Hydration Force Parameters of Phosphatidylcholine Lipid Bilayers as Determined from ^{2}H-NMR Studies of Deuterated Water

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ABSTRACT The continuous decrease of the quadrupolar splitting of deuterated water interacting with phosphocholine lipid bilayers with growing water concentration is analyzed as a function of the water activity. From the apparent linear dependence on water activity a measure for hydration forces is obtained. The forces calculated are in the range of published data using sorption isotherms and osmotic stress technique in combination with SAXS. A simple interaction potential which includes orientational order of water adsorbed on surfaces gives a physical base for these findings. Therefore, deuterium NMR may become a powerful tool for hydration force analysis complementing well-known methods.

INTRODUCTION

Phospholipids organized in bilayers are major components of cell membranes. The lipid-water interface plays an important role in membrane stability and membranes approaching contact on fusion. So- called repulsive hydration forces acting between apposing lipid bilayers for uncharged membranes are of both theoretical and practical interest (Marcelja and Radic, 1976; Harbich and Helfrich, 1984; Cevc and Marsh, 1987; Rand and Parsegian, 1989; Israelachvili and Wennerström, 1992; McIntosh and Simon, 1993; Evans and Parsegian, 1986). There may be several contributions to the short range repulsive forces, including hydration pressure due to the bilayer's influence on water orientation (Marcelja and Radic, 1976; Cevc and Marsh, 1987) and steric interactions between apposing bilayers (McIntosh et al., 1987, 1989; Evans and Parsegian, 1986; Israelachvili and Wennerström, 1992; McIntosh and Simon, 1993). The steric pressures should be due to lipid head group motions, bilayer undulations, and molecular protrusions (McIntosh and Simon, 1993, and references therein). In the latter paper McIntosh and Simon used crystalline bilayers to make the steric contributions relatively small and studied pressure-bilayer distance data by x-ray diffraction to be able to separate different contributions to the repulsive pressure. For short distances (~0.3 nm) there is a sharp upward break in the pressure distance relation not found in the corresponding pressure versus number of water molecules per lipid plot (McIntosh et al., 1987). The break should be due to steric interactions between head groups for short bilayer distances. Several experimental methods are available to monitor the interbilayer forces as a function of bilayer separation and/or water concentration (Rand and Parsegian, 1989; Israelachvili and Wennerström, 1992; Marsh, 1989). There are problems to determine the water layer thickness between the bilayers from the repeat distance in SAXS experiments (McIntosh et al., 1987). These problems are avoided by calculating hydration forces from water sorption isotherms (Marsh, 1989). The progressive hydration of lipids causes drastic changes in structural and dynamic properties of the lipid as well as of the water up to a certain number N of water molecules per lipid molecule (Ulrich et al., 1990; Bechinger and Seelig, 1991; Volke et al., 1994; Ulrich and Watts, 1994b). There is only one study by Ulrich and Watts (1994a) which relates changes of these molecular-level properties to hydration forces. In their study the quadrupolar splitting of headgroup deuterated dioleoyl-phosphatidylcholine (DOPC) was recorded as a function of the water activity a_w, and a linear relationship was found from which the hydration pressure was calculated. We have shown recently (Volke et al., 1994) that the structural and dynamic properties of deuterated water interacting with different phosphocholine lipids exhibit a continuous exponential behavior on lipid hydration. The decay constants for the water quadrupolar splitting, the spin-lattice relaxation rate and the self-diffusion of water on progressive hydration were found to be in the range of those determined from published hydration force measurements (Marsh, 1989). The quadrupolar splitting of deuterated water interacting with diplamitoylphosphatidylcholine (DPPC) and dihexadecylphosphatidylcholine (DHPC) where found to be larger for DHPC than for DPPC and also decrease continuously on lipid hydration (Gawrisch et al., 1992), which was used to interpret the influence of the carbonyl group hydration on hydration forces.

