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Density and bonding profiles of interbilayer water as functions of bilayer separation: a Monte Carlo study

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We have used the Monte Carlo method to calculate the equilibrium properties of water between two interfaces consisting of phosphatidylcholine (PC) headgroups. Using the TIPS2 optimized potential for water–water interactions, and 6–12 and coulombic potentials for water–PC interactions, we have determined the density, orientational, and hydrogen bonding profiles of the water as functions of the location of the water relative to the PC groups. We present here our results for several different studies in which we varied the interbilayer separation, the PC group configurations, and the interbilayer aqueous density. We find that very near each surface there is a layer of water strongly bound to the PC groups, and removing this water from the density profiles reveals a region of reduced water density extending 1–2 Å further into the interbilayer space. In addition when bilayers are very close together all the water is affected by the PC groups, as revealed in the hydrogen bonding profiles.

The surface of a lipid bilayer in aqueous solution must affect the thermophysical properties of the nearby solvent molecules. The detailed nature of this effect and the accompanying interfacial interactions are not known, but they are very important in many biophysical contexts including studies of interbilayer forces, lipid bilayer phase transitions, and cell–cell interactions. Unfortunately it is exceedingly difficult to probe the lipid/water interfacial region experimentally, and analytical theoretical models based upon statistical mechanics are not feasible. Computer studies offer a third approach to obtaining information on complex problems such as this. While all computer simulations suffer from well known deficiencies it is possible to calculate properties of *model* systems which may shed some light on the physical properties in question. For equilibrium studies the Monte Carlo method is an appropriate tool. This method calculates statistical mechanical averages numerically for models of the system under investigation. Because analytical means are not required the models may contain very complex interactions between molecules. This allows realistic theoretical models to be considered, and can provide insights into the manner by which microscopic intermolecular forces lead to certain macroscopic phenomena. The disadvantages are that one must use very small numbers of molecules, and statistical errors may be significant.

In previously published work [1,2] Monte Carlo calculations of several equilibrium properties of models of water molecules between phospholipid bilayers were described. By examining profiles of the aqueous density, average orientation, and hydrogen bonding it was determined that neutral phospholipid interfaces (consisting of phosphatidylcholine (PC) or phosphatidylethanolamine

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(PE), for example) induce changes in the water molecules within 2–4 Å, but the remaining water is basically bulk in nature. Only an unrealistic massive charging of the headgroups [2] led to a longer range dipolar ordering of the interbilayer water. The earlier studies were limited to a single value for the interbilayer separation, 24.5 Å, and a single aqueous density, 1 g/cm^3. In order to test the generality of the results we have now carried out calculations using different head group configurations and at varying interbilayer separations and aqueous densities. In the next section we describe the models used and the numerical method. The third section contains the results, and the final section is a discussion of these results.

Models and Methods

Fig. 1 shows a typical simulation cell as used in our calculations. Each cell contains exactly eight PC headgroups which are positioned in accordance with known configurations of anhydrous PC [3]. This is in contrast to the earlier work of Scott [1,2] in which the headgroups are aligned parallel to the interface plane. While the exact configuration of PC headgroups in bilayers in excess water are not precisely known it must certainly lie within the range considered, that is, between an all-parallel to the plane configuration and the 17°–27° tilt angles of the anhydrous state [3]. In Fig. 1 the 'sawtooth' arrangements of the PC groups in the two interfaces are in phase, so that, for example, choline groups in the different bilayers are directly adjacent. We have also considered arrangements in which the PC groups in one interface are shifted so that choline groups in this interface are adjacent to phosphate groups in the other interface. Starting from these basic configurations we have carried out Monte Carlo calculations at 298 K for various values of the following: (i) interbilayer separations between 6.9 Å and 21.9 Å (as measured between choline groups in each layer in Fig. 1, or between choline and phosphate groups which are closest in adjacent interfaces); (ii) aqueous density varying between 1.0–1.2 g/cm^3 (in order to examine possible changes in the water as two bilayers approach). In all cases periodic boundary conditions were imposed on the cell boundaries not containing the interfaces.

