Molecular dynamics and phase transitions in phospholipid monolayers at liquid—liquid interfaces

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Stable *n*-hexadecane/water and *n*-tetradecane/water macroemulsions containing monolayers of natural (egg yolk lecithin, EY) and synthetic (dimyristoylphosphatidylcholine, DMPC) phospholipids at liquid—liquid interfaces were prepared. The existence of the monolayers was proved by studying the reduction kinetics of a surface-active spin probe with ascorbate anions. Spin labeled derivatives of stearic acid in which the nitroxide group is locared at different distances from the polar head (5-, 12-, and 16-doxylstearic acids) were used to study the temperature dependences of the molecular ordering, rotational mobility, and local polarity in the monolayers in emulsions and also in bilayers in liposomes obtained from the same lipids. In the EY monolayers, the degree of spin probe solubilization is higher, while the order parameters (S) and rotational correlation times (τ) are lower than those in EY bilayers. The differences between these parameters for mono- and bilayers increase with an increase in the distance of the reporter group from the aqueous phase. In the DMPC monolayers, a first-order phase transition was detected by measuring the temperature dependences of S and τ. The temperature region of the phase transition in monolayers is shifted to lower temperatures with respect to that for bilayers and depends on the nature of the oil phase. It was concluded that the phospholipid monolayers in emulsions incorporate hydrocarbon molecules, whose concentration in the DMPC monolayers increases on going from the low-temperature (gel) to the high-temperature (liquid crystal) phase.

Key words: spin probes, molecular dynamics, monolayers, liquid—liquid interfaces, phospholipids, phase transitions.

Many important processes in chemistry and biology (catalysis, extraction, and ion and electron transport) occur at liquid—liquid interfaces. The characteristics of these processes depend mostly on the molecular parameters (molecular ordering, conformations, mobility, polarity of the medium, *etc.*) of the interface rather than on its macroscopic properties. These molecular parameters differ markedly from those in the bulk, owing to the asymmetry of intermolecular interactions at interfaces.[1—3]

In recent years, the development of molecular electronics and bioelectronics has stimulated intense studies dealing with the structure of the Langmuir monolayers formed by surfactants and with phase equilibria at water—air interfaces (see, for example, Ref. 4). Monolayers formed by surfactants, in particular, by phospholipids at interfaces between two nonmiscible liquids, could serve as more adequate models of biological systems (lipid membranes, blood lipoproteins); however, these systems have been studied far less.[5—9] The molecular ordering at a hydrocarbon—water interface has been studied theoretically by molecular dynamics.[5] Experimental studies of the ordering and mobility of surfactant and dye molecules at planar liquid—liquid interfaces have been carried out using generation of the second harmonics,[6] fluorescence depolarization,[7,8] and fluorescence recovery after photobleaching.[9]

The method of spin probes provides detailed information on the molecular dynamics in various chemical and biological systems (see, for example, Ref. 10); however, the potentialities of this method in relation to planar monolayers at a liquid—liquid interface have not yet been exploited, due to the insufficient concentration sensitivity of this method.

It is known that surfactant monolayers at liquid—liquid interfaces can also be formed in oil/water and water/oil emulsions. In recent years, a large number of studies of thermodynamically stable microemulsions have been carried out; in some of them, the spin probe method has been used.[11] However, microemulsions cannot serve as adequate models of planar monolayers or interfaces in biological systems due to both the interface composition (high concentrations of alcohols normally present as co-surfactants) and the large curvatures ($r \leq 50$ Å). Unlike microemulsions, thermodynamically non-equilibrium macroemulsions are characterized by small curvatures with respect to the size of molecules ($r \geq 0.1$ μm) and, simultaneously, by relatively high specific interfacial areas permitting the use of surface active molecular probes. However, virtually no studies of structural and dynamic parameters of macroemulsions at the molecular level have been reported.[12]

Previously,[13—15] using surface active spin probes, we studied the molecular ordering and dynamics in sodium

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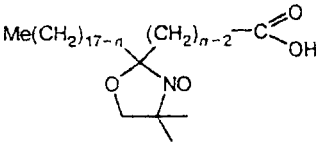
dodecyl sulfate (DDS) monolayers at oil—water interfaces in macroemulsions as functions of the chemical nature of the oil phase, the surface concentration of the surfactant, and the temperature and also the modification of the monolayers with molecules of a co-surfactant or a polymer. In the present study, we prepared for the first time hydrocarbon/water macroemulsions containing monolayers of phospholipids (PL) at the interface. We chose PL, because, on the one hand, they are the major component of biological and artificial membranes (liposomes) and blood lipoproteins in which they occur as monolayers.[16] On the other hand, a gel—liquid crystal temperature-induced phase transition caused by the cooperative disordering of hydrocarbon chains is known to occur in bilayers of one-component PL.[17] It was of considerable interest to find out whether a phase transition of this kind can occur in PL monolayers at liquid—liquid interfaces in an emulsion and also to study its specific features resulting from the effect of the hydrocarbon phase. Therefore, in this work, we studied temperature dependences of the ESR spectral patterns of the spin probes introduced into PL. We used both a PL isolated from biological membranes, which is heterogeneous in the lipid composition (egg yolk lecithin (EY)), and a one-component synthetic PL (dimyristoylphosphatidylcholine (DMPC)). In order to elucidate the specific character of monolayers, these studies were carried out for both monolayers (emulsions) and bilayers (liposomes). As spin probes, we used 5-, 12-, and 16-doxylstearic acids in which the reporter NO group is located at various distances from the aqueous phase.

