

Role of Water in the Hydration Force Acting between Lipid Bilayers

Lalith Perera, Ulrich Essmann, and Max L. Berkowitz*

Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599

Received December 18, 1995. In Final Form: March 7, 1996[®]

To understand the nature of the hydration forces acting between biomembranes, we perform computer simulations on liquid crystalline dilauroylphosphatidylethanolamine (DLPE)/water and compare the results with previously reported dipalmitoylphosphatidylcholine (DPPC)/water systems. From our simulations of both systems we find that the influence of the surface on water properties is detectable only over a short range and that the membrane surfaces are rough on a molecular scale. We find that the hydration force is due to (a) the removal of only one or two layers of solvating water from the membrane surface and (b) steric interactions. The detailed structure of the solvating water is responsible for the difference in the hydration force acting between DLPE membranes compared to DPPC membranes.

Short range exponentially decaying repulsive interactions between electroneutral lipid bilayers were first measured 20 years ago.¹ Similar interactions have been observed in systems such as DNA and polysaccharides indicating an ubiquitous nature of these forces.² They were named hydration interactions because it was believed that they are due to water between membrane surfaces.³ The origin of the hydration force remains the subject of intense study, both in experiment^{2,4–6} and theory.^{7–10}

Since simulations¹¹ and experiments¹² show that membrane surfaces are very rough on a molecular scale, steric interactions must play a part in repulsive interactions when the distances between membranes are below 10 Å. Our recent simulations¹³ on liquid crystalline (lc) and gel phase DPPC lamellar systems showed that water properties are disturbed next to the surfaces. Perturbations beyond one or two layers of solvation were not detectable. Although some facts related to the nature of the hydration force are becoming clearer, the general description is still absent and many questions have yet to be answered. For example, what is the connection between the specific water structure around the membrane headgroups and the nature of the hydration force? Even more compelling and interesting is the question of why the repulsive force acting between membranes with PE (phosphatidylethanolamine) headgroups is shorter ranged compared to PC (phosphatidylcholine) membranes? Why is the hydration behavior of the PC membranes so different from that of PE membranes? Why, for example, can DPPC membranes imbibe up to ~23 waters/lipid¹⁴ while DLPE membranes

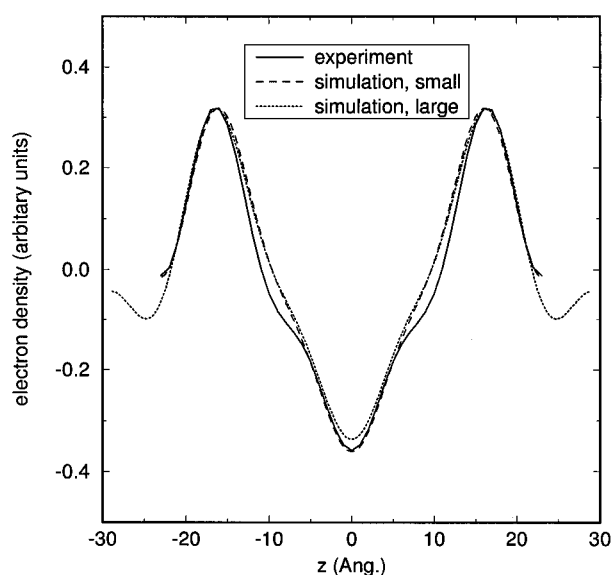


Figure 1. Electron density profiles of the DLPE systems.

Table 1. Conditions of the Four MD Simulations

	small DLPE	large DLPE	small DPPC	large DPPC
area/headgroup [Å ²]	50.4	50.4	61.3	65.8
repeat distance [Å]	46.10	58.15	51.50	56.80
density [g/cm ³]	1.09	1.07	0.98	0.98
temperature [K]	310	310	333	333
no. of waters/lipid	10.3	20.5	11.0	20.5
thickness of water slab [Å]	6.0	17.9	4.9	12.1
width of water interface [Å]	9.2	10.1	10.9	12.2

* Author to whom correspondence should be addressed: e-mail, maxb@gibbs.oit.unc.edu.

© Abstract published in *Advance ACS Abstracts*, May 1, 1996.

(1) LeNeveu, D. M.; Rand, R. P.; Parsegian, V. A. *Nature* **1976**, *259*, 601.