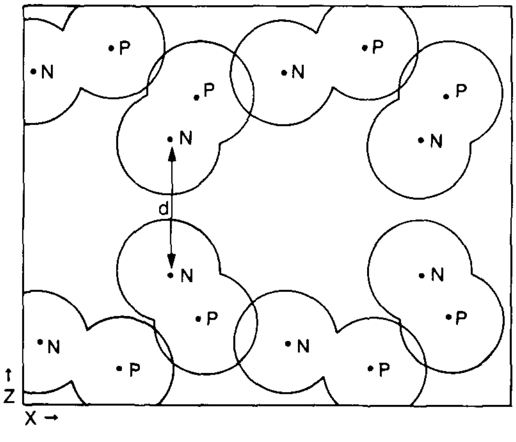


Fig. 1. A projection of the simulation cell on the x-z plane showing the orientation of the P-N dipoles as arranged in the sawtooth anhydrous configuration. The circles at the P and N positions represent Van der Waals spheres of radius 2.7 Å. The small d is the measure of the interbilayer separation used in the text.

The Monte Carlo algorithm employed is identical to that described earlier, as are the intermolecular potentials [1,2]. The water–water interaction used is the TIPS2 potential [4], which is optimized to reproduce many of the structural properties of bulk water including the pair correlation function, several thermodynamic properties, and the density maximum (although the exact location of this maximum is hard to determine numerically). The TIPS2 potential models a water molecule as three point charges and a center for a Lennard-Jones 6–12 potential [4]. The water–headgroup interactions consist of 6–12 potentials and Coulomb potentials between the water molecules and two charged spheres positioned at the choline and phosphate locations. The spheres have an effective charge of ±0.5 e and are also sources of a 6–12 interaction potential, as in earlier work [1,2]. This model for the headgroups is clearly very simple but it is our belief that the features of the molecular potentials responsible for the properties of the interbilayer water which we calculate are included. To model headgroups atom-by-atom, making each a source of 6–12 and/or electrostatic potentials, would require at least an order of magnitude increase in computer time, thereby leading to fewer Monte Carlo steps and less trustworthy results. This is also why headgroup motions and hydrocarbon chains are not explicitly included in the calculations. Doing the calculations for different headgroup configurations is a test of our hypothesis that changes in these configurations need not be explicitly included in each simulation. We have also carried out calculations for model PC groups in which the negative charge is smeared over two phosphate oxygens rather than localized, testing the localized charge assumption. Both tests show that density and bonding profiles are not significantly altered, indicating that headgroup motion and charge localization play secondary roles at least in specifying the properties of the water in the interbilayer region (although headgroup motion may play a larger role in chain packing). An important aspect of the models studied here is that no long range electrostatic corrections are applied to the interactions. All interactions are subject to a 10 Å cutoff using nearest images within the cutoff sphere. Our rationale for not including long range electrostatic effects is that in real systems there is no long range order in the headgroup region, and so no effects of such order should be included in the simulations. We believe that the model used here is reasonably accurate, yet is simple enough that one may obtain reliable Monte Carlo results using an IBM 3081K mainframe machine. Others have used Molecular Dynamics and Monte Carlo methods to study lipid bilayers [5,6] but the detailed profiles of water properties have to our knowledge been examined only by Marcelja and Kjellander [6], who simulate a different model for the interface than ours using Molecular Dynamics. Since an exact theoretical study of PC–water interfaces is not feasible it is important to examine several different model calculations to identify the true molecular properties. The models used in this work and in Ref. 6 are more accurate physically than models which reduce lipids and proteins to points or sets of points on a lattice [7]. At the other extreme, numerical simulation of a lipid bilayer with full interactions between all chain, headgroup, and water molecules is beyond the capabilities of present machines.

Generally systems were equilibrated through 10–15 K Monte Carlo steps per water molecule (representing 1.1–1.9 M total configurations, depending upon the size of the cell) with larger numbers of steps used for systems with smaller numbers of water molecules, and then averages were taken over a further 1.1–1.9 M configurations. Each simulation was begun with the water molecules distributed randomly in position and orientation (with no water molecule located too close to another water molecule or to one of the PC groups). The simulations were carried out in the same way as in Ref. 1, so that the runs in which averages were calculated were broken up into 3–6 sequential shorter runs, and the final results were calculated as averages over the intermediate results.

