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Connectivity of hydrogen bonds in water between lecithin bilayers

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Experimental studies revealed that liquid water bound between lecithin bilayers becomes increasingly viscous with decreasing interbilayer distance. Percolation calculations do indeed show that cluster formation and thus viscosity are favored with decreasing interbilayer distance.

INTRODUCTION

Lecithin bilayers represent models of the biological membrane. It has been found that at least two water molecules per lecithin molecule are necessary for the formation of the lamellar structure. The amount of water between adjacent bilayers may reach up to 45 H_2O molecules per lecithin molecule.[1,2] Various experimental studies have been carried out in order to elucidate the properties of this so-called bound water and its influence on the phase behavior of lipid membranes, for example.[3] Eventually, it was shown that the bound water is more viscous than free water, by measuring the work of removing water from the multilayer lattice formed in water by lecithin[1] and by measuring the dielectric relaxation of the bound water.[2] The work of removal is a continuous function of interbilayer water content and hence of lattice repeat spacing[1] and the same dependence is valid for the dipole relaxation frequency of the bound water.[2] Data of the difference in chemical potential between free and bound water are shown in Fig. 1. They are representative for the lecithins di-myristoyl-phosphatidylcholine (DMPC) (deduced from dielectric experiment) and di-palmitoyl-phosphatidylcholine (DPPC) (deduced from pressure experiments) at temperatures below their main phase transition. At this transition the alcyl chains change their low-temperature conformation from an ordered all-*trans* state to a disordered fluidlike phase, where the chains undergo correlated rapid isomerization rotations between excited *gauche* conformations.[4]

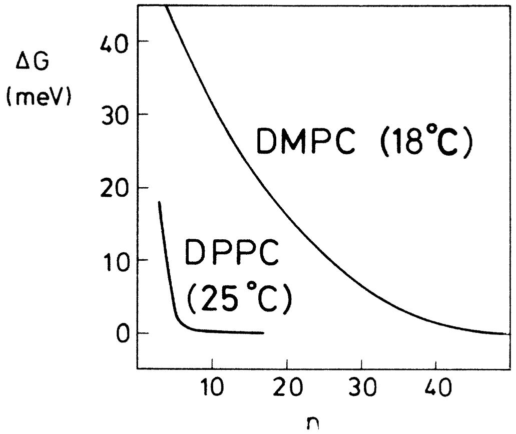

FIG. 1. Increase in chemical potential of water molecules between phospholipid bilayers as a function of hydration. *n* is the number of water molecules per lecithin molecule. Data are deduced from dielectric relaxation for DMPC at 18 °C and pressure experiments for DPPC at 25 °C.

Both experimental methods yielded similar results, an increasing potential with decreasing water content. We interpret this behavior[2,3] as a decrease in entropy due to a higher conformational degree of order of hydrogen-bonded H_2O molecules at the lecithin bilayer surface. This higher order is observed for water concentrations up to 45 H_2O molecules per lecithin molecule in the fluid bilayer phase. The difference in chemical potential ΔG is given by

$$\Delta G = \Delta H - T\Delta S \ , \tag{1}$$

where ΔH is the change of enthalpy, T the absolute temperature, and ΔS the change of entropy.

The hydrogen-bond strength in free water was measured to be $H = 130$ meV.[5] We assume that this does not change for the interlamellar bound water except for the two or three H_2O molecules forming a hydrogen bond to the lecithin head group. The latter bonds are characterized by a higher probability to be intact than the hydrogen bond between two water molecules. Consequently, the observed change in chemical potential ΔG between that of free water and that of bound water is related to a change of entropy ΔS due to a higher conformational order of the interbilayer bound water. In the following, we report on percolation calculations which confirm this model of bound water between lecithin bilayers.

MODEL

We present a model based on correlated site percolation in a simple cubic lattice. It models the behavior of water in lecithin bilayers.

The model is built up in such a way that we get results for free water similar to those of Blumberg, Stanley, Geiger, and Mausbach[6] obtained with their molecular dynamics calculations and with calculations based on an ice lattice.

On an average each H_2O molecule has four intact H bonds to its neighbors. But in the cubic lattice each site has six neighbors. An occupied site at (x,y,z) is interpreted as a H_2O molecule with four intact H bonds if the sites $(x+1,y,z)$, $(x-1,y,z)$, $(x,y,z+1)$, and $(x,y,z-1)$ are occupied, too. Using this definition we have obtained the best numerical agreement with the results of Blumberg *et al.*[6] There were empirical but no theoretical reasons to use this definition. For free water we got the same results using sites in the y direction instead of in the z direction. Because of the boundary conditions we have chosen the z direction. To simulate the bound water between lecithin bilayers the first and last water layer in the z direction were assumed to be fixed to the lecithin molecules. Thus the sites of the first and last water layer were completely occupied.