In this paper we analyze the quadrupolar splitting of deuterated water interacting with palmitoyl-oleoyl-phosphatidylcholine (POPC), egg yolk phosphatidylcholine (EYL), and DOPC as a function of water activity. We will show that for a wide water concentration range close to full hydration of the lipids, the quadrupolar splitting is an apparent linear function of the water activity. By using standard thermodynamics, the hydration pressure as a function of water content is obtained as well as the decay constant. This

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study is therefore complementary to the work of Ulrich and Watts (1994a) for headgroup deuterated DOPC and shows that the local disorder and the dynamics of the lipid headgroups and the water molecules are intrinsically correlated with one another. Because deuterated water may be used for a variety of lyotropic liquid crystalline systems the method presented potentially offers new insight into hydration forces on a molecular level including residual order parameters of the water at interfaces.

MATERIALS AND METHODS

The sample preparation of the lipid water dispersions (POPC, DOPC, and EYL) is described in Volke et al. (1994). The sorption isotherms were determined by the isopiestic method for POPC and EYL as described in Klose et al. (1992) and for DOPC, we used the data of Lundberg et al. (1978). All measurements were done at T = 296 K. The ^{2}H-NMR was performed using a Bruker MSL 300 spectrometer (Bruker, Karlsruhe, Germany) operating at 46.073 MHz.

The effective osmotic pressure P repelling hydrated lipid bilayers is given by $-RT/V_w \ln(a_w)$, where R is the gas constant, T the absolute temperature, V_w the molar volume of water ($18 * 10^{-6}$ m^3), and a_w the water activity. The empirical hydration force (pressure) is $P = P_0 \exp(-N/N_c)$, where P_0 is the pressure extrapolated to zero water concentration ($N = 0$) and N_c a decay constant expressed as a characteristic number of water molecules per lipid (Marsh, 1989).

RESULTS AND DISCUSSION

In a recent paper (Volke et al., 1994) we presented a model which assumed an exponential decaying influence of the bilayer surface on water dynamics as well as on water orientation with increasing hydration. The quadrupolar splitting of deuterated water for POPC, DOPC, and EYL in the lamellar phase (L_α) state, which is used in the present paper, was well described with this model. To connect this microscopic quantity to hydration force, the splitting is plotted as a function of water activity a_w. The results are presented in Fig. 1 *A*, 3 *A*, and 4 *A* for POPC, EYL, and DOPC, respectively. For water activities <0.5, which corresponds to <3 water molecules per lipid molecule in our systems, there may be phase heterogeneities, nonlamellar phase structures, and/or gel phase states (e.g., L_β). Empirically, the quadrupolar splitting is a linear function of a_w up to a water activity of ~0.98. For POPC and EYL, this corresponds to $N = 15$ and $N = 17$ (mole water/mole lipid), respectively. The sorption isotherm of DOPC was taken from the literature (Lundberg et al., 1978) and is not as well recorded as the other data sets.

It was pointed out by Parsegian et al. (1986) that precise water activities above 0.98 are difficult to measure. Errors and consequences of the isopiestic method applied to the hydration of POPC are described in Klose et al. (1992). Nevertheless, the strong change in the slope of the quadrupolar splitting versus water activity at a_w of ~0.98 corresponds to hydration numbers of 15–17 for POPC and EYL, where morphological changes in the liquid-crystalline, lamellar phase structure start to occur. Extended flat lamellae (size of ~1 μm as determined from electron microscopy freeze-fracture technique) show increasing curvature and the number of small size multilamellar liposomes is increasing (Volke et al., 1994; Gawrisch et al., 1985). Therefore, other driving forces for water uptake may dominate, e.g., capillary forces to fill the space between approaching, strong curved liposomes with water.

