REPULSIVE FORCES IN LECITHIN GLYCOL LAMELLAR PHASES

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ABSTRACT The repulsive pressure *vs.* distance for phospholipid bilayers in glycol has been determined from vapor pressure measurements. The magnitude of this pressure is similar to the case when water is present between the lipid bilayers. Hence, an interaction directly corresponding to the previously reported hydration force is shown also for nonaqueous lecithin/solvent systems.

INTRODUCTION

Current theories of the interaction forces between colloidal aggregates in a solution involve three types of forces: van der Waals forces, diffuse double-layer repulsion, and steric interactions. A short-range repulsive interaction that cannot be accounted for by these forces has been experimentally demonstrated in lyotropic lamellar phases formed by phospholipids and water. LeNeveu et al. (1), Parsegian et al. (2), and Lis et al. (3) used a relatively straightforward method to obtain the interbilayer repulsion from measurements of water activities. The additional repulsive interaction was called the "hydration force." A review is given in reference 4.

Three mechanisms have been suggested for the hydration force between phospholipid bilayers in water. Helfrich estimated the effect of steric interactions between out-of-plane fluctuating bilayers to be considerably less than the measured hydration force (5). A mechanism based on the repulsion of dipoles from a region with a lower dielectric constant was proposed by Jönsson and Wennerström (6). Specific interactions between structured water and the phospholipid bilayer surfaces were discussed by Gruen and Marcelja (7).

Forces like the hydration force have so far been demonstrated in aqueous systems only. The existence of a lamellar phase formed by egg lecithin and ethylene glycol has been reported by Moucharafieh and Friberg (8, 9). From measurements of repulsive interactions in similar systems, factors important for the origin of the hydration force may be isolated.

MATERIALS

Two different samples of phospholipids were used. The synthetic dipalmitoyl phosphatidylcholine, L-α-DPPC (99% from Sigma Chemical Co., St. Louis, MO), and a soybean lecithin with highly unsaturated hydrocarbon chains (Epicuron 200 from Lucas Meyer, Hamburg, Federal Republic of Germany, >97% phosphatidylcholine claimed by the manufacturer). In water the soybean and egg lecithin show a similar phase behavior (10). The phospholipids were dried in a vacuum for 3 d before use.

The purity of ethyleneglycol, 1, 2-dihydroxyethane, called glycol below (pro analysi; Merck Chemical Div., Merck & Co., Inc., Rahway, NJ), was determined using component separation in a gas chromatograph. An analysis of the liquid gave >99% glycol according to peak areas. In spite of the high purity of glycol, the vapor phase contained other substances that gave large signals on the chromatograph. Hence the other substances must have high vapor pressures in comparison with glycol. The magnitude of the signal from the other substances was related to the ratio between vapor volume and liquid volume. As an example, the vapor from a freshly supplied 1-l bottle of glycol (purity of liquid >99%) gave peak areas from the other components ~50 times larger than the peak area from glycol. The conclusion is that peak areas from the glycol vapor phase do not correspond to actual liquid phase concentrations. Therefore the glycol in the lamellar phase was considered to be approximately pure (≈99%), although other components could be detected in the vapor phase.

The samples were prepared in 10-ml glass ampules, which were sealed with a rubber septum. About 100 mg DPPC or 1-g soybean lecithin was used for each sample. For equilibration, the DPPC-glycol samples were repeatedly heated to ~100°C for 15 min. Before the measurements the samples were equilibrated at 55°C for 3 h. The soybean lecithin-glycol samples were equilibrated for 1 wk at room temperature.

METHODS

The vapor pressures were measured using a gas chromatograph (4200; Carlo Erba Strumentazione, Milano, Italy) equipped with an automatic sampling system for head space analysis (11). 1-ml vapor, in assumed equilibrium with the lamellar phase, was sampled and injected into a Tenax (Alltech Europe, Applied Science Laboratories, Nazareth, Belgium) column running at 140°C. The signal from a flame ionization detector was plotted from which the relative glycol vapor pressure was measured from the height of the glycol peak.

The samples were thermostatted (±1°C) in a water bath where the

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water level reached almost to the rubber septum. The temperature interval, suitable for vapor pressure determinations, was rather narrow. The relatively low vapor pressure of glycol together with the low sensitivity of the detector for glycol resulted in a lower temperature limit at ~30°C. In samples of high glycol content an experimental problem, probably involving a condensation of liquid on the rubber septum, occasionally gave dramatically erroneous results (omitted). The problems from the assumed condensation were most frequent at higher temperatures, and decreased the possibility of reliable pure solvent vapor pressure determinations above 60°C.

The osmotic (or swelling) pressure of the lamellar phase, π, was conventionally calculated from the measured vapor pressure P_g according to

$$\pi = -(1/v_g)(\mu_g - \mu_g^0) = (1/v_g)RT\ln P_g^0/P_g, \qquad (1)$$

where v_g is the glycol (partial) molar volume, μ_g and μ_g^0 the chemical potentials of glycol in the lamellar phase or approximately pure liquid, respectively, and P_g^0 the vapor pressure of the pure liquid. When $P_g > 0.95\ P_g^0$, corresponding to $\ln\pi = 14.7$, the experimental errors due to the subtraction $\ln P_g^0 - \ln P_g$ in Eq. 1 give large errors in the value of π.