(2) Rand, R. P.; Parsegian, V. A. *Biochim. Biophys. Acta* **1989**, *988*, 351.

(3) Marčelja, S.; Radić, N. *Chem. Phys. Lett.* **1976**, *42*, 129.

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

(5) Gawrisch, K.; Ruston, D.; Zimmerberg, J.; Parsegian, V. A.; Rand, R. P.; Fuller, N. *Biophys. J.* **1992**, *61*, 1213.

(6) McIntosh, T. J.; Simon, S. A. *Ann. Rev. Biomol. Struct.* **1994**, *23*, 27.

(7) Israelachvili, J. N.; Wennerström, H. *Langmuir* **1990**, *6*, 873.

(8) Parsegian, V. A.; Rand, R. P. *Langmuir* **1991**, *6*, 1299.

(9) Israelachvili, J. *Langmuir* **1992**, *8*, 1501.

(10) Parsegian, V. A.; Rand, R. P. *Langmuir* **1992**, *8*, 1502.

(11) Marrink, S.-J.; Berkowitz, M. L.; Berendsen, H. J. C. *Langmuir* **1993**, *9*, 3122.

(12) Wiener, M. C.; White, S. H. *Biophys. J.* **1992**, *61*, 434.

(13) Essmann, U.; Perera, L.; Berkowitz, M. L. *Langmuir* **1995**, *11*, 4519.

take up only less than half of that (~9–10 waters/lipid^{14–16}) at the limit of full hydration? Answers to these questions may provide a key to the resolution of the puzzle of the hydration force.

To find these answers we performed molecular dynamics calculations on two lc DLPE/water systems and compared the results with those of previous simulations¹³ on two lc DPPC/water systems. In the first DLPE/water simulation (referred to as the small DLPE system) we chose the system with 10.3 water molecules per lipid which is the experimentally estimated value in the limit of full hydration.^{15,16} In the second system, there were 20.5 water

(14) Nagle, J. F.; Wiener, M. C. *Biochim. Biophys. Acta* **1988**, *942*, 1.

(15) McIntosh, T. J.; Simon, S. A. *Biochemistry* **1986**, *25*, 4948.

(16) McIntosh, T. J.; Simon, S. A. *Biochemistry* **1986**, *25*, 8474.

