faraday 2* 1983, 79, 19–35.
(5) Kjellander, R. *J. Chem. Soc.* 1984, 80, 1323–1348.
(6) Helfrich, W. *Z. Naturforsch.* 1978, 33a, 305–315.
(7) Rand, R. P.; Parsegian, V. A. *BBA Biomembr. Rev.* 1989, 988, 351–376.
(8) Cevc, G.; Marsh, D. *Phospholipid Bilayers: Physical Principles and Models*; John Wiley & Sons, Inc.: New York, 1987.
(9) Simon, S. A.; McIntosh, J.; Magid, A. D. *J. Colloid Interface Sci.* 1989,
(10) Rand, R. P.; Pangborn, W. A.; Purdon, D.; Tinker, D. O. *Can. J. Biochem. Cell. Biol.* 1975, 53, 189–195.

repeat.[12] Unless the lipid layer thicknesses are very different in these two preparations, there is only a very small difference (3 Å) in the bilayer separation.

(d) A single methylation of an ethanolamine polar group has significant consequences on hydration even while there is less than 6% change in area per molecule. The decay distance and the degree of swelling in excess water both increase, almost by a factor of 2.[13] The linear work of removal of a molecule from the bilayer, developed as the α parameter introduced by IW, should be negligibly different due to this small change in a polar group. (They speak of the energy of protrusion as a linear function, αz, in the length of protrusion z.) One would have to think of a way of connecting such a change in the work of protrusion with the chemistry of the headgroup without invoking hydration. There is nothing in the IW logic to suggest that a singly methylated amine will be likely to have a different coefficient α for moving into water.

(e) Hydration forces are strikingly insensitive to other changes in mechanical properties of lipid bilayers. Adding cholesterol has complex effects on the imbibing of water between bilayers. In small quantities it can extend interbilayer separations by tens of angstroms between frozen bilayers, distances well beyond molecular lengths precluding the possibility of molecular contact across that space.[14] Cholesterol increases lateral stiffness and bending modulus many times,[15,16] yet in many (but not all) circumstances it shows no corresponding change in the forces between bilayers, except for interactions near contact where the spreading of polar groups by cholesterol is said to allow polar groups to interdigitate from opposite surfaces.[17]

(f) In a larger context, one cannot ignore the results emerging from direct measurements of forces between other types of molecules. The Marcelja approach gives some rational basis for the similar features of forces seen in lipids,[7] DNA polyelectrolytes,[18,19] and polysaccharides[20] as measured by the osmotic stress method.[21] The IW model applies only to liquid-crystalline lipid phases. In the case of DNA, measured forces have been compared with the predictions of non-linear Poisson–Boltzmann double layer theory which was found to be qualitatively inadequate;[18] with the simultaneous measurement of molecular motion with the force vs. distance relation, it was found possible to distinguish the contributions of electrostatic double layer, mechanical undulatory and hydration forces.[19] Hydration forces are clearly apparent and in these polyelectrolyte systems there is not the possibility of molecular protrusion that would be analogous to those postulated by IW to act to create 3-Å exponential repulsion.

(3) In Undertaking Quantitative Comparison with Experiment, One Must Justify More Carefully the

(11) Gruner, S. M.; Tate, M. W.; Kirk, G. L.; So, P. T. C.; Turner, D. C.; Keane, D. T.; Tilcock, C. P. S.; Cullis, P. R. *Biochemistry* **1988**, *27*, 2853–2866.

(12) Gruner, S. Personal communication.

(13) Rand, R. P.; Fuller, N. L.; Parsegian, V. A.; Rau, D. C. *Biochemistry* **1988**, *27*, 7711–7722.

(14) Rand, R. P.; Parsegian, V. A.; Henry, J. A. C.; Lis, L. J.; McAlister, M. *Can. J. Biochem. Cell. Biol.* **1980**, *58*, 959–968.

(15) Evans, E.; Needham, D. *J. Phys. Chem.* **1987**, *91*, 4219–4228. Needham, D. *Biophys. J.*, in press.

(16) Evans, E. A.; Rawicz, W. *Phys. Rev. Lett.*, in press.

(17) McIntosh, T. J.; Majid, A. D.; Simon, S. A. *Biochemistry* **1989**, *28*, 17–25.

(18) Rau, D. C.; Lee, B.; Parsegian, V. A. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 2621–2625.

(19) Podgornik, R.; Rau, D. C.; Parsegian, V. A. *Macromolecules* **1989**, *22*, 1780–1786.

(20) Rau, D. C.; Parsegian, V. A. *Science* **1990**, *249*, 1278–1291.

(21) Parsegian, V. A.; Rand, R. P.; Fuller, N. L.; Rau, D. C. *Methods Enzymol.* **1986**, *127*, 400–416.

