Multilayer of Phospholipid Membranes on a Hydrosol Substrate

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The molecular structure of a multilayer of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) adsorbed on the surface of the hydrosol of silica nanoparticles has been studied by the synchrotron radiation scattering method. According to the reflectometry data, the multilayer is formed by planar phospholipid bilayers with a thickness of (69 ± 1) Å and its total thickness is about 400 Å. Grazing incidence diffraction indicates that the bilayers are in the crystal state with an area of (41.6 ± 0.7) Å^2 per molecule.

Phosphatidylcholine molecules in an aqueous medium form microscopic aggregates, vesicles and liposomes, whose shell is formed by molecular bilayers and is often considered as a model of a biological membrane [1]. Detailed information on the structure of a lipid bilayer is important for the solution of a large number of biophysical problems. The characteristic spontaneous curvature radius of a phospholipid membrane in the aqueous medium is smaller than 10 μm; for this reason, the membrane samples for structural investigations are prepared on planar solid substrates made of crystalline silicon, glass, quartz, or polymer [2–7]. In this paper, a method for obtaining macroscopically planar lipid membranes on a liquid substrate made of the aqueous solution of amorphous silica particles is reported.

The film samples of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC, see Fig. 1) were prepared and studied in a fluoroplastic dish with a diameter of about 100 mm. Using a 25-μl syringe (Hamilton), a 5- to 10-μl drop of the solution of phospholipid in chloroform (~0.03 mol/l) is deposited on the surface of the liquid substrate; the amount of the substance in the drop is enough to form a multilayer consisting of more than ten lipid monolayers. In this case, the spreading of the drop on the surface is accompanied by a decrease in the surface tension γ of the air–hydrosol interface from 74 to 35–40 mN/m, which was detected by the Wilhelmy method using an NIMA PS-2 surface pressure gauge. Then, the equilibrium of the sample was reached inside an air-tight single-stage thermostat at $T = 298$ K for about 12 h.

For comparison, an adsorbed DSPC monolayer was similarly prepared on the aqueous substrate (pH ≈ 12), which is a NaOH solution (99.95% in the metal content, Sigma-Aldrich) in deionized water (Barnstead UV). In this case, γ ~ 50 mN/m.

A powder of synthetic 1,2-distearoyl-sn-glycero-3-phosphocholine was purchased from Avanti Polar Lipids and chloroform (~99.8%) was purchased from Sigma-Aldrich. Monodisperse hydrosols of amorphous silica particles with a diameter of about 220 Å were delivered by Grace Davison. These solutions (pH ≈ 9) with a density of (1.30 ± 0.01) g/cm^3 (Ludox
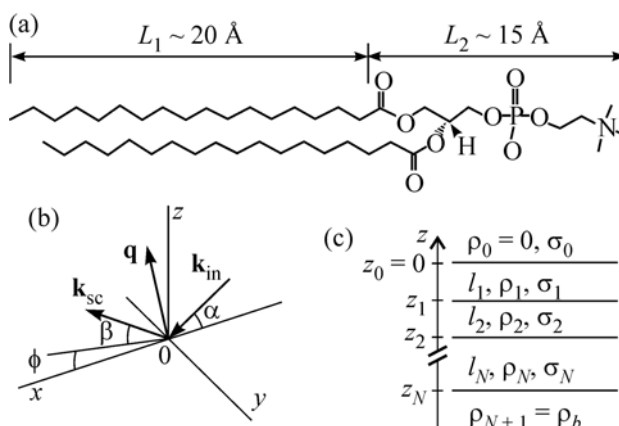
Fig. 1. (a) Structure of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); (b) the kinematics of scattering on the surface of a liquid, where $\mathbf{k}_{in}$ is the wave vector of the incident wave, $\mathbf{k}_{sc}$ is the wave vector of the wave scattered in the direction of the observation point, and $\mathbf{q} = \mathbf{k}_{in} - \mathbf{k}_{sc}$ is the scattering vector; and (c) the parameterization of the interface electron density $\rho(z)$. The adsorbed layer is divided into N layers (slabs) with thickness l_j and electron density ρ_j (slab model); $\sigma_j = \sigma_0$ is the standard deviation of the position of the jth interface from the nominal value z_j. The layer adjacent to the substrate is formed by polar groups of glycero-3-phosphocholine. The surface and bulk densities are $\rho_N = \rho_w$ and $\rho_{N+1} = \rho_b$, respectively. The contribution from the transient layer to the coefficient of reflection at $q_z > 0.1$ Å^{-1} is neglected ($\sigma_N \gg \sigma_0$) [16].