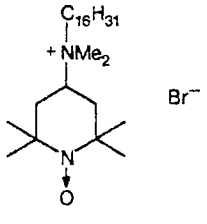
Experimental

Materials. Phospholipids (EY and DMPC) and spin probes. *n*-(4,4-dimethyloxazolidin-*N*-oxyl)stearic acids with n = 5, 12, 16 (probes **1**, **2**, and **3**, respectively) were received from "Sigma."



n = 5 (**1**), 12 (**2**), 16 (**3**)

In addition, in special experiments dealing with the determination of the proportions of emulsions and liposomes in the dispersions, surface-active radical cation **4** (synthesized in the prof. L. Packer's laboratory, USA) was used.



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Hexadecane and tetradecane of the "pure" grade were distilled *in vacuo*. The other reagents were of the "chemically pure" grade.

Preparation of emulsions and liposomes. Variation of the composition of the mixture and the conditions of mixing and dispersion as well as investigation of the stabilities of the resulting emulsions made it possible to choose the following preparation procedure. A mixture of bidistilled H_2O, a hydrocarbon (hexadecane or tetradecane), and a phospholipid taken in a ratio of 20 : 1 : 0.1 (w/w) with a total volume of ~5 mL was dispersed using a UZDN-2 ultrasonic disperser (22 kHz) at 0 °C by three consecutive exposures 2 min each and with 2 min intervals between the exposures. According to the data of dynamic light scattering, the resulting emulsions consisted of particles with an average diameter of ~0.1 mm and were stable over a period of 48 h.

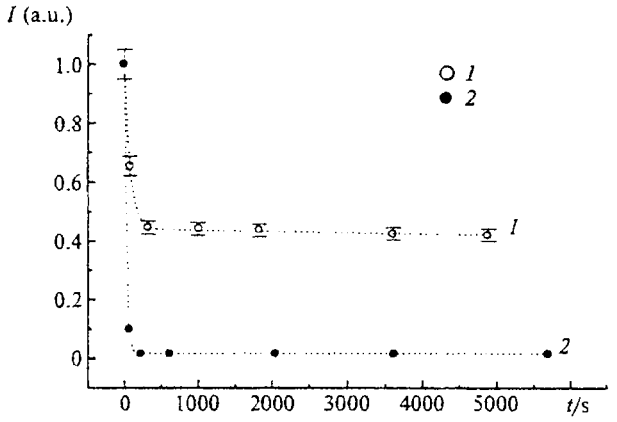

Fig. 1. Kinetics of the reduction of probe **4** in DMPC liposomes (*1*) and in hexadecane/DMPC/H_2O emulsions (*2*). Temperature 2 °C, pH 10.2; concentration of the probe 10^{-4} mol L^{-1}, concentration of the ascorbic acid $3 \cdot 10^{-3}$ mol L^{-1}. The dashed line shows the simulation of the kinetic curves by exponents using nonlinear least-squares method. The pseudo-first-order rate constants and the contributions of the fast (k_1, A_1) and slow (k_2, A_2) steps for liposomes: $k_1 \approx 1.17 \cdot 10^{-2}$ s^{-1}, $A_1 \approx 0.56$ and $k_2 \approx 10^{-5}$ s^{-1}, $A_2 \approx 0.44$; for emulsions: $k_1 \approx 3.8 \cdot 10^{-2}$ s^{-1}, $A_1 \approx 0.984$ and $k_2 \approx 5 \cdot 10^{-6}$ s^{-1}, $A_2 \approx 0.016$.

Liposomes from both phospholipids were also prepared by sonication with a UZDN-2 instrument at a H_2O : phospholipid ratio of 200 : 1 by weight; the conditions of dispersion were similar to those described above. According to dynamic scattering and ESR (see below), the average diameter of liposomes was 0.05—0.1 mm.

ESR measurements. Spin probes **1**—**3** (final concentration $5 \cdot 10^{-5}$ mol L^{-1}) were introduced into the prepared emulsions or liposomes from their solutions in EtOH. The resulting bulk concentration of EtOH in the sample did not exceed 0.1% and had no effect on the ESR spectral parameters. In special experiments (see below), probe **4** was introduced into a mixture of the components prior to dispersion.

ESR spectra were recorded on a Bruker ER-220 instrument using flat quartz cells; the temperature was maintained constant with an accuracy of ±0.5 °C. The temperature was measured using a copper—constantan calibrated thermocouple, which was placed, together with the sample, at a distance of 1.5 cm from the center of the cavity. The ESR spectra were recorded at an incident microwave power of 13 dB. The modulation amplitude did not exceed 30% of the width of the narrowest ESR line. The ESR spectra were collected on an IBM AT-386 computer using 2—9 accumulations.

The anisotropy of the spin probe molecular rotation was characterized by the distance between the outer extrema in the ESR spectrum ($2A'_{\parallel}$, Fig. 1) and by the order parameter S:

$$S = \langle 3\cos^2\theta - 1\rangle/2 = a_N(A'_{zz} - A'_{xx})/a'_N[A_{zz} - (A_{xx} + A_{yy})/2],$$

where A'_{xx}, A'_{yy}, and A'_{zz} are components of the hyperfine coupling (HFC) tensor ($A'_{xx} = A'_{yy}$) averaged over the molecular motion, which are found from the experimental splittings $2A'_{\parallel}$ and $2A'_{\perp}$ using calibration dependences;[18] A_{ii} ($i = x, y, z$) are the principal values for the tensor components in the absence of molecular rotation; a_N, a'_N are the isotropic HFC constants calculated from the traces of the A and A' tensors, $a_N = (1/3)Sp(A_{ii})$ and $a'_N = (1/3)Sp(A'_{ii})$, or, in the case of fast isotropic rotation, from the distance between the HFC components.