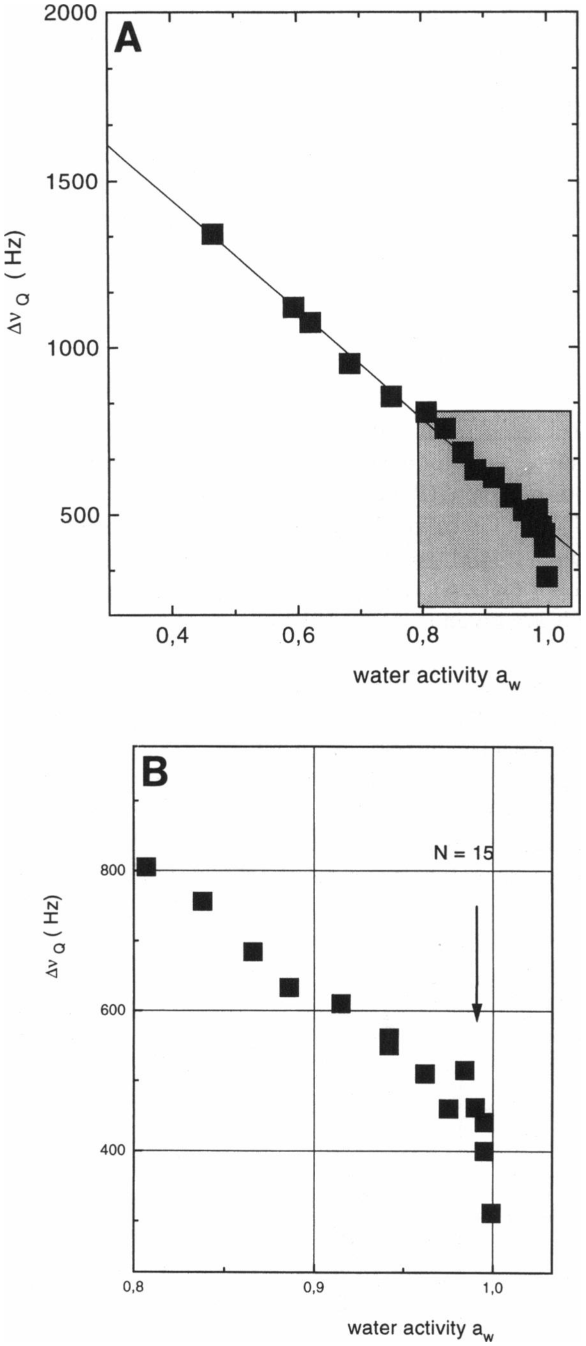

FIGURE 1 (*A*) Quadrupolar splitting of deuterated water as a function of water activity for POPC at 296 K. The solid line is a linear fit of the data. (*B*) Expanded region of *A*. The arrow indicates the onset of morphological changes of the bilayers on hydration.

From the apparent linear dependence of $\Delta\nu_Q$ on a_w we have a measure to express the water activity in terms of a quadrupolar splitting, a quantity related to the mean order S of the water molecules with respect to bilayer director orientation (Volke et al., 1994; Gawrisch et al., 1978).

Therefore:

$$\Delta\nu_Q = A + Ba_w \tag{1}$$

where A and B are constants determined from a fitting procedure. This allows the calculation of the osmotic pressure P:

$$P = \left(-\frac{RT}{V_w}\right)\ln\left(\frac{\Delta\nu_Q - A}{B}\right) \tag{2}$$

In Figs. 2, 3 *B*, and 4 *B* the pressure is presented in dependence on N for the three lipids for direct comparison with published data (Rand and Parsegian, 1989; Israelachvili and Wennerström, 1992; Marsh, 1989; McIntosh and Simon, 1993). The data can be fitted by the equation $P = P_0 \exp(-N/N_c)$. The parameter P_0 and N_c for the three lipids are given in Table 1. Errors are the 95% confidence limits.

These values are well within the range of fitted parameters for many different amphiphilic systems (Rand and Parsegian, 1989; Israelachvili and Wenerström, 1992; Marsh, 1989; McIntosh et al., 1993). Similar results were obtained by Ulrich and Watts (1994a) analyzing the quadrupolar splitting of headgroup deuterated DOPC as a function of the water activity. Therefore, the whole lipid-water interface reacts in a common way on progressive hydration, and the deuterium NMR may be used in parallel to conventional hydration force measurements. New motional mechanism and/or morphological changes of the membrane structure start at a water