Statistical errors were estimated from the standard deviations in these data. In general errors were very close to those reported in Ref. 1, so only representative error bars are shown in Figs. 2b, 4, 5b, and 6b in this paper. The larger statistical fluctuations in the center of the cells in for widely separated layers means the profiles in this region may be smoothed out for larger systems and/or longer runs. This is also true for the polarization profiles at all separations. In all other cases the peaks and valleys are stable, although the heights and the exact locations of the extrema in the *centers* of the profiles vary in different runs. This suggests to us that the TIPS2 water in this region is not well layered or structured. The peaks and valleys in the profiles nearest each interface do stand out well beyond the estimated errors for this region, indicating these are *not* due to statistical fluctuations or finite size effects. As a further check identical simulations were performed for some of the models studied using different random number sequences, different numbers of steps, and/or different initial configurations. In all cases considered the results of the extra runs were within the error limits for the reported runs, indicating that the systems were well equilibrated and stable. In calculating the profiles the height of the simulation cell along the z axis was divided into layers of equal width, which were used as bins in the profile counting procedure. For consistency, all bin widths in the reported results are set at 1.0 Å. Smaller bin sizes led to greater uncertainty in the counting.

Results

(i) Density profiles

Fig. 2 shows density profiles for varying interbilayer separations. The profiles all rise to an initial peak which is followed by a minimum and a series of peaks and valleys. Ideally profiles should be symmetric, and the lack of symmetry is due to the statistical error in the results. As discussed above it is likely that many of the peaks and valleys in the central regions of the simulation cells would be smoothed out if much larger systems could be studied, but the overall shapes of all the plots including the peaks closest to the interfaces are not likely to be affected by system size. Fig. 2a shows that the density profiles are not qualitatively altered as the interbilayer spacing is changed at constant aqueous density of 1 g/cm^3. Also shown on this figure is a profile calculated after moving the top headgroups in Fig. 1 by 6 Å laterally to align cholines in one interface with phosphates in the other. This displacement should most strongly affect the profiles at the smallest interbilayer separation. It is evident from the figure that outside of the statistical fluctuations in the middle region of the cell the profiles retain the same overall appearance. This suggests that the relative positions of the PC groups in opposing interfaces has a fairly small influence on the interbilayer water. Fig. 2b shows the estimated statistical errors for one of the profiles in Fig. 2a. As in Ref. 1 the fluctuations are largest in the central part of the simulation cell and considerably smaller within 4–5 Å of each interface. These errors are typical of all calculated density profiles. All profiles in Fig. 2 were calculated using the same water density of 1 g/cm^3. Fig. 3 shows profiles in which the interbilayer separation was reduced from 21.9 Å without changing the total number of molecules in the cell, thus increasing the density. At the highest density, 1.22 g/cm^3, we observe a rounding of the peaks closest to the interfaces and a smoother profile in the middle of the cell, indicating a more random positioning of the molecules. This is of course a very unrealistic density. At 1.1 g/cm^3 the profile appears very similar to that of 1 g/cm^3 water.

(ii). Orientation profiles

The one property of the interbilayer water which should be most strongly affected by the PC headgroup orientations is the molecular orientation of those waters nearest the interfaces. Fig. 4 shows a molecular dipole orientation profile for the component of the molecular dipolar moment parallel to the component of the PC dipole vector in the interface plane (the x axis) at the closest interfacial separation; 6.9 Å. Fig. 5a shows similar