After vapor pressure determinations, the repeat distances, d, of the samples were determined using x-ray low-angle diffraction. At room temperature, the rubber septum was removed and a small portion of the sample was pulled into a thin glass capillary. The position sensitive x-ray detector system and the thermal unit (±2°C) used have been described previously (12). For calculation of the structural parameters d_g (= d · glycol volume/total volume) and A_l (= bilayer area for lecithin molecule), the densities of the lipids are taken equal to $\rho = 1.00$ g/cm^3. The molecular weight of the soybean lecithin is estimated to be 773 g/mol (10). Together with known data these assumptions give for the molecular volumes: soybean lecithin, 1.283 nm^3; DPPC, 1.219 nm^3; and glycol, 0.093 nm^3.

RESULTS

The vapor pressures and x-ray repeat distances from lamellar phases formed by phosphatidylcholine and glycol have been determined. The phosphatidylcholines used were synthetic dipalmitoylphosphatidylcholine, DPPC, and soybean lecithin. In Table I, measured and calculated parameters for various compositions of the DPPC-glycol lamellar phase are shown. The glycol vapor pressures, P_g, were determined twice[1] at 54°C using the head space gas chromatograph. From the average vapor pressure values, the osmotic pressure of the lamellar phase, π, was, calculated. The measured x-ray repeat distances, d, at 54°C, were used to calculate d_g, a measure of the glycol layer thickness, and A_l, the bilayer area per lecithin molecule. According to x-ray diffraction patterns the lipid alkyl chains were liquidlike at glycol concentrations higher than 8.4%. At 8.4% glycol, x-ray diffraction patterns from two phases were observed.

In Table II, information similar to that in Table I, is shown for the soybean lecithin-glycol lamellar phase. The glycol vapor pressures were determined N times,[1] allowing for error estimations in each measured value (95% significance, Student's t test). X-ray repeat distances, d, were in agreement with values reported previously for egg lecithin-glycol lamellar phases (8, 9). Only liquidlike alkyl chain disorder was found in the samples, even at 5°C, according to x-ray diffraction patterns in the 0.3–0.5 nm region.

The osmotic pressures, π, of the lecithin-glycol lamellar phases are plotted against the solvent layer thickness, $d_{solvent}$, in Fig. 1. For some compositions d_g have been interpolated or extrapolated from values shown in Tables I and II. For comparison the corresponding magnitude of π in an egg lecithin-water lamellar phase, reported by Parsegian et al. (2) is shown as a dotted line in Fig. 1. Their results are in accordance with data obtained from the soybean lecithin-water lamellar phase using the headspace

TABLE I

MEASURED X-RAY REPEAT DISTANCES, d, AND RELATIVE VAPOR PRESSURES P_g/P_g^0 FOR THE LAMELLAR PHASE FORMED BY DIPALMITOYLPHOSPHATIDYLCHOLINE AND GLYCOL AT 54°C

% Glycol	d	d_g	A_l	P_g/P_g^0	π^0
wt/wt	*nm*	*nm*	*nm*^{2}		*MPa*
64.3	—	—	—	1.0 1.0	0
60.2	—	—	—	0.99 0.90	3
55.9	—	—	—	1.03 0.99	0
51.4	—	—	—	1.03 1.02	0
47.3	—	—	—	1.06 0.96	2.2
44.7	5.22	2.20	0.81	1.06 0.92	4.0
42.0	—	—	—	0.917 0.860	5.8
39.1	4.98	1.83	0.77	0.844 0.826	8.8
33.3	—	—	—	0.728 0.849	11.3
30.0	—	—	—	0.719 0.712	16.1
28.9	4.44	1.19	0.75	0.708 0.710	16.5
23.6	—	—	—	0.583 0.599	25.2
19.4	—	—	—	0.444 0.455	38.4
17.1	4.57	0.71	0.63	0.242 0.373	56.6
14.4	—	—	—	0.302 0.329	55.4
8.4	4.79	0.37	0.55	0.222 0.221	72.4
6.3	—	—	—	0.214 0.248	70.3

The bilayer thickness, d_g, and bilayer area per lecithin molecule, A_l, are calculated from the repeat distance. The average relative vapor pressure is used to calculate the osmotic pressure of the lamellar phase, π. (1 MPa = $10^6\ N/m^2$ = 10 bar.)

[1]Upon repeated sampling from the same ampule, no effect from the reduced absolute pressure in the ampule was detected.