TM-40, ~40 wt % of SiO_2) and (1.40 ± 0.01) g/cm³ (Ludox TM-50, ~50 wt % of SiO_2) were stabilized by sodium hydroxide (~0.2 mol/l).

The X-ray diffraction data were obtained on the X19C station at the National Synchrotron Light Source (NSLS) [8]. The transverse and in-plane structures of the lipid films were investigated by the methods of reflectometry and grazing incidence diffraction of a focused beam (~10^{11} photons/s) with a photon energy of 15 keV ($\lambda = 0.825 \pm 0.002$ Å), respectively.

The kinematics of scattering on the surface of the liquid is naturally described in the system of coordinates with the xy plane coinciding with the surface and the z axis normal to the surface and opposite to the gravitational force (see Fig. 1b). The triangles in Fig. 2 present the coefficient of reflection R as a function of $q_z = (4\pi/\lambda)\sin\alpha$ for the DSPC monolayer adsorbed on the surface of water. The squares and circles in Fig. 2 present the coefficient of reflection of the phosphocholine films adsorbed on the surface of hydrosol with silica concentrations of 40 and 50%, respectively. For a more convenient representation of the $R(q_z)$ dependence, it is normalized to the Fresnel function $R_F(q_z) \approx (q_z - [q_z^2 - q_c^2]^{1/2})^2/(q_z + [q_z^2 - q_c^2]^{1/2})^2$, where $q_c = (4\pi/\lambda)\sin\alpha_c$. At the glancing angles smaller than $\alpha_c \approx \lambda\sqrt{r_e\rho_b/\pi}$ (where $r_e = 2.814 \times 10^{-5}$ Å is the classical electron radius and ρ_b is the average electron density in the substrate), the incident ray undergoes total reflection; i.e., $R \approx 1$. The electron density of water $\rho_w \approx 0.333$ e/Å³ (where e is the elementary charge); the electron densities of Ludox TM-40 and Ludox TM-50 silica hydrosols are $\rho_b \approx 1.2\rho_w$ and $\rho_b \approx 1.3\rho_w$, respectively.

The period of oscillations in $R(q_z)$ for the aqueous substrate is about twice as long as that for the hydrosol substrate. Moreover, the $R(q_z)/R_F(q_z)$ dependence of the surface of hydrosol contains a set of sharp peaks, whereas the structure factor of the monolayer on the surface of water is characterized by wide oscillations.