We investigated the behavior of the clusters, built of next-neighbor water molecules, with decreasing bilayer thickness, as a function of the probability p for an intact H bond. For that purpose we have calculated the weight function of the cluster size, $W_s(p)$, and the second moment of the cluster size distribution without the biggest cluster, $C(p)$. $W_s(p)$ is calculated from

$$W_s(p) = sn_s(p) \ , \qquad (2)$$

where s is the number of water molecules in a cluster and n_s is the number of clusters of size s per occupied site. The percolation threshold is determined from the maximum of $C(p)$; further simulation methods are given elsewhere.[7,8] We suggest that one interprets the percolation threshold as the phase transition from the fluid to the ice phase of the water between lecithin bilayers. The transition to thin layers is realized by a decrease of the "thickness" of the z plane. The bonding of the water molecules to the lecithin bilayers is simulated by occupation of all border sites. Thus the border is built up from a network of water molecules with intact H bonds. We can interpret this as ice.

PERCOLATION RESULTS

The results for free water, which are compared with the calculations for bound water, were found in a system of size $L_x = L_y = L_z = 60$. For the examination of the thin layers we have enlarged the x,y extension up to $L_x = L_y = 120$ sites and lowered the z extension from $L_z = 20$ to $L_z = 4$. This corresponds to a layer thickness from 18 to 2 H_2O molecules.

We have found that with decreasing thickness of the layer the increase of the number of clusters with more than two sites is stronger than the increase of the water molecules which have no or only one water neighbor (see Fig. 2). Thus one can say that the system changes to higher order with decreasing thickness of the layer. This behavior can be interpreted as a decrease of the conformational entropy. With decreasing layer thickness the water in the lecithin bilayers becomes more icelike and thus more viscous. A rather strong influence of the boundary conditions up to a thickness of 12 molecules is found. For more than 18 molecules between the layers the influence of the boundary becomes negligible.

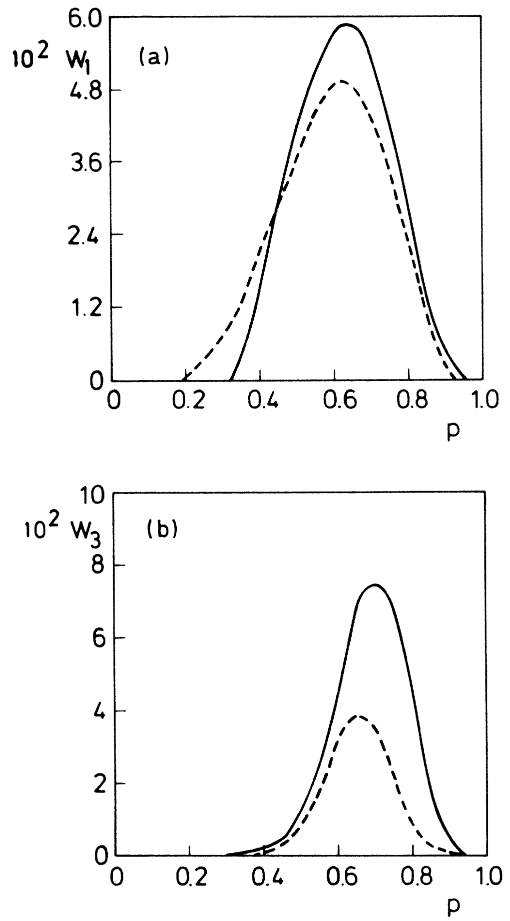

FIG. 2. (a) Weight function W_1 of water monomers for free water (dashed line) ($L_x = 60$, $L_z = 60$) and for bound water with two water molecules per lecithin molecule (solid line) ($L_x = 120$, $L_z = 4$) as a function of the probability for intact H bonds. (b) Weight function W_3 including the clusters of from four up to seven water molecules for free water (dashed line) ($L_x = 60$, $L_z = 60$) and for bound water with two water molecules per lecithin molecule (solid line) ($L_x = 120$, $L_z = 4$) as a function of the probability p for intact H bonds.

CONCLUSION

Experimental studies on the properties of water bound between lecithin bilayers have yielded a higher chemical potential[1,2] and a stronger viscosity[2] (i.e., a longer electric dipole relaxation time of the bound water) compared with free water. We interpreted this behavior as due to a higher conformational order of water bound between lecithin bilayers than in free water. Percolation calculations indeed have now confirmed this model. The calculations show an increase of water cluster size with decreasing interbilayer distance. The distance having an effect on the water configuration is in good agreement with that found in experimental studies.[1,2,9]

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[1] L. J. Lis, M. MacAlister, N. Fuller, R. P. Rand, and V. A. Parsegian, Biophys. J. **37**, 657 (1982).

[2] A. Enders and G. Nimtz, Ber. Bunsenges. Phys. Chem. **88**, 512 (1984).

[3] G. Nimtz, A. Enders, and B. Binggeli, Ber. Bunsenges. Phys. Chem. **89**, 842 (1985).

[4] A. Enders and G. Nimtz, Phys. Rev. A **32**, 2521 (1985).

[5] C. J. Montrose, J. A. Bucarno, J. Marshall-Coakley, and T. A. Litovitz, J. Chem. Phys. **60**, 5025 (1974).

[6] R. Blumberg, H. E. Stanley, A. Geiger, and P. Mausbach, J. Chem. Phys. **80**, 5230 (1984).

[7] W. Weiss, Staatsexamensarbeit, Cologne University, 1985 (unpublished).

[8] D. Stauffer, *Introduction to Percolation Theory* (Taylor and Francis, London, 1985).

[9] M. Grünert, L. Börngen, and G. Nimtz, Ber. Bunsenges. Phys. Chem. **88**, 608 (1984).