The correlation time for the probe rotation in the rapid rotation region ($\tau \leq 3 \cdot 10^{-9}$ s) was determined in terms of the isotropic rotation model from the following formula:

$$\tau = 6.65 \cdot 10^{-10} \cdot \Delta H_{+1}(I_{+1}/I_{-1} - 1)^{1/2},$$

where I_{+1} and I_{-1} are the intensities of the HFC components with $m = \pm 1$, and ΔH_{+1} is the peak-to-peak line width of the low-field component with $m = +1$ (in G).

Results and Discussion

Determination of the proportion of bi(multi)layered structures in phospholipid emulsions. Since the emulsions were prepared by dispersing mixtures of PL, a hydrocarbon, and water, liposomes can also be formed, together with the emulsions. The formation of liposomes and their concentration cannot be established by standard methods used to study dispersions, for example, by dynamic light scattering. This is also difficult to accomplish by optical microscopy, because most of the dispersed particles are smaller than 0.5 μm.

To determine the concentration of the liposomes formed during the dispersion, we used surface-active cation **4**, whose radical fragment is located in the aqueous phase. This approach is based on the idea that the rate of flip—flop transitions of this probe between the two monolayers in liposomes should be much lower than the rate of chemical reduction of its NO fragment by ascorbic acid in the aqueous phase. Consequently, the kinetics of the probe destruction in liposomes should consist of two steps, and the slow step should be limited by the transition between the inner and outer monolayers. In the case of emulsions containing only one monolayer at the interface, it should be expected that the ESR signal of the nitroxide would rapidly decay in one step as a result of its reduction by ascorbate ions.

To ensure a uniform initial distribution of radical **4** in PL layers, we introduced it in a concentration of 10^{-4} mol L^{-1} into a mixture of the components prior to its dispersion. Ascorbic acid was added to the resulting dispersions of liposomes or emulsions at 2 °C, pH 10.2, and at a final concentration of $3 \cdot 10^{-3}$ mol L^{-1}; after that, the dispersion was immediately (over a period of 30 s) transferred to the cavity of an ESR spectrometer maintained at the same temperature.

The time variations of the amplitude of the central ESR component for the liposomes and emulsions containing DMPC are shown in Fig. 1. As expected, in the case of liposomes, reduction occurs in two steps. Although the number of experimental points is insufficient to draw a reliable conclusion about the order of the reaction, it is known from the literature[19] that in the presence of excess ascorbate, the reaction obeys pseudo-first-order kinetics, *i.e.*, both steps can be described by exponents. The fast step with the characteristic time $t \approx 82$ s corresponds to ~56±3% of radicals, while the second step is characterized by $t \approx 10^5$ s and 44±3% of radicals. The characteristic times for the fast and slow steps are close to the corresponding values for the reduction of nitroxides in the outer and inner lipid layers in other membrane systems.[19,20]

The ratio of the numbers of radicals in the outer and inner monolayers of liposomes makes it possible to estimate the average size of the liposomes. In the case of ultrasonic dispersion with irradiation doses used in our study, bilayered liposomes are mostly formed.[21,22] Assuming that liposomes are spherical, the bilayer is ~50 Å thick, and the concentrations of the probes in the inner and outer layers are equal, we can estimate that the average diameter of liposomes is ~750 Å. This is in agreement with the dimensions of liposomes determined by dynamic light scattering (see Experimental).

The most important result of the kinetic measurements is the fact that in the case of emulsions, the fast step of the reduction proceeds almost completely: the intensity of the residual signal is ~1.5% of the initial intensity (see Fig. 1). Similar results were obtained for the EY-containing liposomes and emulsions. Thus, it can be concluded that hexadecane—PL—water ternary mixtures of the specified compositions dispersed under the conditions used in our experiments can form emulsions containing monolayers of PL with fairly low proportions (1—1.5%) of bilayer structures.

Some researchers[23,24] have attempted to obtain microemulsions in hydrocarbon—PL—water mixtures; however, these attempts were successful only in the presence of a relatively large quantity of a fourth component, namely, a low-molecular-weight alcohol (ethanol, propanol, or butanol). The average size of dispersed particles in the emulsions obtained in our experiments is ~0.1 mm (see Experimental), *i.e.*, they should be classified as macro("midi")emulsions, which are fairly stable.

Location of spin probes 1—3 in emulsions and liposomes. Previously, it has been shown for hydrocarbon—DDS—water emulsions that at pH ≥ 10, molecules of **1** are localized in the DDS monolayer at the interface.[13,14] It can be seen from Fig. 2 that the ESR spectra of this probe in hydrocarbon—EY—water and hydrocarbon—DMPC—water emulsions, like those for DDS monolayers, correspond to anisotropic rotation of the probe (see, for example, Ref. 18), *i.e.*, to its anisotropic environment; this indicates that the probes are located at the interface.