Magnitude of the Parameters Used. IW use the example of escape of SINGLE-chain compounds from micelles as their point of departure. And they use a surface energy parameter, γ, of 20 dyn/cm. (They take this number 20 from work by one of us (ref 22) and use it in a way quite disconnected from its original definition.) However, their model of uncorrelated fluctuations envisages creation of two interfaces—oil/water and oil/vacuum—for the independent uncorrelated protrusions they consider. Uncorrelated protrusions of single molecules imply an absence of "back-filling" so that the emergent molecules necessarily create cavities whose cost, the creation of an oil/vacuum interface, must be included. A value for γ then for their uncorrelated fluctuations should be 70 dyn/cm = 50 (o/w) + 20 (o/v) dyn/cm.

IW assume that one may take the diameter of the phospholipid to be that of a single hydrocarbon chain (3–5 Å) rather than the 8–9 Å that correspond to the area of a phospholipid or at least the 6–7 Å equivalent to pulling out two neighboring chains.

With these numbers of 70 dyn/cm and 6–9 Å, which we judge from experiment to be those applicable, their estimated decay distances go down by about an order of magnitude. For example, taking $kT = 4 \times 10^{-14}$ erg, diameter = d = 6–9 Å, one obtains $\alpha = \pi\gamma d \simeq (1.3$ to $1.9) \times 10^{-5}$ dyn and decay distance $\lambda = kT/\alpha \simeq 0.2$ to 0.3 Å, far different from the 2 Å found by IW, and nearly an order of magnitude less than those estimated experimentally.[7]

If one insisted on using a single-chain diameter, 3–5 Å, rather than 6–9 Å, the energy would be $\simeq 1/2$ as much, to give decay distances of 0.8 to 1.2 Å, still smaller than most of what is seen experimentally.

In this connection, one must point out that there is not the ambiguity in measured hydration force that IW imply exists for individual phospholipids. The force vs volume of water per lipid, the physical measure of the work done, leads to excellent agreement among all laboratories making direct measurements on bilayer–bilayer interactions (discussed in detail in ref 7). The actual difference in the measured work of dehydration in the two eggPC data sets cited by IW is less than 10%! Differences in hydration force coefficients are due mainly to differences in definition of the "zero" of bilayer contact. In the IW paper, there is a serious misrepresentation of the directly measured forces between eggPC bilayers (see IW Figure 2). McIntosh et al.[23] were careful to point out that they were using a different convention from ours for the "zero" of separation and for the thickness of the bilayer. (See refs 23 and 6 for detailed consideration of this matter.)

If one has a decay constant λ of 1–3 Å and is fitting forces to the form $P_0 \exp(-d/\lambda)$, then a change in the reference point for d by 10 Å can easily have great consequence on the apparent P_0. Israelachvili and Wennerstrom have drawn a graph that makes the difference seem like a factor of nearly 100! An amusing consequence of their misrepresentation is that by forcing their line to go midway between the "experiments", IW guarantee that they are off by about 10 times either way from either data set. They have enforced apparent disagreement with the very experiments the theory is supposed to explain.

(4) One Must Undertake Careful Critical Comparison with Other Probes and Theories. The molecular dynamics simulations of Kjellander and Marcelja[24]

(22) Parsegian, V. A. *Faraday Discuss. Chem. Soc.* **1966**, *62*, 848.

(23) McIntosh, T. J.; Simon, S. A. *Biochemistry* **1986**, *25*, 4058–4066.

cited by IW were made on rigid surfaces for times on the order of ~ 10 ps, surfaces neither free enough nor times long enough to compare either with thermal motions or the 10^{-5} s times of nuclear magnetic resonance or with the thermodynamically long times of force measurements and undulatory force formulations. The ~ 500 cal/mol accuracy of computer simulation is far less than the relevant $\sim$ cal/mol activity of water seen as hydration forces.

The connection made by IW between what is termed a "minor" perturbation of water as seen in NMR and the force measurement implies that one knows how to relate the one-valued time-and-space-average NMR order parameter to the thermodynamics of water rearrangement by a surface. Such a relation has in fact not been realized.

The Marcelja order parameter formalism, even with its lack of specificity about the physical source of the perturbation, does provide a rationale for an exponentially varying force and, with inclusion of surface features, of variations in decay rate. For example, the difference in the decay rates of PE and PC of the same or similar hydrocarbon chain composition has been rationalized in this way. Likewise, the Marcelja formalism provides a ready picture for the effects of lateral ordering through chain freezing or through the difference between homo-geneous and heterogeneous chains.[13,25] It provides some way to connect the energetic changes attendant upon polar group methylation simultaneously affecting hydration forces and membrane lipid phase transitions.[8] To be successful, any protruding molecule picture would have to match these achievements.

In sum, molecular protrusion seems an implausible cause of hydration forces.

V. Adrian Parsegian*

National Institutes of Health,
Bethesda, Maryland 20892

R. Peter Rand

Brock University,
St. Catherines, Ontario, Canada

(24) Kjellander, R. and Marcelja, S. *Chem. Scr.* **1985**, *25*, 73; *Chem. Phys. Lett.* **1985**, *120*, 393.

(25) Kornyshev, A. A.; Leikin, S. *Phys. Rev. A: Gen. Phys.* **1989**, *40*, 6431–6437.