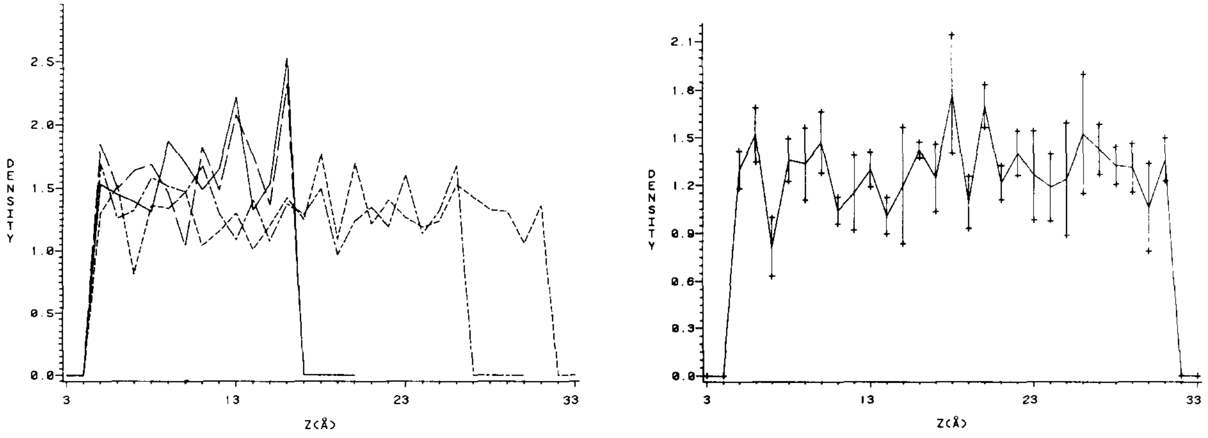
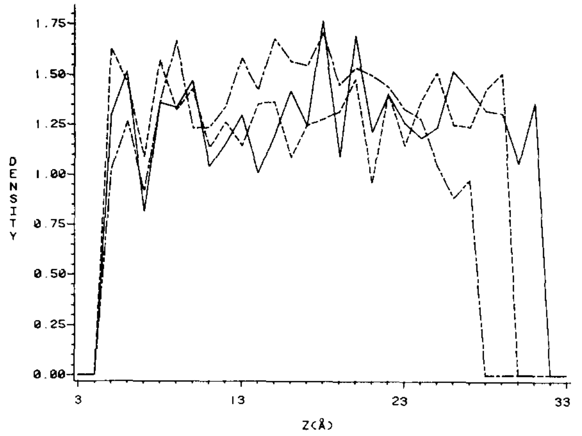


Fig. 2. (a) Density profiles for interbilayer water at aqueous density 1 g/cm^3 as a function of z, the coordinate perpendicular to the PC interfaces, for $d = 6.9$ Å (solid line); $d = 6.9$ Å with top headgroups laterally displaced by 6 Å (wide-dashed line); $d = 16.9$ Å (dot-dashed line); and $d = 21.9$ Å (narrow-dashed line). At 1 g/cm^3 the cell with $d = 6.9$ Å contains 73 TIPS2 molecules, the cell with $d = 16.9$ Å contains 152 TIPS2 molecules, and the cell with $d = 21.9$ Å contains 191 TIPS2 molecules. (b) Same plot as narrow-dashed line in Fig. 2a, but with error bars shown.



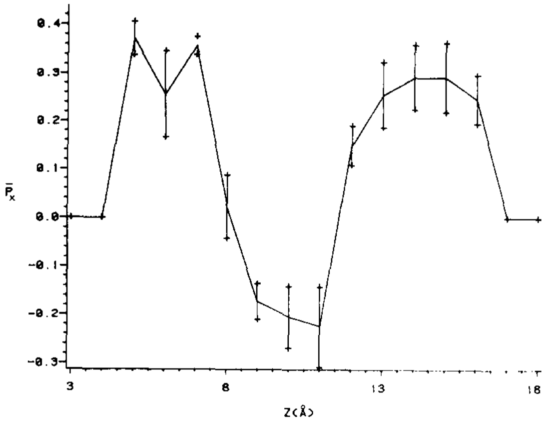


Fig. 3. Density profiles of water at different densities; 1 g/cm^3 (solid line); 1.1 g/cm^3 (dashed line); 1.22 g/cm^3 (dot-dashed line). All data are for cells with 191 TIPS2 molecules, but with $d = 21.9$, 19.9, and 17.9 Å, respectively.

Fig. 4. Profile of the average x-component of the TIPS2 molecular dipole vector, $\bar{P}_x(z)$ for $d = 6.9$ Å, and density 1 g/cm^3.

profiles for larger separations and at varying densities. These profiles are all substantially different from Fig. 3 of Ref. 1 in which the PC headgroups were aligned parallel to the interfaces. At the larger separations the induced polarization falls off very rapidly while at the closest separation the decay is, as expected, slower. From Fig. 5 we also conclude that increased aqueous density does not strongly affect the magnitude of the induced polarization. Error bars shown in Fig. 4 and Fig. 5b point out that fluctuations are greater for the orientation profiles than for the density profiles. We have also calculated the averages of $P_2(\cos\theta)$ where θ is the angle between the molecular dipole axis and the tumbling axis. These data are not shown but they indicate rapid tumbling about the z axis (perpendicular to the interfaces) and restricted rotational motion in the plane of the interfaces. The results are consistent with earlier calculations [1,2] so that headgroup configuration does not seem to affect this motion.