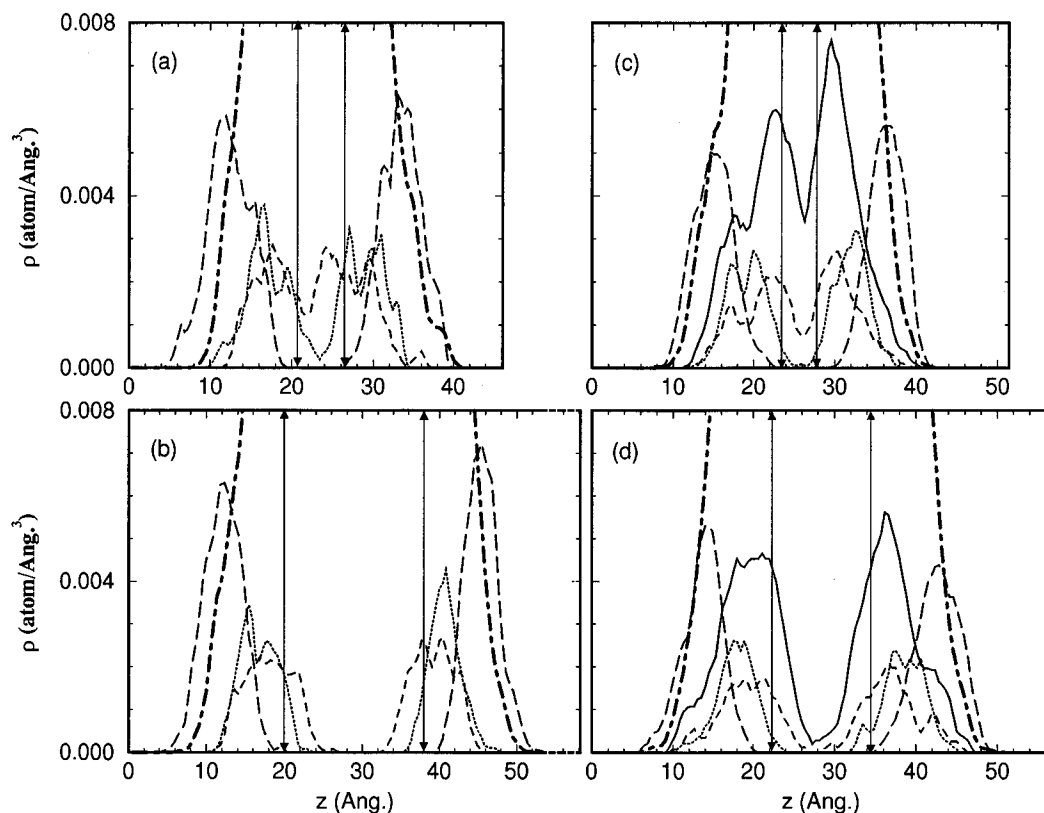


Figure 2. Distributions of atom (or group) densities along the bilayer normal: (a) small DLPE system; (b) large DLPE system; (c) small DPPC system; (d) large DPPC system (dotted-dashed line, water; dotted line, P; short dashed line, N, long dashed line, carbonyl C; solid line, methyl groups on N in the DPPC systems). Vertical lines represent the lipid-water boundaries which are located 4 Å (for DLPE) and 5 Å (for DPPC) away from the maxima in the electron density profiles toward the water layer.

molecules/lipid and since the number of waters in this system is larger than what is needed to achieve the hydration limit, one expects to find a larger number of water molecules displaying bulk properties. The above choices of the number of water molecules per lipid allowed us to make a direct comparison between the results from the present simulations and our previous simulations¹³ on the DPPC/water systems with 11 and 20.5 waters per lipid.

The general methodology for the preparation of samples and the algorithms used in the current simulations are similar to the ones described in detail for the DPPC/water system in our previous work (see ref 13). For the small DLPE system the box dimensions were determined by using the experimental data on bilayer repeat distance and the area per headgroup.^{15,16} In the large DLPE system, the area per headgroup was kept at the same value as the small DLPE system, and the repeat distance was evaluated by adding water with density 1.0 g/cm³. All parameters are presented in Table 1 along with those of the two simulated DPPC/water systems. We used a modified version of the program AMBER 3a¹⁷ in which long range interactions were calculated through the particle mesh Ewald method.¹⁸ The interaction parameters were selected from the Amber force field by the united atom approach except that dihedral interactions within the hydrocarbon tails were modeled using the Ryckaert-Bellemans potential.¹⁹ Charges for DLPE were reevaluated using the CHELP module of the Gaussian 92 program.²⁰

To compare the results from our simulations with some of the experimental data, we evaluated the electron density profiles using the method described previously.¹³ The calculated density profiles are given in Figure 1 along with the corresponding experimental profiles.⁴ In general, overall agreement between the experimental and simulated electron density profiles for the DLPE/water systems is reasonably good.

Having achieved a reasonable agreement with the experimental electron density profiles, we established some confidence in the force fields used in the simulations. Therefore, we further analyzed the lipid and water structure in order to obtain a detailed description regarding the solvation behavior which may not be directly accessible from experiment.

The distributions of atoms in the direction of the surface normal have been commonly used in the literature to characterize the spatial arrangement of such lipid/water systems. In Figure 2, we display the distributions of NH₃, phosphorus, carbonyl carbons, and water obtained from our two simulations of DLPE/water systems. In addition, we present the distributions obtained from our previous simulations of DLPC/water systems. In these figures, the distributions of atoms in the small DLPE/water and DPPC/water systems look very similar. In all cases the water distributions are extended up to the carbonyl positions as has been found in the experiment.^{6,21}

(19) Ryckaert, J.-P.; Bellemans, A. *Chem. Phys. Lett.* **1975**, *30*, 123.

(17) Singh, U. C.; Weiner, P. K.; Caldwell, J. W.; Kollman, P. A., *AMBER (Version 3.1)*, Department of Pharmaceutical Chemistry, University of California, San Francisco, 1988.

(18) Darden, T. A.; York, D.; Pedersen, L. G. *J. Chem. Phys.* **1993**, *98*, 10089.

(20) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Roplogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *Gaussian 92, Revision E.2*; Gaussian, Inc.: Pittsburgh, PA, 1992.

(21) Wong, P. T. T.; Mantsch, H. H. *Chem. Phys. Lipids* **1988**, *46*, 213.