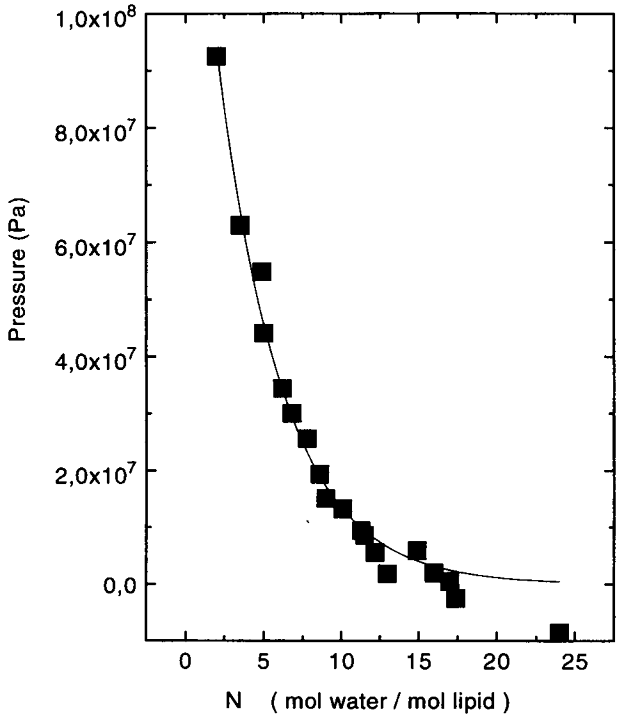

FIGURE 2 Hydration pressure calculated from the quadrupolar splitting of water as a function of water concentration (mol water/mol lipid). Data shown for POPC.