The quantity $R(q_z)$ contains information on the transverse profile of the electron density $\rho(z)$ averaged over a large area (~0.5 cm²). The density $\rho(z)$ is parameterized in the standard (slab) model of the multilayer (see Fig. 1b). The model profile is constructed on the basis of the error function under the assumption that σ_j is the standard deviation of the position of the jth interface of the multilayer from the nominal value z_j [9]. The lower limit of the parameters σ_j is determined by the capillary width, $\sigma_{cw}^2 = (k_B T/2\pi\gamma)\ln(Q_{max}/Q_{min})$ (where k_B is the Boltzmann constant), which is specified by the short-wavelength limit in the spectrum of capillary waves $Q_{max} = 2\pi/a$, where $a \approx 5$ Å is the intermolecular distance, and $Q_{min} = q_z^{max}\Delta\beta$ ($2\Delta\beta \approx 6 \times 10^{-4}$ rad is the angular resolution of the detector and
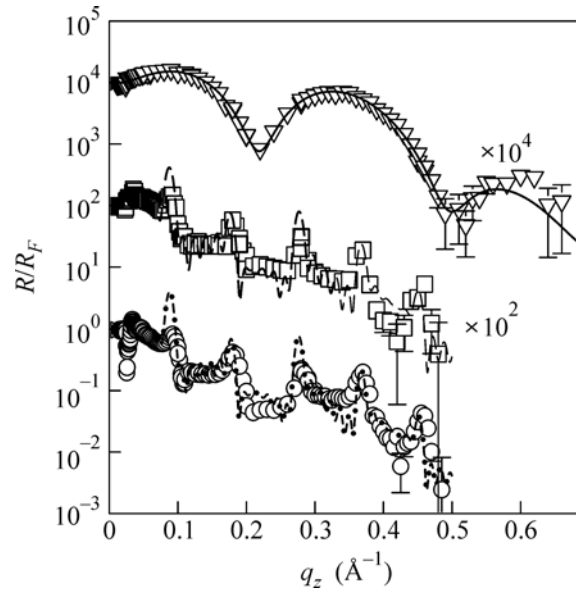
Fig. 2. Ratio $R(q_z)/R_F(q_z)$ for (triangles) the DSPC monolayer on the surface of water; the solid line is the two-layer model of the monolayer and (squares and circles) the lipid multilayer on the surface of hydrosol with a silica concentration of 40 and 50 wt %, respectively; the dashed line is the model of six bilayers on the substrate with $\rho_b = 1.21\rho_w$ and $\sigma_0 = 4.4$ Å; and the dash–dotted line is the model of seven bilayers on the substrate with $\rho_b = 1.3\rho_w$ and $\sigma_0 = 4.2$ Å.

$q_z^{max} \approx 0.5$ Å⁻¹) [10–13]. Under the assumption that $\sigma_j = \sigma_0$ for all j values, the structure factor in the first Born approximation has the form [14]

$$\frac{R(q_z)}{R_F(q_z)} \approx \left| \frac{1}{\rho_b} \sum_{j=0}^{M} (\rho_{j+1} - \rho_j) e^{-iq_z z_j} \right|^2 e^{-\sigma_0^2 q_z^2}. \quad (1)$$

Analysis of the data presented by triangles in Fig. 2 indicates that DSPC molecules are adsorbed as a monolayer on the surface of water; the coefficient of reflection $R(q_z)$ of this monolayer is well described by the two-layer model with five fitting parameters. The variation of the parameters in the monolayer model is in agreement with the structure of the DSPC molecule (see Fig. 1a). The first layer of the thickness $L_1 \sim 18$ Å is formed by hydrocarbon chains and has the density $\rho_1 = 1.01\rho_w$, which corresponds to the densest packing of saturated hydrocarbons in the γ phase [1]. The second layer of the monolayer, which is in direct contact with water, is formed by polar hydrophilic groups of glycerophosphocholine and has the density $\rho_2 = 1.38\rho_w$ and the thickness $L_2 \sim 9$ Å. The calculated value $\sigma_{cw} = 3.4 \pm 0.2$ Å coincides within the errors with the fitted value $\sigma_0 = 3.6 \pm 0.2$ Å. The solid line in Fig. 2 is the model structure factor with the parameters presented in the table. The $\rho(z)$ profile of the two-layer

Model parameters of the electron-density profile (see Fig. 4)

Substrate	L_1, Å	L_2, Å	ρ_1/ρ_w	ρ_2/ρ_w	σ_0, Å
Water	17.5 ± 3/−0.5	9 ± 1/−5	1.005 ± 0.025/−0.006	138 ± 0.3/−0.05	3.6 ± 0.2/−0.1
Hydrosol	20 ± 1	15 ± 1	0.97 ± 0.05	0.80 ± 0.02	4.2 ± 0.2

Note: L_1 is the thickness of the layer of hydrocarbon tails with the density ρ_1, L_2 is the thickness of the layer of polar groups with the density ρ_2, and σ_0 is the width of the interfaces; the densities are given in units of the density of water $\rho_w = 0.333$ electrons/Å^3. The errors of the determination of the parameters were obtained using the standard χ^2 criterion for a confidence level of 0.9.

model of the monolayer is shown in Fig. 4a (see below) and is in agreement with the previous results [15].