(iii). Hydrogen bonding profiles

The property which we have found to be most revealing of the water–interface interactions is the average number of hydrogen bonds per molecule,

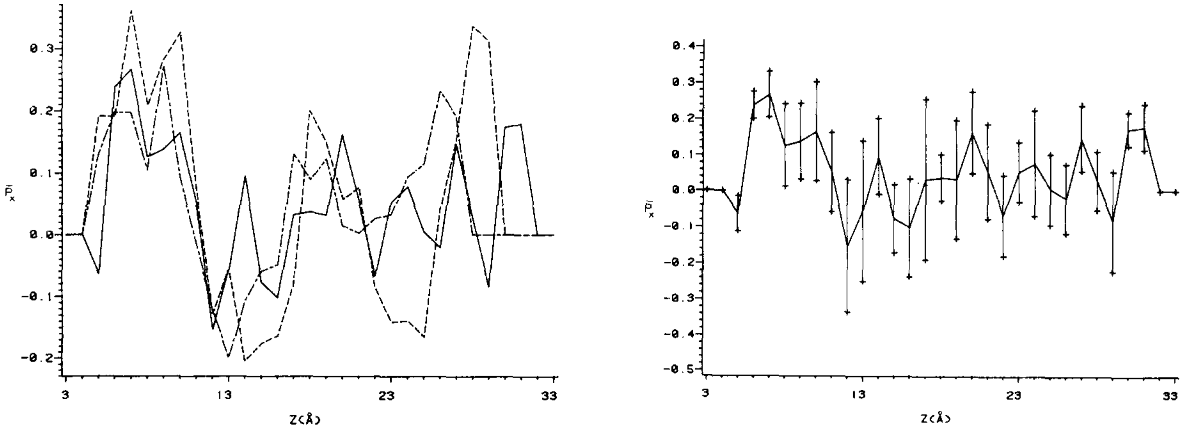


Fig. 5. (a) Profile of $\bar{P}_x(z)$ for varying aqueous densities; 1 g/cm^3 (solid line); 1.1 g/cm^3 (dashed line); 1.22 g/cm^3 (dot-dashed line). Cell parameters are as in Fig. 3. (B) Same plot as solid line in Fig. 5a, but with error bars shown.

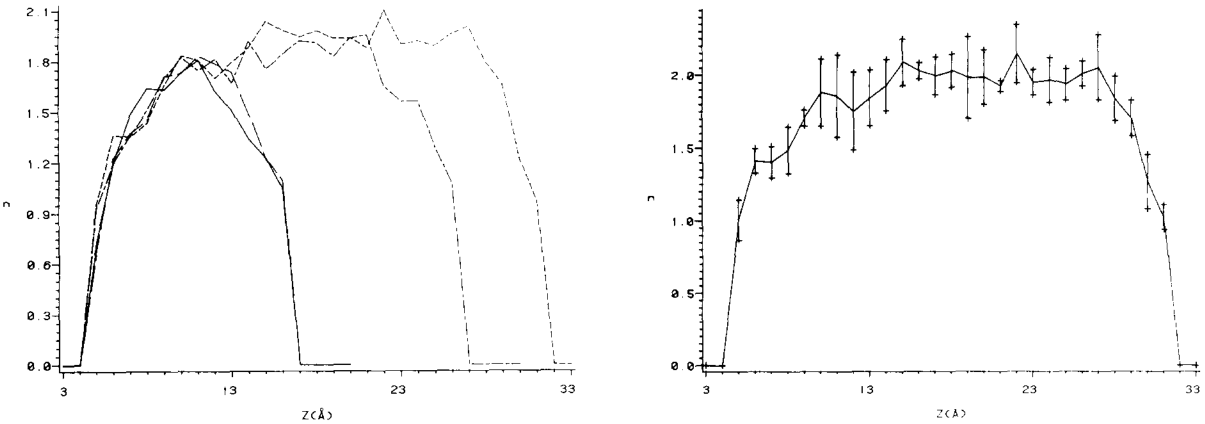


Fig. 6. (a) Profiles of n, the average number of hydrogen bonds per molecule at density 1 g/cm^3 for varying separations; $d = 6.9$ Å (solid line); $d = 6.9$ Å with top headgroups displaced laterally by 6 Å (wide-dashed line); $d = 16.9$ Å (dot-dashed line); $d = 21.9$ Å (narrow-dashed line). Cell parameters are as in Fig. 2. (b) Same plot as narrow dashed line in Fig. 6a, but with error bars shown.