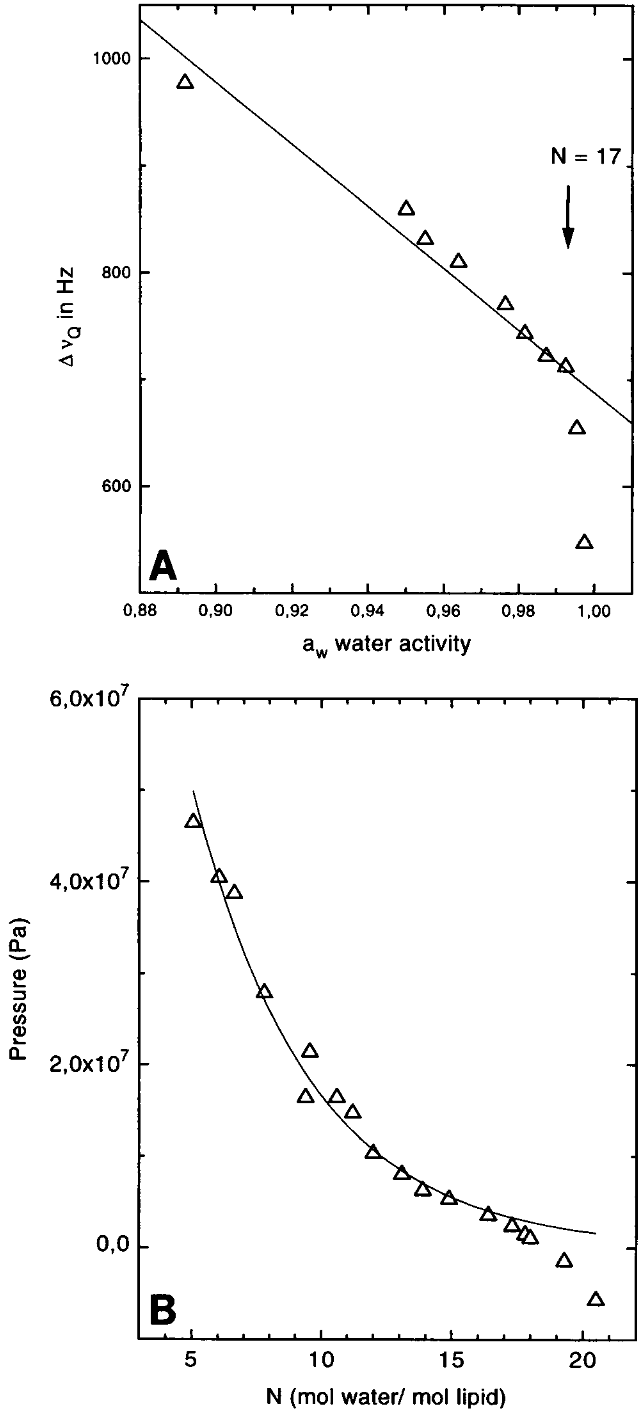

FIGURE 3 (*A*) Quadrupolar splitting of deuterated water as a function of water activity for EYL at 296 K. The solid line is a linear fit of the data. (*B*) Hydration pressure calculated from the quadrupolar splitting of water as a function of water concentration (mol water/mol lipid). Data shown for EYL.

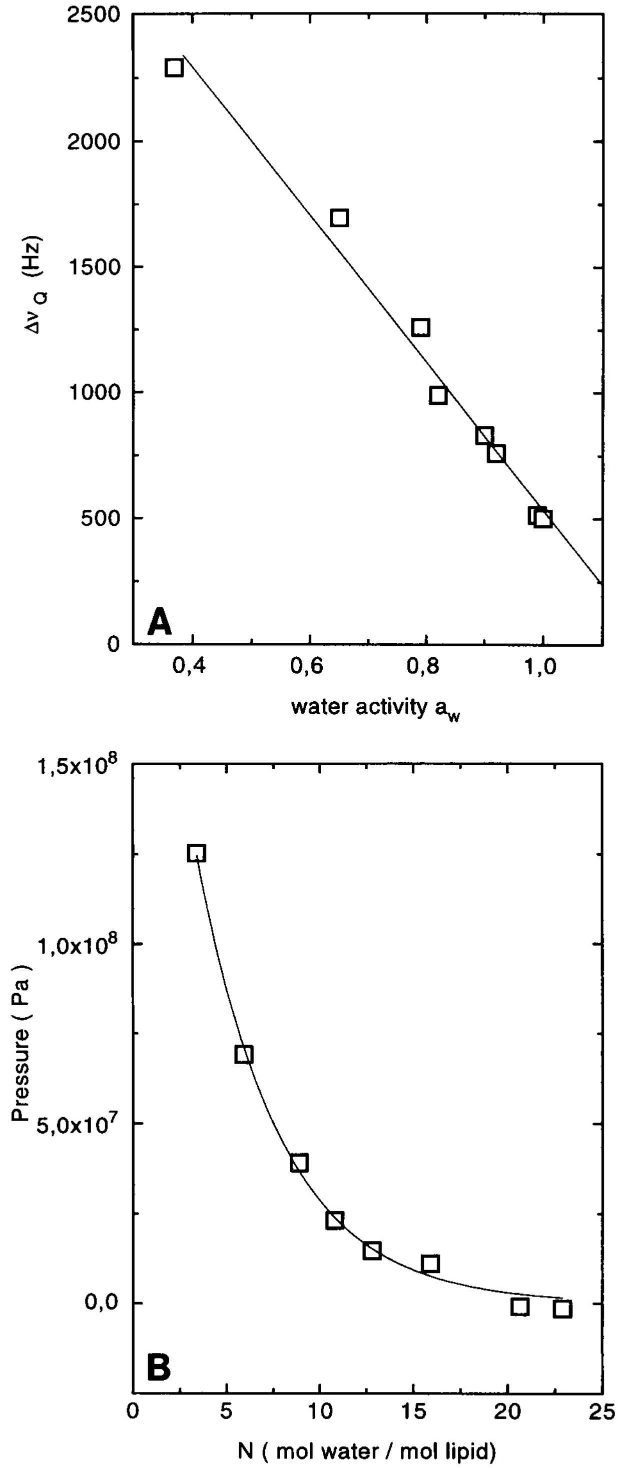

FIGURE 4 (*A*) Quadrupolar splitting of deuterated water as a function of water activity for DOPC at 296 K. The solid line is a linear fit of the data. (*B*) Hydration pressure calculated from the quadrupolar splitting of water as a function of water concentration (mol water/mol lipid). Data shown for DOPC.

activity of ~0.98 (N = 15–17) and lead to a drastic decay of the residual quadrupole splitting of the water increasing hydration.