The spectra of probes **2** and **3** at pH 10.2 in liposomes and, to a markedly lesser extent, in emulsions normally consist of two components (Fig. 3). The narrow signal with $a_N \approx 16$ G corresponds to the probes in

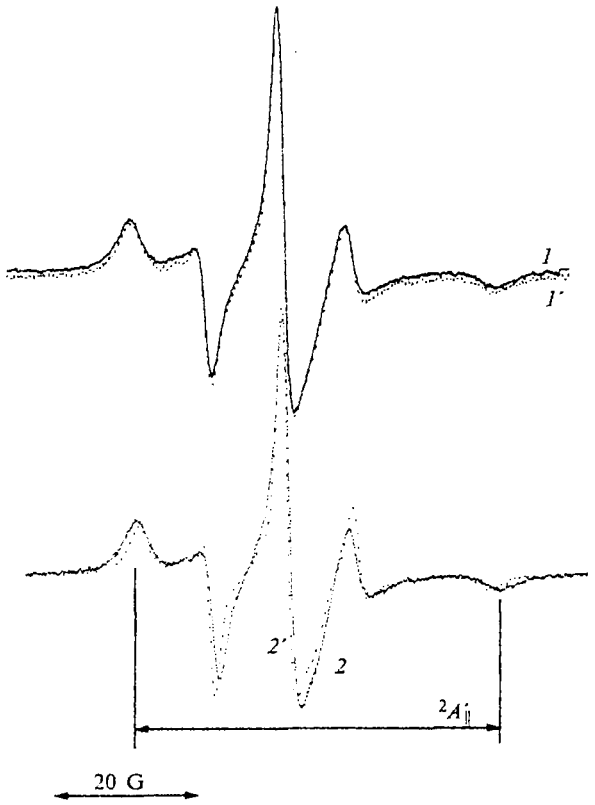

Fig. 2. ESR spectra of probe **1** in EY liposomes (*1*) and in hexadecane/EY/water emulsions (*1'*) at 20 °C; in DMPC liposomes (*2*) and hexadecane/DMPC/water emulsions (*2'*) at 25 °C, pH 10.2.

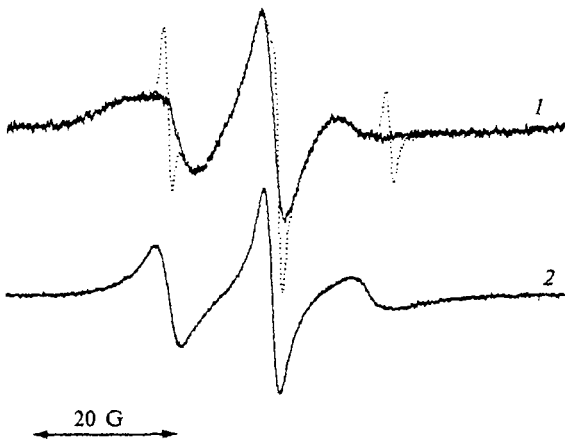

Fig. 3. ESR spectra of probe **2** in liposomes (*1*) and in emulsions (*2*) of DMPC at 20 °C. The dashed lines show the initial signals, and the solid lines correspond to the signals obtained by subtracting the ESR signals of the probes in the aqueous phase.

the aqueous phase. It can be seen from Fig. 3 that this signal can be completely removed from the spectrum by subtracting the reference signal of this probe in an

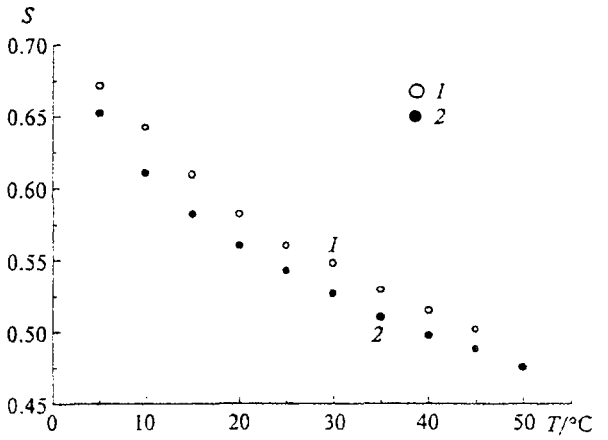

Fig. 4. Temperature dependences of the order parameter (*S*) for probe **1** in EY liposomes (*1*) and in hexadecane/EY/water emulsions (*2*).

Table 1. Distribution coefficients (*K*) of probes **2** and **3** in hydrocarbon—water mixtures at 20 °C, pH 10.2

Probe	Hydrocarbon		
	Decane	Tetradecane	Hexadecane
2	0.28	0.18	0.115
3	0.23	0.21	0.17

aqueous solution recorded at the same temperature. The broad triplet characterized by a_N = 14—15 G and corresponding to slightly anisotropic or isotropic rotation is due to the probes located in the PL layer.

However, in the case of emulsions, the possibility that the probes located in microvolumes of the hydrocarbon phase also contribute to this ESR signal cannot be *a priori* ruled out. To estimate this contribution, we measured the coefficients of distribution of all the probes between the hydrocarbon and water bulk phases: $K = I_{hc}/I_w$, where I_{hc}, I_w are double integrals of the spectra of the probes located in these two phases (Table 1). An emulsion can be represented as a three-phase system[11] in which the coefficient of distribution between the two bulk phases does not depend on the presence of the third phase (monolayer). It can be seen in Table 1 that at pH 10.2, the K values for probes **2** and **3** in hexadecane—water and tetradecane—water mixtures amount to 0.1—0.2. Since the hydrocarbon : water ratio in the emulsions under study is 1 : 20, the integral intensity of the signal from the spin probes located in the hydrocarbon microvolumes does not exceed 0.5—1% and, hence, the major contribution to the observed ESR spectrum is made by the spin probes located in the monolayer.