The phospholipid film adsorbed on the surface of the hydrosol is a multilayer. At $q_z > 0.1$ Å^{-1}, scattering on the inhomogeneities of the electron density, which are attributed to the transverse distribution of 22-nm silica particles, makes a small contribution to the reflected power [16]; i.e., $R(q_z)$ at these angles is determined only by the structure of the lipid layer. The periodicity of peaks $\Delta Q_z \sim 0.09$ Å^{-1} in $R(q_z)$ in Fig. 2 corresponds to the layered structure along the z axis with the period $L \sim 2\pi/\Delta Q_z \sim 70$ Å, which is the thickness of the double layer of DSPC molecules. A relatively small width of the peaks, $\delta q_z \sim 1.5 \times 10^{-2}$ Å^{-1} in $R(q_z)$ is due to the interference of reflected rays in the layer about 400 Å in thickness, which can contain $\Delta Q_z/\delta q_z \sim$ 6−7 lipid bilayers.
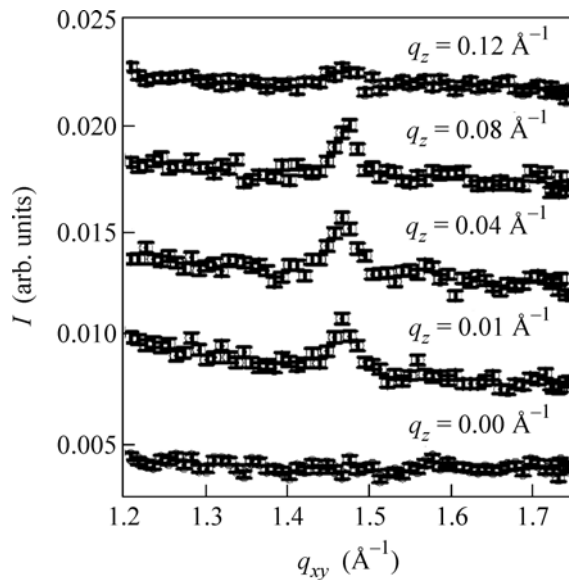

Fig. 3. Intensity of grazing incidence diffraction from the lipid multilayer at the air−hydrosol interface. The Bragg peak structure along the z axis was measured at the glancing angle $\alpha \approx 0.07°$ using the vertical position-sensitive detector (Ordela). The horizontal angular resolution of the detector, which determines the width of the diffraction peak, is about 0.003 rad or $\Delta q_{xy} \approx 0.02$ Å^{-1}. The angular resolution of the detector in the vertical plane is about 0.002 rad or $\Delta q_z \approx 0.02$ Å^{-1}.

The presence of only one peak in the grazing diffraction (see Fig. 3) indicates the highly symmetric hexagonal packing of hydrocarbon tails in the multilayer. Its position at $q_{xy} = 1.48 \pm 0.01$ Å^{-1} ($q_{xy} = (q_x^2 + q_y^2)^{1/2}$) corresponds to the triangular lattice of the hydrocarbon chains with the period $a = 4.91 \pm 0.04$ Å. The area per chain, $S = 20.8 \pm 0.5$ Å^2, within the measurement error coincides with the value in the hexagonal bulk phase $P_{\beta'}$ of phosphocholine crystals [1].