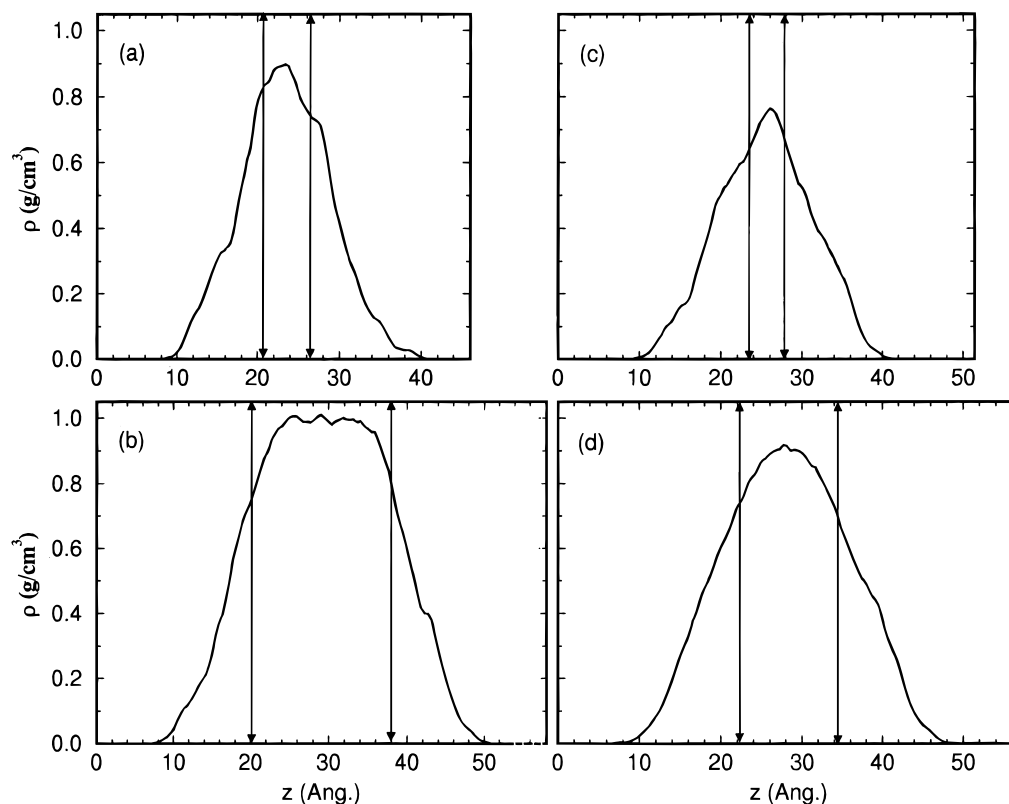


Figure 3. Distributions of water densities along the bilayer normal: (a) small DLPE system; (b) large DLPE system; (c) small DPPC system; (d) large DPPC system. Vertical lines represent the boundaries of lipid water interface as described in the caption of Figure 2.