Monolayers and bilayers of EY. The ESR spectra of probe **1** (see Fig. 2) indicate that its motion in the EY monolayers formed at hexadecane—water interfaces, like

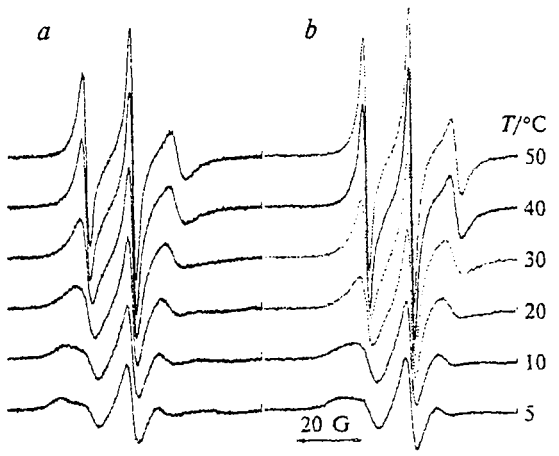

Fig. 5. Temperature variations of the spectral patterns of probe 2 in EY liposomes (*a*) and in hexadecane/EY/water emulsions (*b*).

that in bilayers, is essentially anisotropic and the magnitudes of the order parameter S are somewhat smaller than those found for bilayers (Fig. 4). Meanwhile, the S values for EY monolayers are much greater than those for DDS monolayers at the same hexadecane—water interface[13] (for example, at 5 °C, these values are equal to 0.65 and 0.52, respectively); apparently, this is due to the fact that the non-charged PL molecules are more closely packed in a monolayer. The temperature dependences of S in EY mono- and bilayers are similar; for both systems, the S value decreases monotoneously as the temperature increases in the 5—50 °C range (see Fig. 4).

The ESR spectral patterns for probe 2 in mono- and bilayers are similar (Fig. 5); however, the τ values for monolayers are 1.2—1.4 times smaller than those for bilayers (Fig. 6). The τ values for mono- and bilayers, like the parameter S of probe 1, decrease monotoneously with increase in temperature. A specific feature of the temperature variation of the ESR spectral pattern of probe 2 in liposomes and in EY emulsions is that the I_{+1}/I_{-1} ratio of the HFC components depends only slightly on the temperature, *i.e.*, all the changes in τ are caused by the change in the line width. This character of temperature variations is apparently due to the fact that the probe molecules in mono- and bilayers are distributed over various conformations characterized by different a_N and τ values, and the width of this distribution decreases as the temperature increases.

A more pronounced difference between mono- and bilayers can be observed in the spectra of probe 3. The motion of this spin probe in both systems is almost isotropic and corresponds to the "rapid rotation" region; the ratio between the τ values in liposomes and emulsions (τ_L/τ_E) changes from 1.4 to 1.8 (Fig. 7).

Unlike the mobilities, the local polarities characterized by the parameter a_N for spin probes 2 and 3 in EY monolayers at the hexadecane—water interface and in EY bilayers are virtually identical (see Fig. 7).

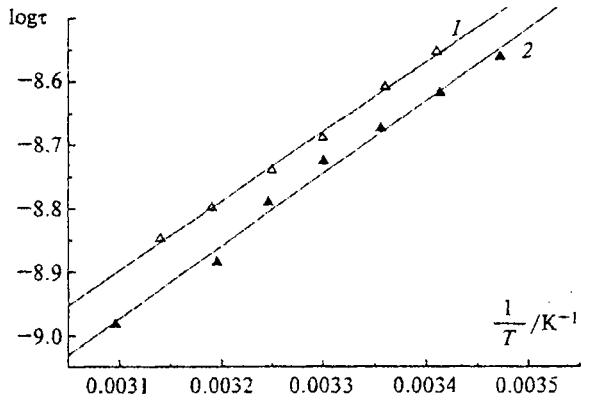

Fig. 6. Temperature dependences of the correlation time of rotation (τ) of probe 2 in EY liposomes (*1*) and in hexadecane/EY/water emulsions (*2*).

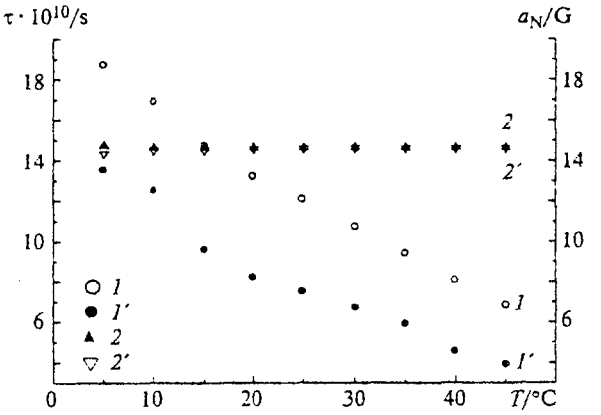

Fig. 7. Temperature dependences of the correlation time of rotation (τ) (*1*, *1′*) and the polarity parameter a_N (*2*, *2′*) for probe 3 in EY liposomes (*1*, *2*) and in hexadecane/EY/water emulsions (*1′*, *2′*).