TABLE 1 Hydration pressure P_o extrapolated to zero water content and decay constant N_c, expressed in terms of number of water molecules

Lipid	POPC	EYL	DOPC
P_o (Pa)	$(1.5\pm0.1)10^8$	$(1.5\pm0.2)10^8$	$(2.6\pm0.1)10^8$
N_c	4.2 ± 0.2	4.5 ± 0.3	4.5 ± 0.2

What could be the physical relationship of the apparent linear change of the quadrupolar splitting with water activity? In a crude first approximation, the work to adsorb water from the gas phase is given by Brunauer (1945):

$$\epsilon = RT\ln(1/a_w) \qquad (3)$$

The two assumptions are 1) the gas vapor obeys the ideal gas law; and 2) the liquid in the adsorbed phase is incompressible. On the other hand, the quadrupolar splitting is given by: $\Delta\nu_Q = \frac{3}{4}\varkappa S$, where $\varkappa$ is the quadrupole coupling constant (222 kHz for deuterated water (Volke et al., 1994; Gawrisch et al., 1978)) and S a mean order parameter. The quadrupolar splitting may be used to obtain directly the mean orientational order parameter $S = \langle P_2(\cos\Theta)\rangle$ for the angle Θ between the O—^{2}H bond direction and the bilayer director, and P_2 is a Legendre polynomial of order 2. The validity of this treatment was proven for the Lα phase using oriented bilayers (Arnold et al., 1979; Gawrisch et al., 1978). Though the order parameter is very small, it indicates some residual anisotropy of the water interacting with phospholipid bilayer surfaces. The residual S arises from the specific arrangement and motion of water associated with the lipid headgroup which itself changes conformation and dynamics on hydration (Volke et al., 1994; Ulrich and Watts, 1994b). The steric restriction of headgroup motion on bilayer approach, lipid protrusions and bilayer undulations should be reflected therefore in the measured residual quadrupolar splitting of the water. Although we have no detailed knowledge of these interactions, we think that there is an orientation dependence (dependent on order parameter) in the intermolecular potential. An interaction potential that contains the minimum necessary feature was published by Kobayashi (1971) and used by Wojtowicz (1974) for smectic-A liquid crystals:

$$V = U(r) + W(r)\langle P_2(\cos\Theta)\rangle = U(r) + W(r)S \qquad (4)$$

where the functional dependence of U and W on r is not specified, and the average over P_2 is the mean order parameter S as determined from the NMR data above. In the liquid crystalline phase state the whole bilayer including the interacting water shows a P_2 symmetry, the molecular motions are axially symmetric, the axis of symmetry being the normal of the bilayer (Gawrisch et al., 1978; Arnold et al., 1979). The work to adsorb water at an interface is than partially used to orient water in a not yet specified way (probably only hydrogen bonds with the lipid).

Therefore

$$\epsilon = RT\ln(1/a_w) = V = U + WS \qquad (5a)$$

and

$$S = (RT/W)\ln(1/a_w) - U/W \tag{5b}$$

where U is the work to form liquid surface water and W an orientation energy which makes the water different from bulk water formed from the vapor phase. The dependence of V on water concentration is assumed to due to S only; the values of S obtained from the quadarupolar splitting, as described above, decreases as the water concentration is increased.

The measured quadrupole splitting $\Delta\nu_Q$ is divided by $\frac{3}{4}\chi$ to get the order parameter S, and the data are fitted using Eq. 5b. Fig. 5 presents the fit of the POPC data to Eq. 5b. They are equally well described as using a linear fit (compare Fig. 1 *A*), especially in the range of water activities usually used for hydration force measurement. Taking into account the crudeness of the model used, it depicts the main features of the measurement reasonably well.