The positions and intensities of the peaks in $R(q_z)$ can be satisfactorily described by the electron density profile specified by only five parameters, which can be easily determined using the X-ray diffraction data for phosphocholine crystals. The volume occupied by the $-CH_2-$ group in the hexagonal phase is $V_0 \sim 25-26$ Å^3 [1, 17]. Thus, the thickness of the layer of hydrocarbon tails $CH_3-(CH_2)_{16}$ of DSPC is $L_1 \sim 17 V_0/S \approx 20$ Å and the electron density of this layer is $\rho_1 \approx \rho_w$ (137 electrons in the volume $L_1 S$). Since $L = 2(L_1 + L_2)$, the thickness of the layer formed by polar groups is $L_2 = L/2 - L_1 \sim 15$ Å and its density is determined by 164 electrons of the glycerophosphocholine group $\rho_2 = 164e^-/2SL_2 \approx 0.8\rho_w$ (the $C_{44}H_{88}NO_8P$ molecule contains 438 electrons).

The dashed and dash−dotted lines in Fig. 2 correspond to the structure factors given by Eq. (1) for the model of the multilayer formed by the six and seven bilayers, respectively (see Fig. 4b, table). In these experiments, a decrease in $R(q_z)$ at large q_z values is well described by the calculated value $\sigma_0 = 4.2 \pm 0.2$ Å. A better quantitative agreement of the model with the experimental data at $q_z > 0.05$ Å^{-1} can apparently be achieved by taking into account the presence of topological defects of the occupation of the multilayer at the interface with air. However, in this case, the certainty of the description will require exact knowledge of the morphology of the surface.

Thus, DSPC molecules are adsorbed as a monolayer on the surface of water, whereas they form a multilayer of membranes on the surface of hydrosol. The estimate shows that the areas per lipid molecules both in the monolayer on the surface of water and in the bilayer are approximately the same, 42 Å^2. However, according to the parameters of the models presented
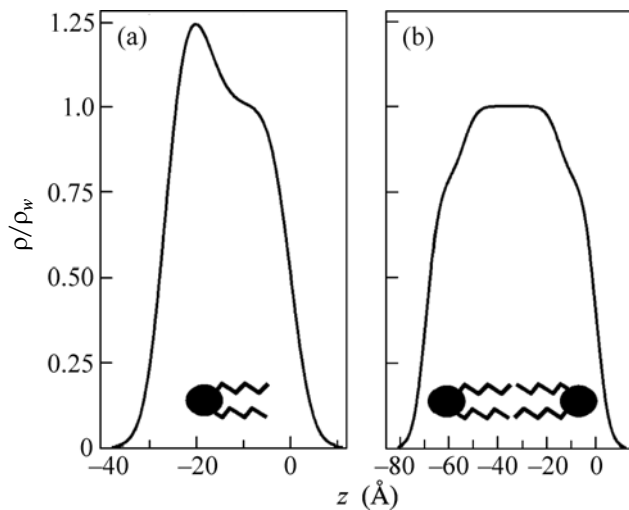
Fig. 4. Model profiles $\rho(z)$ normalized to $\rho_w = 0.333\,e^-/\text{Å}^3$ for the (a) lipid monolayer on the surface of water and (b) the lipid bilayer in the multilayer on the surface of the hydrosol. The interfaces of the layers with air and the substrate are at $z = 0$ and $z < 0$, respectively.

in the table, the layer of polar groups in the monolayer is ~50% denser and ~50% thinner than that in the bilayer. This indicates that the configurations of glycerophosphocholine groups are different in these layers. This is possibly due to the hydration of the polar group of the DSPC molecule on the surface of water, whereas lipid molecules in the multilayer apparently remain dry [18, 19].

To conclude, the observed thickness of the adsorbed DSPC layer, ~400 Å, corresponds to the Debye screening length in the hydrosol bulk (~1000 Å) and to the width of the transient layer on its surface, which appears due to the difference between the potentials of the electrical image forces for Na^+ cations and negatively charged nanoparticles (macroions) [20]. The spontaneous formation of the lipid multilayer is apparently attributed to the unique boundary conditions on the surface of hydrosol on which the electric field orienting molecular dipoles reaches ~10^9–10^{10} V/m [16]. A high concentration of Na^+ ions on the surface of hydrosol, ~10^{19} m^{-2}, can also promote the formation of the multilayer. The ability of sodium ions to penetrate deep into phospholipid membranes and, thus, to form a positive surface potential, was discussed earlier in [21, 22].

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