Hydrated EY layers are known to undergo no phase transitions in the 5—50 °C range; however, the oil phase (hexadecane) crystallizes in macrovolume at 18 °C. It can be seen from Figs. 6 and 7 that the temperature dependences of times τ for both spin probes (like that of the parameter S for probe 1) in the monolayer are monotonic in the 5—50 °C range. Since the reporter group of probe 3 is located near the boundary between the monolayer and the oil phase, this result can be explained by the fact that the oil phase does not crystallize near the monolayer, probably because the structure of the liquid in the near-surface layer is perturbed and a long-range order cannot arise. We have observed a similar absence of crystallization in the case of DDS monolayers formed at the interfaces in emulsions of various hydrocarbons.[14]

Thus, the obtained results make it possible to conclude that the molecular dynamic parameters of spin labeled lipids in polar regions of EY monolayers at a hexadecane—water interface are close in magnitude to

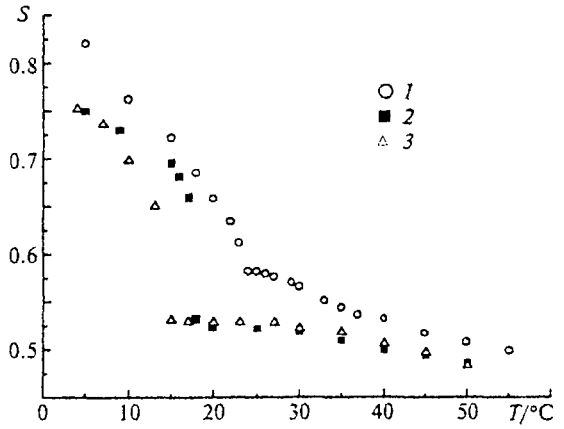

Fig. 8. Temperature dependences of the order parameter (S) of probe **1** in DMPC liposomes (*1*), hexadecane/DMPC/water emulsions (*2*), and in tetradecane/DMPC/water emulsions (*3*).

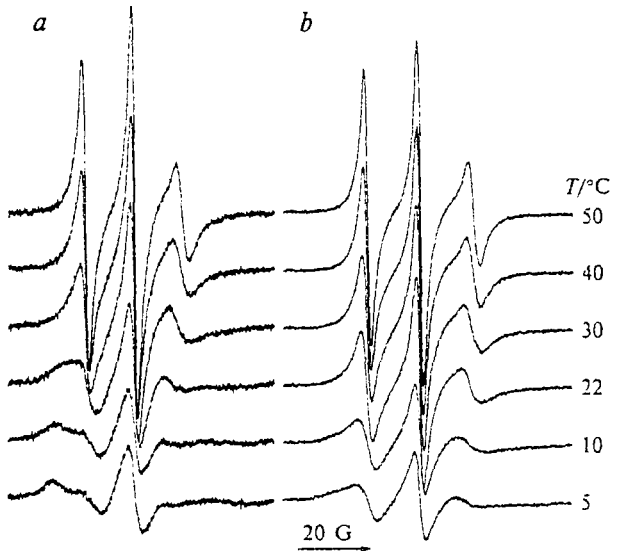

Fig. 9. Temperature variations of the spectral patterns of probe **2** in DMPC liposomes (*a*) and hexadecane/DMPC/water emulsions (*b*).

the corresponding parameters of EY monolayers in bilayer membranes. As the distance from the aqueous phase increases, the orientational ordering in the monolayers of emulsions decreases, while the rotational mobility increases to a greater extent than that in a bilayer. This is probably due to the fact that molecules of the oil phase are incorporated into the emulsion monolayer, whereas mutual intercalation of PL monolayers in bilayer membranes in the aqueous environment virtually does not occur.[25]

DMPC monolayers and bilayers. The ESR spectral patterns of probe **1** both in monolayers and in bilayers of DMPC imply an anisotropic local environment (see Fig. 2); the magnitudes of S are larger than those for EY (Fig. 8).

In the temperature dependences of the order parameter for bilayers, a sharp change in the S value and a change in the slope of the $S(T)$ dependence can be observed (see Fig. 8). The temperature T of this change corresponds to the gel—liquid crystal phase transition in hydrated DMPC bilayers (23 °C). It is significant that a fairly sharp change in the parameter S is also observed for DMPC monolayers in hydrocarbon/water emulsions (see Fig. 8). This transition occurs at a lower temperature than that in liposomes, and, besides, its position depends on the nature of the oil phase. For hexadecane emulsions, the abrupt change in S occurs at 16—18 °C, *i.e.*, near the melting point of hexadecane. However, this transition is not associated with melting of the hydrocarbon microvolumes in the emulsions, because it is not observed in emulsions with EY (see Fig. 4); in addition, for tetradecane-containing emulsions, this transition occurs at T = 10—15 °C, whereas the melting point of tetradecane is 5.5 °C.

It can be seen in Fig. 8 that the S values for monolayers are smaller than those for bilayers at temperatures both below and above the phase transition temperature and that the largest difference is observed in the transition region. These differences can be explained by assuming that hydrocarbon molecules are incorporated into the monolayer both in the solid (gel) and mesomorphic phases.

