

THE FREE ENERGY, ENTHALPY, AND ENTROPY OF HYDRATION OF PHOSPHOLIPID BILAYER MEMBRANES AND THEIR DEPENDENCE ON THE INTERFACIAL SEPARATION

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The phenomenological theory of water binding to non-charged phospholipid bilayer membranes is presented and the free energy, enthalpy, and entropy of hydration are calculated as a function of interfacial separation. These estimated hydration quantities agree with our experimental data provided the interfacial effects are described in terms of the surface water-orienting field of constant strength.

It is now agreed that the structuring of water plays an important role in determining the properties and behaviour of aqueous solutions of inorganic and organic compounds (ions, biomolecules, membranes, cell aggregates) [1]. But whilst the hydration of simple molecules has been experimentally well explored and can be described within the framework of standard quantum chemical physics, hardly any relevant experimental data [2,3] and hardly any suitable theories of hydration of larger, more complex systems have been available. Here we report results of the first measurements of the enthalpy, entropy and free energy of hydration of multibilayer membranes (from the non-charged phospholipid phosphatidylethanolamine) as a function of the interfacial separation down to 0.2 nm, i.e. to the lipid monohydrate. We describe a simple phenomenological theory of the water interactions with a polar or non-polar interface which bears no net charge, based on the concept of the interfacial water-orienting fields and show that such a model provides a correct description for the interfacial water binding beginning with the mono-

hydrate. Within the framework of this theory, we derive expressions for the free energy, enthalpy, and entropy of hydration of two dissimilar, opposing interfaces as a function of their separation. We also determine the right boundary condition for the water order induced by the presence of neutral interfaces. The excellent agreement between our calorimetric or X-ray data and the theoretical predictions indicates that the model is adequate for describing both the microscopic and macroscopic bilayer hydration parameters down to the smallest experimentally achievable interfacial separations.

The enthalpy and entropy of hydration, H^{hyd} and S^{hyd} , were determined calorimetrically as a function of the water content in multilayer dispersions of the non-charged lipid, phosphatidylethanolamine. The inter-bilayer separation d was estimated from the relative water concentration by using the lipid molecular areas (determined separately in X-ray experiments) and the molar volume of water. The free energy of hydration, G^{hyd} , was calculated from H^{hyd} and S^{hyd} and is thus subject to somewhat larger experi-

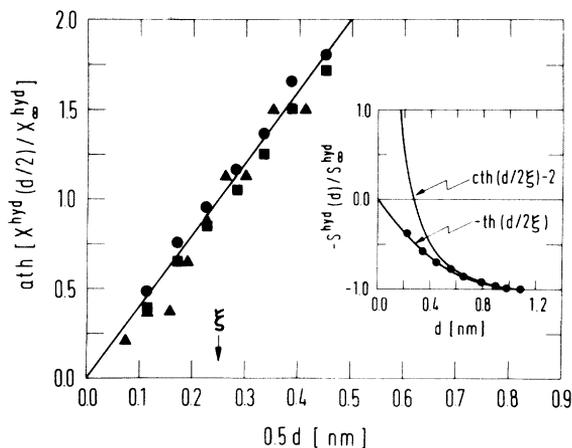


Fig. 1. The inverse hyperbolic tangent of the calorimetrically determined values of the enthalpy ($X = H$, ●), entropy ($X = S$, ■) and free energy ($X = G$, ▲) of hydration of multibilayer phosphatidylethanolamine dispersions in water relative to the values of these quantities at maximal hydration, i.e. at maximal interbilayer separation, (H_{∞}^{hyd} , S_{∞}^{hyd} , G_{∞}^{hyd}), and as a function of the half-distance between the interfaces, $0.5d$. The solid line denotes the calculated dependence according to the present theory and ξ the water-order correlation length. The insert shows the corresponding experimental ratio $S^{\text{hyd}}(d)/S_{\infty}^{\text{hyd}}$ as a function of the total interbilayer separation d , together with the calculated dependence assuming a fixed value of the surface water polarization ($\text{cth}(d/2\xi)-2$) or a fixed value of the surface orienting field strength ($-\text{th}(d/2\xi)$) and $\xi = 0.26$ nm. $G^{\text{hyd}}(d)/G_{\infty}^{\text{hyd}}$ and $H^{\text{hyd}}(d)/H_{\infty}^{\text{hyd}}$ also behave similarly.

mental errors. The details of these experiments will be given elsewhere [4].