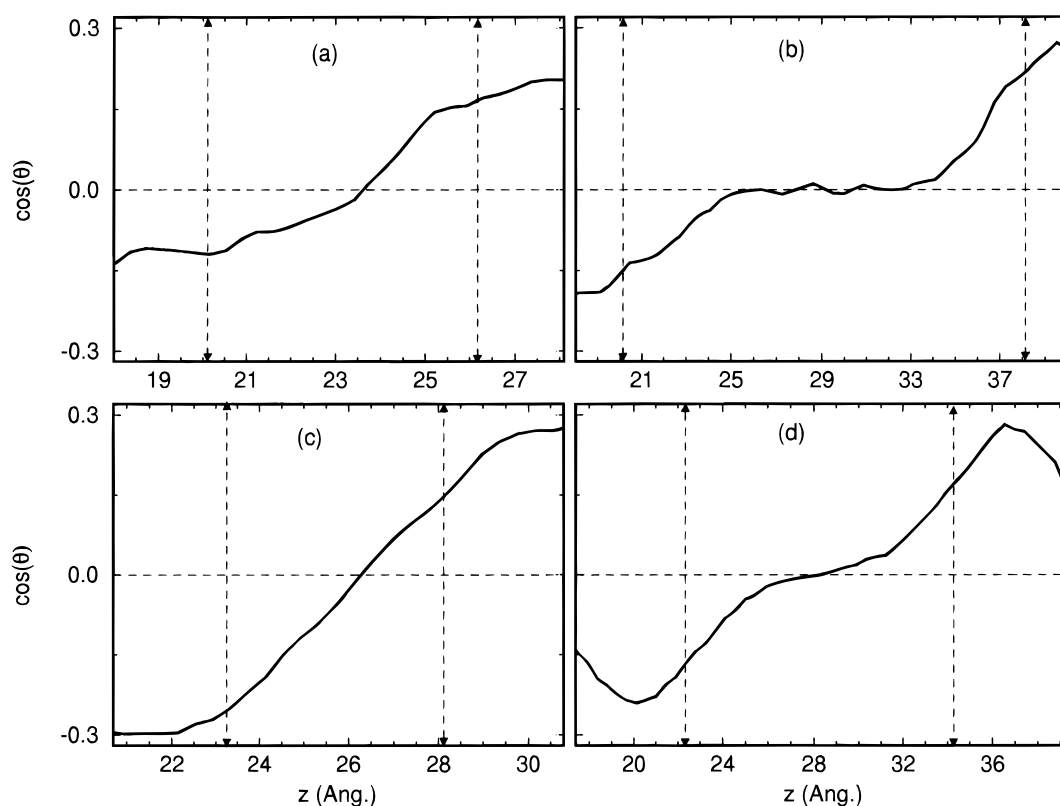


Figure 4. Orientational polarization profiles: (a) small DLPE system; (b) large DLPE system; (c) small DPPC system; (d) large DPPC systems. Vertical dotted lines represent the boundaries of lipid water interface as described in the caption of Figure 2.

The distance for which the water density decreases from 90% of its maximum value to 10% provides a quantitative measure for the size of the interface. The water densities in g/cm^3 are displayed in Figure 3. From this figure we obtained interfacial thicknesses of 9.2 and 10.1 Å for small

and large DLPE systems, respectively. Note here that for the DPPC/water systems with similar amounts of water the interfacial thicknesses are 10.9 and 12.2 Å. We also observed from the water density in the large DLPE system that the water molecules in the middle of the water lamina