where we define a hydrogen bond as an interaction energy of -4 kcal/mol or less between pairs of molecules [8]. Fig. 6a shows hydrogen bonding profiles for three different interbilayer separations all at aqueous density of 1 g/cm^3. At the closest separation the rise from the interface to the peak is slower and the height of the peak is slightly lowered compared to the larger distances in which the profiles rise rapidly to values similar to those of bulk water [2,8]. Also shown in this figure is a profile at 6.9 Å separation in which the PC groups are translated laterally in the top interface as in Fig. 3. This translation clearly has little effect on the average bonding patterns, apart from a shift in the assymmetry. Fig. 6b contains a typical hydrogen bonding profile with error bars included, and it is evident that the fluctuations in these data are considerably smaller than in the density and orientation profiles. Fig. 7 shows the effect of increased density on the hydrogen bonding profiles. There is a gradual but distinct lowering of the average number of hydrogen bonds at all points on the profiles as the density increases which must be due to increased packing constraints at the larger densities.

The data become more meaningful if strong interactions between water and PC groups are monitored at the same time as the inter-water hydrogen bonds. Since headgroup–water interactions need not be as strong as water–water interactions we have constructed separate density profiles in which we remove all water molecules bonded to head groups with energies less than -4.0, -3.0, and -2.0 kcal/mol, respectively. The results for $d = 21.9$ Å are shown in Fig. 8 and the results for $d = 6.9$ Å are given in Fig. 9. For the larger separations the sharp peaks in the density profiles nearest each interface now consist entirely of water bound to PC groups. This is not surprising, but it is somewhat surprising to see that a substantial fraction of the water molecules which comprise the second density peak at each interface are also bound to the PC groups. When these are subtracted away the density of free water

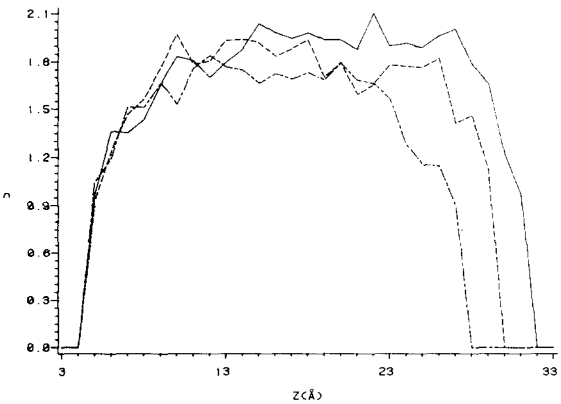
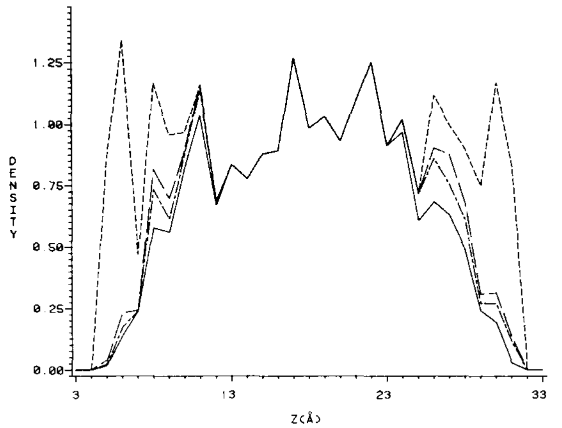


Fig. 7. Hydrogen bonding profiles for water molecules at varying densities; 1 g/cm^3 (solid line); 1.1 g/cm^3 (dashed line); 1.22 g/cm^3 (dot-dashed line). Cell parameters are as on Fig. 3.