The fit parameters U and W are given in Table 2. There are several interesting aspects in these data beside the very basic model used. The energy to hydrate (dehydrate) lipids is mainly stored in the orientational energy term WS. It reflects therefore the restriction of motion of the water by the bilayer surface as well as conformational changes of the lipid headgroups which influences the residual order of interacting water itself. At present we are not able to separate steric repulsive pressure from headgroup mobility from hydration repulsive pressure by water orientation. The pressure versus number of water molecules per lipid plot shows no upward break at low hydration as it was reported by McIntosh and co-workers (McIntosh et al., 1987; McIntosh and Simon, 1993). Nevertheless, on a molecular level, it seems that both contributions are interdependent-coupled. The total energy ($U + W$, for $S = 1$) is well within the range of reported energies of hydration (Cevc, 1993). For water activities $a_w = 1$, $S = U/W$. For the three systems studied U/W corresponds to water activities of ~0.92 or 12–14 mol water/mol lipid. Again, this is in accordance with the onset of morphological changes in the samples on hydration. Additional motional processes reduce the splitting (and therefore order parameter) for higher hydration. These processes are probably undulations (Evans and Parsegian, 1986; McIntosh et al., 1989; McIntosh and Simon, 1993) or at least the onset of remarkable curvature in the bilayers which reduce the residual splitting due to fast lateral diffusion of water along the curved surface. Furthermore, this corresponds roughly with the change in the slope of the splitting versus water activity as seen in Fig. 1 *A*. While these contributions will have an influence on the quadrupolar splitting and therefore S, they are not yet considered in terms of energies in Eq. 4.

TABLE 2 Energy-fit parameters U and W according to Eq. 5b as determined from order parameter measurements as a function of water activity

Lipid	POPC	EYL	DOPC
U (kJ/mol)	−1.0 ± 0.2	−0.5 ± 0.1	−0.9 ± 0.2
W (kJ/mol)	300 ± 25	129 ± 15	234 ± 20

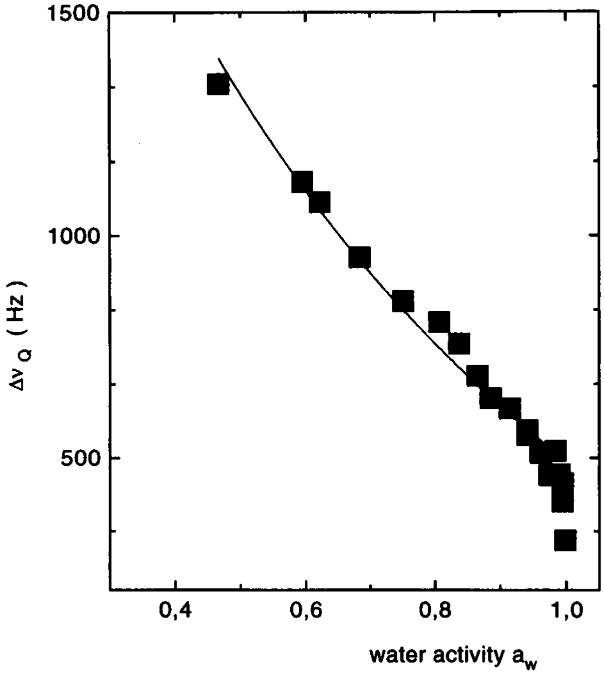

FIGURE 5 Data as in Fig. 1 *A* but fitted (*solid line*) using Eq. 5b.

We are currently improving these calculations to get a better understanding of the interaction terms in the light of recent hydration force discussions. Similar studies on macroscopically oriented, solid state supported bilayers are in progress to reduce the influence of undulations and curvature. For such systems the maximum uptake of water for $a_w = 1$ is less than for unoriented bilayers used in this study (unpublished observations).

CONCLUSIONS

The quadrupolar splitting of deuterated water interacting with phospholipid bilayers was shown to be useful for hydration force studies. The main aim of this paper was to give an explanation for the linear dependence of the quadrupolar splitting on water activity. This is explained in terms of a very simple orientation potential and adsorption work of water on surfaces. The procedure works well for water activities up to ~0.92. For higher water activities morphological changes which are connected with membrane curvature lead to a different hydration behavior of the lipids. Because deuterated water may be used in a variety of amphiphilic molecules (Volke, 1984) and biological membranes, it is worthwhile to test the above method for these systems.

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