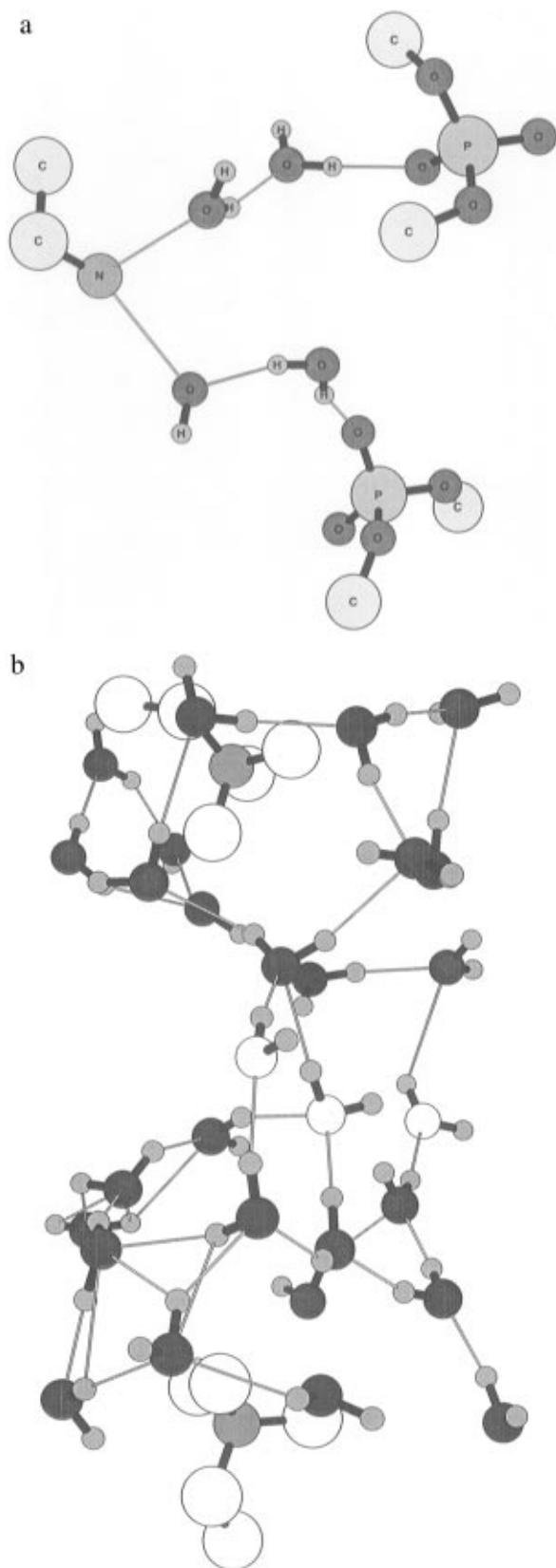


Figure 5. (a) Snapshot showing water bridges between opposing headgroups from the small DLPE system. (b) Snapshot from the large DPPC system depicting the clathrate cages around $\text{N}(\text{CH}_3)_3$ groups from two lipid headgroups from opposing bilayers. Water molecules which bridge clathrate cages are shown with white oxygen centers.

retain the density of 1 g/cm^3 for a segment of about 10 \AA . In contrast, for the DPPC counterpart the density is yet to attain its bulk value in the middle region.

The radial distribution functions (rdf) of the water around N atoms can be used to study the solvation behavior of the NH_3 groups. Our rdfs are similar to the ones obtained previously by Damodaran and Merz.^{22,23} Since the NH_3 groups are positively charged and the size of the groups is relatively small, the water oxygen centers are closer to the amine group of the membrane molecules than the hydrogen centers. No prominent water shells beyond the first solvation shell of the NH_3 groups are observed. This behavior is different from the solvation of choline headgroups where water molecules create a clathrate-like structure around the positively charged $\text{N}(\text{CH}_3)_3$ groups.^{22,23} We have calculated the number of waters/lipid required to solvate the polar headgroups in each system (solvation number) by counting the number of water molecules that do not fall into the first peak of the rdfs of any lipid atom and subtracted that from the total number of water molecules. In the calculations for the DPPC membranes we considered each $\text{N}(\text{CH}_3)_3$ group as one entity. On the average nine waters/lipid were found in the first solvation shell of both DLPE systems. This number corresponds closely to the hydration limit of $8.8\text{--}10.3$ for the DLPE system.^{14–16} For the large DPPC system which is close to its full hydration limit of 23 waters/lipid,¹⁴ we found 15.5 waters/lipid in the first solvation shell.

As discussed in the previous work,¹³ the smooth polarization profiles of water arise from the rough interface. Even though the headgroup in PE is less bulky compared to that of PC membranes, it could still create a rough interface thus giving rise to a smooth water polarization profile. We calculated the orientational polarization profiles for both DLPE systems and present them in Figure 4 with the profiles of DPPC systems. Comparing Figure 2 and Figure 4, we observe no polarization of water beyond one layer of the furthest extension of nitrogen atoms in the larger DLPE system with excess water. The extra water region in the center of the bilayer displays bulklike properties and therefore attractive forces will expel this water and maintain the waters that are needed for hydration.

How can our molecular dynamics simulations explain the difference in the hydration force observed for membranes with PE and PC headgroups? Let us start with the previously mentioned observation that the first hydration shell of a DLPE headgroup contains only 9 water molecules, while the shell around a DPPC headgroup contains 15.5 water molecules. This is mostly due to the difference in the size of the headgroups as well as the large area per headgroup in DPPC membranes. In the limit of hydration, DLPE membranes contain around $9\text{--}10$ waters/lipid,^{14–16} while DPPC membranes contain around 23 waters/lipid,¹⁴ showing that both DLPE and DPPC membranes have their hydration shells completed. While DPPC membranes contain ~ 7 additional waters/headgroup, DLPE membranes have only one extra water/headgroup. That means that in the lamellar phase PE membranes are separated on the average by just two water layers, whereas PC membranes on the average contain more than two water layers. A possible explanation that accounts for this difference has its origin in the different solvent structures around PE and PC headgroups. In PE, water molecules can create hydrogen-bonded bridges that connect positively charged NH_3 groups with negatively charged oxygens attached to phosphorus. These water bridges serve as a "glue" that keeps opposing membrane surfaces together. The existence of bridging waters has

(22) Damodaran, K. V.; Merz, K. M. *Langmuir* **1993**, *9*, 1179.