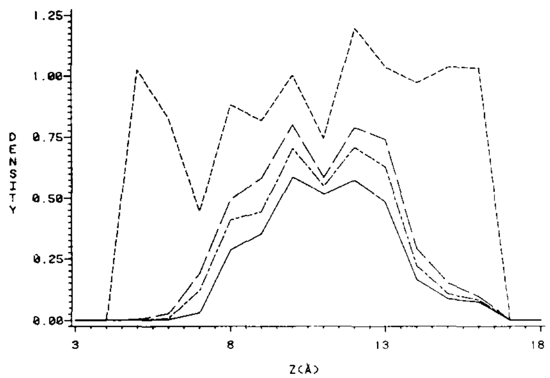


Fig. 8. Density profiles at overall density 1 g/cm^3 and $d = 21.9$ Å with all water molecules removed which have interaction energies with a PC group less than −2.0 kcal/mol (solid line); −3.0 kcal/mol (dot-dashed line); −4.0 kcal/mol (wide-dashed line). The narrow-dashed line is the density profile for $d = 21.9$ Å with no molecules subtracted out.

Fig. 9. Same as Fig. 8 with $d = 6.9$ Å.

is reduced below the bulk value for a distance of 1–2 Å from each interface. At smaller interbilayer separation this reduction in unbound water density is even greater, as the head groups are now very close. It is important to point out that there is free water present in the reduced density regions but its density is less because of the bound water in these regions.

Discussion

We have studied a model system consisting of phosphatidylcholine headgroups and water, with the goal of learning more about the states of the water molecules at close interbilayer distances and with the PC groups in configurations corresponding to the anhydrous state. We find that the anhydrous orientation of the PC has little effect on density profiles when compared to Ref. 2, but the average water dipolar orientation near the surfaces is altered in direction, reduced in magnitude, and decays less rapidly when the interbilayer distance decreases, compared to the data of Refs. 1 and 2. Surprisingly this makes little difference in the other properties studied. Subtraction of the water molecules bound to PC groups from the density profiles allows differentiation between the free and bound water. We find a region parallel to the bilayer plane and extending from about 1 to 3 Å beyond the center of the choline groups which are most extended in the anhydrous state (see Fig. 1) in which there is a mixture of bound and bulk water. The bound water is for the most part attached to the protruding cholines, but is otherwise surrounded by free water. This is almost certainly a consequence of the anhydrous configurations used in the calculations. In the earlier calculations of Scott [2] in which the PC were forced to align parallel to the bilayer plane hydrogen bond profiles rose more sharply to the bulk water value, as all bound water was in a narrower region near the surfaces. Allowance for headgroup motions should produce hydrogen bonding profiles which are intermediate between the earlier work [2] and the present data.

Experimental work related to the present simulations include the interbilyaer force measurements [9,10] and studies of molecular volumes [11] in bilayers. It is not possible in this type of Monte Carlo study to calculate the interbilayer force directly, but we can examine the effect of decreasing interbilayer distance on the water molecules for clues pertaining to the molecular mechanisms involved. At larger separations (21.9 and 16.9 Å) the density, orientational, and bonding profiles are qualitatively identical, indicating little change other than displacement of water has occurred. At the closest separation, 6.9 Å, the Van der Waals spheres of the protruding choline groups may be only 1–2 Å apart, and the interbilayer water is now much more strongly ordered orientationally, and interacts more strongly with the PC groups. This water should now be much harder to displace than the bulk water so that further decreases in interbilayer distance will require larger force to be applied.

The experimental conclusions of White and King [11] may also be examined in the light of the data presented here. They find that molecular volumes for PC headgroups rise linearly with hydration levels with a change in the slope as the level passes 100% hydration. If the change in volume were due to more water molecules associated with the PC groups this would affect the hydrogen bonding profiles even at large interbilayer distances. As was described above the bonding profiles change only when the interfaces are very close, and the profiles at $d = 16.9$ Å and $d = 21.9$ Å are very similar. What seems more likely is that as one enters the excess water region headgroups gain some limited conformational freedom and this is not considered in this work.

Finally we point out again that the models studied here are not exact replicas of actual PC groups in water. However, they contain many of the complex 6–12 and coulombic interactions characteristic of the real systems, and our tests of several of the assumptions regarding conformation and charge localizatioin of the head groups suggest that we have included enough detail to obtain accurate bonding and density profiles. The orientational profiles are more sensitive to PC configurations. Since it is impossible to perform analytical statistical mechanical calculations on models even as complex as those considered here it is important to do Monte Carlo and Molecular Dynamics calculations on models to gain better insights into how the electrostatic and Lennard-Jones forces produce observed patterns of molecular behavior. This then allows one to begin to construct useful analytical theories.

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