Similarly, the rotational mobility of probe **2** in monolayers is larger than that in bilayers at temperatures above and below T_c. In fact, as can be seen in Fig. 9, the region of fast rotations ($\tau \leq 3 \cdot 10^{-9}$ s) in bilayers corresponds to $T \geq 30$ °C, whereas in the case of monolayers at a hexadecane—water interface, it corresponds to $T \geq 10$ °C. For identical temperatures higher than T_c (in the 30—50 °C range), the τ_L/τ_E ratio is 1.2—1.4, *i.e.*, it is approximately equal to that found for EY; the τ values for the two lipids are also close to each other.

The temperature dependence of τ for spin probe **2** in monolayers also has a bend near 18 °C; however, it is not as sharp as in the case of probe **1** (Fig. 10).

The spectra of probe **3** in DMPC monolayers in emulsions (like those for EY monolayers) correspond to the rapid rotation region over the whole temperature range, 5—50 °C. The difference between the rotational mobilities of this probe in liposomes and emulsions is much larger than that in the case of probe **2** (see Fig. 10); in fact, in the 30—50 °C temperature range, the τ_L/τ_E ratio is 1.6—1.8, while at 10 °C, it reaches 4. It is reasonable to explain the different τ_L/τ_E ratios for probes **2** and **3** by a higher concentration of hydrocarbons in the hydrophobic part of the monolayer in which the nitroxide group of probe **3** is localized.

The $\tau(T)$ dependence for probe **3** in liposomes exhibits a clear-cut bend at the point of phase transition (~23 °C). It can be seen from Fig. 10 that a weak bend

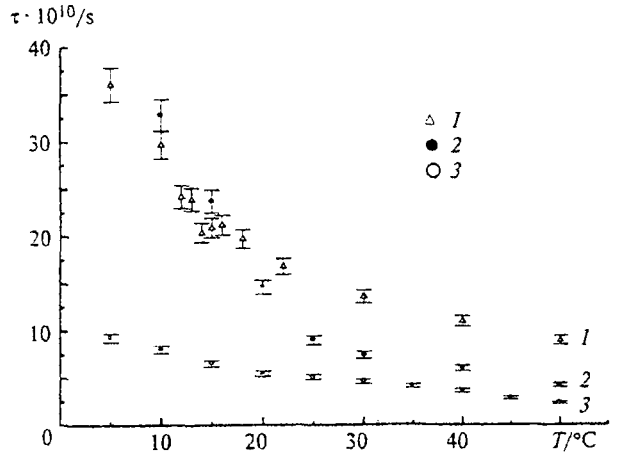

Fig. 10. Temperature dependences of the correlation time of rotation (τ) of probe **2** in hexadecane/DMPC/water emulsions (*1*) and of probe **3** in DMPC liposomes (*2*) and hexadecane/DMPC/water emulsions (*3*).

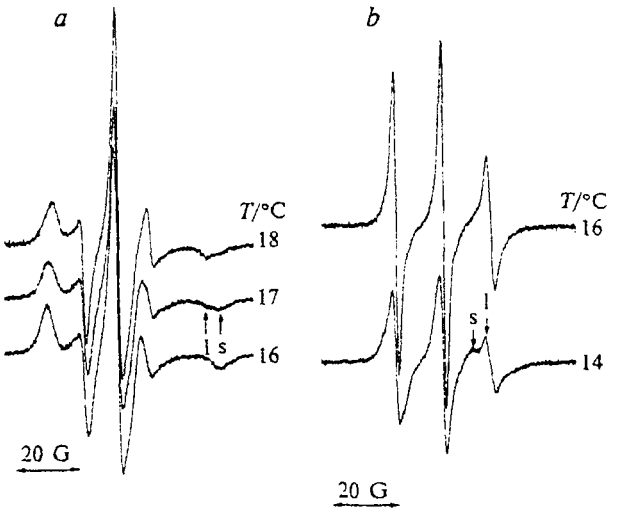

Fig. 11. Spectral patterns for probes **1** (*a*) and **3** (*b*) in the region of the gel—liquid crystal phase transition in the DMPC monolayers at the hexadecane/water interface of emulsions. The arrows mark the ESR signals due to the gel phase (s) and the liquid-crystal phase (l).

at 15—18 °C also occurs in the case of emulsions (both bends can also be observed in the $\tau(T)$ dependences plotted in the Arrhenius coordinates, which are not shown in the figure). Thus, the phase transition occurring in the DMPC monolayer at a hydrocarbon—water interface involves all areas of the monolayer; however, the degree of structural changes varies depending on the distance from the aqueous phase: it is the greatest in the polar area and decreases on passing to the hydrophobic area of the monolayer.

Analysis of the ESR spectral patterns for probe **1** and, especially, probe **3** indicates that near the phase transition, the signals due to the low-temperature and high-temperature phases overlap (Fig. 11), *i.e.*, the phase transition occurs over a certain temperature range. (Fig. 11 shows the spectrum of probe **3** recorded at 14 °C in which the signals corresponding to both phases can be clearly seen; however, at this temperature, the integrated intensity of the signal from the mesomorphic phase is much lower than that of the signal from the gel phase. The intensities of both signals are comparable at 16 °C.) The coexistence of the two phases in a certain temperature range can be explained by the fact that in the presence of a hydrocarbon, the monolayer is a two-component system and, according to the Gibbs phase rule, it has two degrees of freedom (the mole fraction of the hydrocarbon and the temperature), which allow coexistence of the gel and the liquid crystal in a certain temperature range.