(23) Damodaran, K. V.; Merz, K. M. *Biophys. J.* **1994**, *66*, 1076.

been proposed previously^{15,24} and we can directly observe them in the simulations. (See for example Figure 5a depicting some of such water bridges.)

To analyze the number of hydrogen bonds in the DLPE system at the hydration limit, we used purely geometric criteria. Following the definition used by Luzar and Chandler,^{25,26} we define two water molecules to be hydrogen bonded if the oxygen–oxygen distance is less than 3.4 Å and the angle between the O–O connecting vector and one of the O–H bonds is less than 30°. The same definition was used for a pair involving an oxygen of a phosphate group and a water molecule. For a nitrogen–water pair this definition is not possible and therefore we called all nitrogen–water pairs to be hydrogen bonded if the water molecule belongs to the first hydration shell, i.e., if the N–O distance is less than 4 Å. Even though these criteria are arbitrary, they provide some hints to the relative importance of the number of hydrogen bonds. With the above criteria, we found that on average a lipid forms an 0.8 hydrogen bond bridge to the opposing membrane via one water molecule: 61% of these bridges are of the form amine group–water–phosphate bridge or phosphate–water–amine group bridge; 30% of these bridges are of the form amine group–water–amine group; 9% are of the form phosphate–water–phosphate. In addition we also counted how many hydrogen bond bridges are formed involving two water molecules. In this case we find that on average 1.5 bridges/lipid of the types amine group–water–water–phosphate or phosphate–water–water–amine group are formed. With a frequency of 0.9 bridge/lipid we find bridges of the type amine group–water–water–amine group and with a frequency of 0.26 bridge of the type phosphate–water–water–phosphate, amounting to a total of 2.7 bridges/lipid.

In PC, the first solvation shells of the $\text{N}(\text{CH}_3)_3$ groups have a clathrate-like structure. The opposing clathrates will not hydrogen bond effectively, therefore one needs extra water molecules between them (see Figure 5b). However, the difference between the solvation number and the true hydration limit is about 7 waters/lipid in the DPPC case and corresponds to only two water layers. A substantial fraction of those waters could also be accom-

modated in voids within the very rough membrane surfaces. Other evidence for the existence of more than two water layers between DPPC membranes stems from the comparison of the number of water molecules in the gel phase DPPC system¹³ and the small lc DLPE system, both at the limit of hydration. These systems have similar areas per headgroup (47 vs 50.4 Å) and also the same solvation numbers (9 waters/lipid). However, in DLPE, the hydration limit is reached with 9–10 waters/lipid, whereas DPPC can imbibe 4 more waters/lipid showing that the clathrate structures around the DPPC headgroups are separated by additional waters.

For both PE and PC membranes, removal of water from the solvation layers between opposing membrane surfaces costs free energy and, therefore, contributes to the short range repulsive force. On further dehydration, the very rough nature of the membrane surfaces brings the headgroups from opposing membrane surfaces into close contact. Direct interactions may be present even if the definition of the water slab d_f from the electron density profile still indicates a substantial thickness. Due to the roughness of these surfaces, individual pairs of lipid molecules approach each other and can make close contact even at earlier stages of dehydration.

Both the experiment and our simulations indicate that it is extremely hard to separate the contributions of solvent and steric interactions to the total repulsive force. However, the nature of this force has become clearer. In the picture that emerged from the current simulations, the hydration force is due both to the removal of one or two layers of the solvating water (from each side of the membrane) and to steric interactions of the headgroups. The simulations also revealed that the difference in the structure of the solvation shell is responsible for the difference in the range of the hydration force of PE vs PC membranes.

Acknowledgment. We acknowledge Dr. T. A. Darden at NIEHS for providing the PME code, Professor T. J. McIntosh at Duke University for valuable discussions and for providing the experimental electron density data, and Ms. L. S. Sremaniak and Dr. K. Schweighofer for careful reading of the manuscript. The simulations were performed at the North Carolina Supercomputer Center. This work was supported by the Office of Naval Research.

LA9515534

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

(25) Luzar, A.; Chandler, D. *Nature* **1996**, *379*, 55.

(26) Luzar, A.; Chandler, D. *Phys. Rev. Lett.* **1996**, *76*, 928.