Our main interest was to study the dependence of the thermodynamic membrane hydration parameters on the interbilayer separation. In excess water, when the molar water/lipid ratio ≥ 9 , $S^{\text{hyd}}(d)$, $H^{\text{hyd}}(d)$, and $G^{\text{hyd}}(d)$, as well as the interlamellar water layer thickness, $d = d_{\infty}$, cease to depend on the water concentration. In the insert to fig. 1 we present the inverse ratio of the absolute value of $S^{\text{hyd}}(d_{\infty}) = S_{\infty}^{\text{hyd}}$ (H_{∞}^{hyd} and G_{∞}^{hyd} behave similarly) and the corresponding quantities measured for $d < d_{\infty}$. These data indicate that whenever $d \rightarrow 0$, $H^{\text{hyd}}(d)$, $S^{\text{hyd}}(d)$, and $G^{\text{hyd}}(d)$ tend to zero approximately as the hyperbolic tangent. In fig. 1 we have therefore also plotted $\text{ath}(X^{\text{hyd}}(d/2)/X_{\infty}^{\text{hyd}})$, where $X = S, H, G$ and found that all the experimental points fall roughly onto one straight line which goes through the origin and has slope $\xi = 0.26$ (1 ± 0.13) nm.

The only theory of membrane hydration proposed so far is due to Marčelja and co-workers. These authors assumed that the water structuring caused by an interface can be described by an average water order parameter which they have shown to fall-off as the hyperbolic sine with the distance from the interface. By using the fixed value of this parameter at the surface as a free parameter they successfully explained the nature of the water-mediated interlamellar repulsion [5,6]. However, because of the assumption that the surface order parameter has a fixed value, the validity of their approach remained confined to large interfacial separations. Moreover, the question as to the right choice of the boundary condition was raised [1]. For these reasons, and also because of the lack of unambiguous experimental verification [3,7], their ideas have been less widely acknowledged than they deserve [8].

Here we generalize their concept, to become applicable for the description of the hydration of arbitrary, non-charged, polar or non-polar interfaces and for the calculation of the hydration energies at all separations $> \xi$. We represent the order of the water molecules within the interlamellar space by an orientational order parameter $P(x)$, which is a function of the distance x measured from the midpoint between the two interfacial planes, which are located at $\pm d/2$. In order to make the problem of determining $P(x)$ reasonably tractable, we neglect the discrete origin of the water binding sites. Instead, we replace their orienting field by its effective average $F(x)$. The changes of the free energy caused by the partial orientation (polarization) of the interbilayer water, $G^{\text{hyd}}(d)$, can now first be written in terms of $P(x)$, its derivatives, and $F(x)$:

$$G^{\text{hyd}}(d) = (1/2A) \int [P^2(x) + \xi^2(dP(x)/dx)^2 - 2AF(x)P(x)] dV, \quad (1)$$

where $A = A(T)$ and ξ , the water-order correlation length, is a parameter with lesser, by assumption negligible, temperature dependence. It can be shown that $G^{\text{hyd}}(d)$ written in such form includes all the free-energy terms neglecting the fourth and higher orders [9]. The requirement for $G^{\text{hyd}}(d)$ to be minimum then leads to the Euler-Lagrange equation

$$\xi^2 [d^2P(x)/dx^2] - P(x) = -AF(x). \quad (2)$$

We confirm ourselves to the most simple case when all the sources of $F(x)$ are located solely on both interfaces or

$$F(x) = f_1 \delta(x + d/2) - f_2 \delta(x - d/2), \quad (3)$$

where f_1 and f_2 denote the strengths of the orienting fields at $x = -d/2$ and $x = d/2$, respectively, and δ is the delta function. The appropriate boundary conditions for $P(x)$ can be found by integrating eq. (2) across the boundary at $x = -d/2$ and at $x = d/2$, respectively. Assuming that P is finite for $-d/2 < x < d/2$ and identically zero outside this interval, one obtains

$$Af_1 = -\xi^2 \left. \frac{dP}{dx} \right|_{x=-d/2},$$

$$Af_2 = -\xi^2 \left. \frac{dP}{dx} \right|_{x=d/2}.$$

Moreover, because of the simple form of $F(x)$, eq. (1) can be arranged (by integration by parts of the $(dP/dx)^2$ term) to consist of only surface terms

$$G^{\text{hyd}}(d) = -\frac{1}{2} S [f_1 P(x = -d/2) + f_2 P(x = d/2)], \quad (4)$$

where S is the bounding surface area.