TABLE II
STRUCTURAL PARAMETERS AND MEASURED RELATIVE VAPOR PRESSURES, P_g/P_g^0, FOR THE LAMELLAR PHASE FORMED BY SOYBEAN LECITHIN AND GLYCOL AT 35°C

% Glycol	d	d_g	A_l	P_g/P_g^0	N	π
wt/wt	*nm*	*nm*	*nm²*			*MPa*
100	—	—	—	1.00 ± 0.016	53	0
60.0	—	—	—	1.02 0.104	4	0
39.9	4.84	1.84	0.85	0.955 0.057	6	2.1
35.8	4.68	1.59	0.83	0.912 0.036	7	4.2
30.4	4.57	1.31	0.79	0.745 0.018	7	13.3
24.9	4.30	1.01	0.78	0.765 0.021	7	12.2
20.6	4.21	0.81	0.76	0.686 0.014	7	17.1
18.3	—	—	—	0.594 0.016	7	23.6
16.4	4.01	0.61	0.76	0.480 0.020	7	33.3
13.6	3.96	0.50	0.74	0.331 0.006	7	50.0
12.5	—	—	—	0.314 0.012	7	52.4
11.4	—	—	—	0.248 0.007	7	63.0
10.1	—	—	—	0.186 0.005	7	76.2
9.3	—	—	—	0.132 0.003	7	91.2
8.8	—	—	—	0.143 0.004	7	88.0
7.2	—	—	—	0.099 0.004	7	105
6.5	—	—	—	0.058 0.002	7	129
4.9	—	—	—	0.040 0.002	7	146
4.2	—	—	—	0.018 0.002	7	181
3.0	—	—	—	0.013 0.001	7	198

95% confidence intervals in P_g/P_g^0 are shown. The average value is used to calculate the osmotic pressure of the lamellar phase, π.

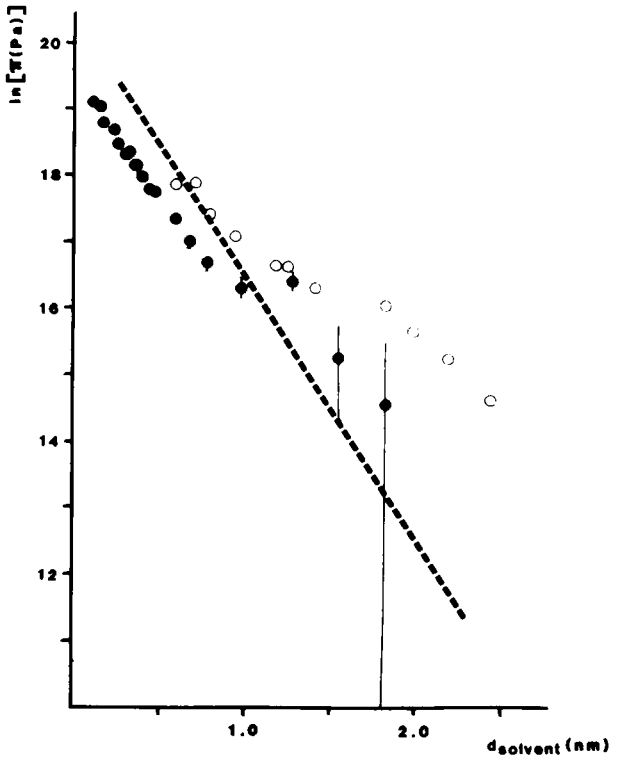

FIGURE 1 The logarithm of the osmotic pressure π in lecithin-glycol lamellar phases v interbilayer distance d solvent. ●, soybean lecithin at 35°C (95% confidence intervals shown); ○, synthetic dipalmitoyl phosphatidyl choline at 55°C. The dotted line shows the corresponding results from reference 2, when water instead of glycol is added between egg lecithin bilayers.

gas chromatograph (Bergenståhl, B., unpublished results). As seen in Fig. 1, similar magnitudes of the osmotic pressures are found for the two solvents glycol and water, respectively.

The molecular solubility of soybean lecithin in glycol was estimated from visual inspection of laser-light scattering and foaming properties. Between 20 and 70°C, a sample containing a weight fraction lecithin/total = C_l = 5.8×10^{-4} scattered light and showed foaming after shaking. In contrast, a sample containing $C_l = 5.5 \times 10^{-5}$ did not show these signs of molecular association. The molecular solubility is hence concluded to be $< C_l = 5.8 \times 10^{-4}$.

DISCUSSION

As shown by Parsegian et al. (2) and Guldbrand et al. (13), the osmotic pressure of a lamellar lyotropic mesophase can be used to calculate interbilayer interaction energies. The osmotic pressure is equal to the repulsive force per unit bilayer area, i.e., $\pi = -(1/A_l)\ [\partial G'/\partial\ (d_g/2)]$ (2, 13), where G' represents the free energy of the interactions in the lamellar phase.

The experimental results, represented in Fig. 1, clearly show that an interaction resulting in an interbilayer repulsive force is present in lecithin-glycol lamellar phases. The magnitude of the force is similar to what is found when water is the solvent. The results consequently imply that a specific water structure is not necessary for the presence of the hydration force.

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