Molecular dynamics and phase transitions in mono- and bilayers. It is normally assumed that the interaction between the phospholipid monolayers in a bilayer is weak, and the gel—liquid crystal phase transition is determined by interactions inside the monolayers (see, for example, Ref. 17). These views are in agreement with some experimental data for the Langmuir monolayers formed by PL at water—air interfaces and for PL monolayers supported on solid substrates with grafted hydrocarbons.[17,26,27] In particular, the temperatures of phase transitions in layers of the latter type are close to the corresponding values for bilayers. This is apparently due to the fact that the intercalation of the hydrocarbon (octadecane) into the PL monolayer is weak or totally lacking, because the monolayers were applied in the compressed ("crystalline") state, and diffusion of grafted hydrocarbons into the monolayer was hampered.

The monolayers studied in the present work are formed on a free surface of emulsions without external surface pressure. Apparently, the most important feature of these layers determining the difference between their phase transitions and those in bilayers is that these monolayers incorporate molecules of the oil phase.

A number of studies dealt with the effect of admixed alkanes on the gel—liquid crystal transition in multibilayer membranes (see, for example, Ref. 28). It was found that in the case of DMPC, the addition of long-chain alkanes ($n \geq 12$) results in an increase in T_c, whereas the addition of hydrocarbons with $n < 12$ decreases T_c. However, the data obtained in our study indicate that in the case of monolayers formed at emulsion interfaces, long-chain alkanes (tetradecane and hexadecane) cause a decrease rather than an increase in the T_c value.

The effect of hydrocarbons on the phase transition in a monolayer can also be considered in terms of the theory of two-component solutions. It is known that for two-component mixtures of PL in membranes, two

extreme cases can be distinguished: (1) non-miscibility of two solid phases (so-called lateral phase separation) and (2) complete mixing both in the liquid and solid phases.[29] We consider a more complex situation when a DMPC monolayer is in contact with the hydrocarbon phase in emulsion microvolumes, and the concentration of hydrocarbons in the monolayer can change on melting. However, the main experimental results, *viz.*, the decrease in the phase transition temperature, the presence of a temperature range in which the solid and liquid phases coexist, and the noticeable difference between the molecular dynamic parameters and solubilization of spin probes for mono- and bilayers of phospholipids, can be explained qualitatively by the fact that the monolayers in the emulsions contain molecules of the organic phase.

In fact, the broadening of the phase transition range in the monolayers compared to that for the bilayers is caused by the fact that cooperativity areas become smaller in the presence of hydrocarbon molecules. Since the average T_c value is determined by the $T_c = \Delta H_c/\Delta S_c$ ratio, where ΔH_c and ΔS_c are the enthalpy and the entropy of the transition, respectively, the decrease in T_c is due either to an increase in ΔS_c or to a decrease in ΔH_c. When a monolayer contains a hydrocarbon, the entropy of mixing contributes to the total entropy. If the amount of the hydrocarbon in the high-temperature (mesomorphic) phase of the monolayer is greater than that in the low-temperature (gel) phase, the ΔS_c value increases. The sign of the change in ΔH_c caused by the intercalation of hydrocarbon molecules into a monolayer is not so unambiguous. The further decrease in T_c observed on going from hexadecane to tetradecane can be attributed to the increase in the mole fraction of the hydrocarbon in the monolayer, which leads to an increase in the entropy of mixing.

The effects of the intercalation of the oil phase molecules on the dynamic parameters of spin probes are different at various depths within the monolayer; they also depend on the nature of the phospholipid and on the temperature. Apparently, a hydrocarbon is present in the lowest concentration in the more ordered near-surface region of the monolayer (in which the NO groups of spin probe **1** are located), whereas in the region of relatively disordered hydrocarbon tails of the acyl groups, its concentration is the highest.

According to this model, in the case of EY, the relative differences between the dynamic parameters of spin probes in mono- and bilayers increase on moving from the polar to the hydrophobic part of the layer. These differences depend only slightly on the temperature, which can be explained by the fact that the concentration of hydrocarbons in an EY monolayer also slightly depends on the temperature.

In the case of mono- and bilayers of DMPC, the difference between the dynamic parameters of probe **1** and, especially, probe **3** is quite pronounced even at temperatures below the phase transition temperature range (see Figs. 8 and 11). The patterns of the ESR spectra recorded for these probes in mono- and bilayers at $T < T_c$ indicate the absence of substantial spin-spin coupling, *i.e.*, the absence of clusterization of the probes in the gel phase, which is observed, for example, during crystallization of pure hydrocarbons. Therefore, the differences mentioned above can be explained by assuming that in the monolayer, DMPC and a hydrocarbon form a solid solution in which the spin probes are distributed more or less uniformly. The decrease in the difference between the S and τ parameters for mono- and bilayers following an increase in the temperature is apparently due to the fact that away from the phase transition, the difference between the dynamics of the hydrocarbon chains in PL and alkanes becomes less pronounced.

Thus, in the present study, we prepared monolayers of phospholipids at hydrocarbon—water interfaces of macroemulsions and studied the molecular-dynamic parameters of the monolayers at various distances from the aqueous phase using spin probes. It was shown that molecules of the dispersed oil phase of the emulsion can be incorporated into the monolayers formed from the natural and synthetic phospholipids. A phase transition in the monolayers of dimyristoyllecithin was found; the temperature of this transition is lower than that found for bilayers and depends on the nature of the oil phase.

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