The use of the appropriate solution of eq. (2) together with eq. (4) finally leads to the expression for the total free energy of hydration of two mutually interacting, dissimilar interfaces as a function of f_1 , f_2 and d :

$$G^{\text{hyd}}(d) = -(AS/2\xi) [(f_1^2 + f_2^2) \text{cth}(d/\xi) - 2f_1 f_2 \text{csch}(d/\xi)] \quad (5)$$

and includes the self-energy terms

$$G_{\text{self}}^{\text{hyd}} = -(AS/2\xi) (f_1^2 + f_2^2)$$

and the corresponding interaction energy

$$G_{\text{int}}^{\text{hyd}}(d) = -(AS/2\xi) \{ (f_1^2 + f_2^2) [\text{cth}(d/\xi) - 1] - 2f_1 f_2 \text{csch}(d/\xi) \}. \quad (6)$$

We note that these expressions may also be used in the case of small interfacial separations despite their phenomenological character. This is so because $[\xi/P(d = \infty)] (dP(x)/dx)_{x=d/2} \rightarrow 1 \Leftrightarrow d \rightarrow 0$ due to the interfacial coupling. The conditions for the validity of the series expansion implicit in eq. (1) are thus fulfilled even when the hydrated surfaces come close together.

If $f_1 = f_2 = f$, eq. (5) simplifies to

$$G^{\text{hyd}}(d) = -(ASf^2/\xi) \text{th}(d/2\xi) = G_{\infty}^{\text{hyd}} \text{th}(d/2\xi), \quad (7)$$

which is precisely the dependence on the interfacial separation found in our experiment. The Marčelja model [5], on the other hand, predicts the free energy of hydration to increase as $\text{cth}(d/2\xi)$. For large x , $-\text{th} x \approx \text{cth} x - 2 \approx 2 \exp(-2x) - 1$ and the deviations between the concept of the fixed surface water-order polarization and fixed surface orienting field is immaterial (insert in fig. 1). But when $d \rightarrow 0$, the model using $P(\pm d/2)$ as a parameter becomes inadequate since it does not account for the mutual disturbance of the surface water order caused by the approaching interfaces; hence it leads to the non-physical singularity of the free energy $G^{\text{hyd}} \times \text{cth}(d \rightarrow 0/2\xi) \rightarrow \infty$.

From eq. (7) we can also calculate the entropy of hydration, $S^{\text{hyd}}(d)$:

$$S^{\text{hyd}}(d) = -\partial G^{\text{hyd}}(d)/\partial T \approx -(\partial G_{\infty}^{\text{hyd}}/\partial T) \text{th}(d/2\xi) = S_{\infty}^{\text{hyd}} \text{th}(d/2\xi)$$

and the enthalpy of hydration

$$H^{\text{hyd}}(d) \approx (G_{\infty}^{\text{hyd}} + TS_{\infty}^{\text{hyd}}) \text{th}(d/2\xi),$$

to see that in both cases the dependence on the interfacial separation is given approximately by $\text{th}(d/2\xi)$.

It seems that the discreteness of the water binding sites and the precise nature of the bilayer surface play only a minor role, at least in the case of phosphatidyl-ethanolamine. This is indicated by the excellent agreement between our experiment and the theory (fig. 1) and also by the fact that our model fits to within 11% (average error 4%) the phosphatidyl-ethanolamine bilayer hydration data obtained in the Monte Carlo simulation procedure when the detailed molecular structure was taken into account [10]. (Because the bilayer-bilayer interactions were neglected in the Monte Carlo study, the optimal value for ξ is in this case somewhat larger, 0.35 nm.) The phenomenological description of the interfacial hydration proposed in this work and in ref. [5] is thus quite accurate. However, the prerequisite for the general applicability of such a theory is that the presence of the interface is described by its orienting field, rather than by the surface value of the water polarization. The fact that such a model agrees with both the

microscopic and the macroscopic experimental data can be seen by comparing the water-order correlation length determined in our calorimetric measurements, $\xi \approx 0.26$ nm and in the X-ray experiments, $\xi \approx 0.25$ nm (see also refs. [3,7,8]). It is worth restating that our results also provide an extension into the region of small bilayer-bilayer separations of the previous studies of the interlamellar forces since the interbilayer repulsive pressure is given simply by $S^{-1} [\partial G^{\text{hyd}}(d)/\partial d]$.

In summary, we have determined the entropy, enthalpy and free energy of hydration of non-charged polar phospholipid bilayers as a function of the interlamellar separation. By generalizing the recent theory we have developed a simple phenomenological description of the interfacial hydration, based on the use of the surface orienting field density which can be used even in the case of small interfacial separations. We have clarified the question of the appropriate boundary condition for the water-order parameter (in case of membranes with no net charge) and shown that good agreement between both the microscopic and macroscopic experimental data and the theory can

be obtained provided the interfacial orienting fields are taken to be surface distributed.

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