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SPIN PROBE ESR STUDY OF THE HYDRATION OF ORIENTED EGG YOLK PHOSPHATIDYLCHOLINE BILAYERS

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The method of parallel-beam spattering enables the preparation of well oriented egg yolk phosphatidylcholine (EYPC) multilayers on the solid surface. The increasing hydration of these multilayers induces a decrease in the order parameter S_z and an increase in the probability of gauche conformers in the EYPC acyl chains as detected by DPPC molecules spin labeled on the 12th or 16th carbon of the acyl chain. The dependences of S_z on the number of incorporated water molecules contain the information equivalent to the sorption isotherm. Using the 16-DPPC and 12-DPPC spin probe data, the nominal pressure P_0 and exponential decay factor ν have been obtained for the hydration repulsion between the bilayers.

1. Introduction

Considerable interest has focussed on the structure and physical properties of lipid bilayers following realization that they are a common component of biological membranes. A range of bilayer preparations formed from both natural and synthetic lipids have been widely used as simplest models of biological membranes. Phases of greatest interest have been shown to be lyotropic liquid crystalline phases of amphiphilic lipids wherein lipid layers are separated by layers of water [1]. Many physical studies of lyotropic phases require oriented samples, i.e. ones in which many lipid layers are stacked parallel to one another. As a result, several techniques have been developed by which oriented samples may be produced. Except micellar lyotropic liquid crystals which orient in applied magnetic field [2-5], all the other techniques make use of the orienting effect of solid surfaces on the lipid layers. Probably the best oriented multilayers are produced by Langmuir-Blodgett technique [6,7]: the layer by layer deposition onto a solid surface is made by raising and lowering the solid through the lipid monolayer at the air-water interface while keeping the surface pressure constant. Second technique

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involves smearing and/or pressing the lipid sample between two parallel surfaces [8-10]: the lipid bilayers slide on each other practically without friction at higher water concentration, so that the shearing forces and the contact with solid surfaces arrange the bilayer domains in parallel layers on the supporting surfaces. A modification of the smearing technique is a prolonged heating of the lipid sample compressed between two solid surfaces [11,12]. This seems to be the most succesful technique in terms of the maximum area and thickness of the obtained oriented sample. Orienting effect of the solid surface is used also in the fourth technique - deposition from evaporation of lipid solution on the solid surface and subsequent hydration [13-17]. All four described techniques suffer from several drawbacks. For instance, the Langmuir-Blodgett technique needs rather complicated apparatus, the smearing techniques work well for highly hydrated samples but not for samples with low level of hydration, where the interaction between bilayers is strong. The samples prepared by the compression techniques disorder upon increase of temperature due to undulative instability - an effective dilative strain caused by the decrease of bilayer thickness, deposition from evaporation yields very often a mixed orientation of the bilayers because of the rounded edges of spots forming during evaporation of the solvent.

Kawano et al. [18] have developed a new improved technique termed the parallel beam spattering (PBS) method for depositing lipid bilayers on solid surfaces. This technique involves atomizing the lipid solution with a stream of gas and passing this atomized mixture through orifices before deposition on the solid surface. The authors claim that the PBS method yields highly reproducible results.

The main aim of our work is to reproduce the PBS method preparing oriented bilayers from egg yolk phosphatidylcholine (EYPC) and to investigate effects of hydration on the spin probe ordering in bilayers using electron spin resonance spectroscopy (ESR). EYPC is the naturally occuring and readily available phospholipid and is in the physiologically relevant liquid crystalline bilayer phase over broad temperature and hydration ranges [19]. Effects of hydration on phospholipid properties has been studied earlier by ESR spin probe method by Jost et al. [20] and by Korstanje et al. [21]. They have found that the order parameter of spin probes located in the lipid bilayer hydrophobic region decreases with the increase of lipid hydration. The second aim of our work is to study these effects in more detail using well oriented samples.

2. Material and methods

Phosphatidylcholine from hen egg yolks (EYPC) was prepared and purified according to Singleton et al. [22]. Its purity was checked by a thin-layer chromatography. The content of peroxides determined spectrophotometrically according to paper [23] was less than 1%. Dipalmitoylphosphatidylcholine labeled with the paramagnetic dimethyloxazolidinyl (doxyl) group on the m-th carbon atom of the sn-2 acyl chain (m-DPPC) was kindly provided by Dr. K. Ondriaš (Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava). The other chemicals were of analytical grade (Lachema Brno, The Czech Republic). The glass and quartz plates for the sample deposition had surfaces polished to the optical quality. We found that the plates had to be initially scrupulously clean and free of defects, cracks, and scratches to obtain

reproducible results. Cleaning of the plates involved several steps as suggested in [24]: 1. dipping and swabbing in benzene, 2. dipping and swabbing in acetone, 3. dipping in boiling aqua regia for 5 minutes, 4. washing with redistilled water, and 5. drying under the stream of N_2. After this procedure the surface was hydrophilic. Kuo and Wade [24] suggested to treat the surface with p-xylene or to dip it several times in 1% solution of N-hexadecyl-N,N,N-trimethylammonium bromide in chloroform according to an US patent [25] to make the surface hydrophobic. However, we obtained satisfactory results with hydrophilic surfaces, so that we did not hydrophobize them.

The EYPC + m-DPPC mixture to be applied to the solid surface was dissolved in ethanol or in methanol at the concentration of 125 mg/ml. The molar ratio of m-DPPC:EYPC was less than 1:100. The lipid deposition on the surface of the glass/quartz plate was conducted as depicted in Fig.1. The EYPC+m-DPPC solution contained in a needle of Hamilton microsyringe HS was atomized by a stream of nitrogen gas expired from a Pasteur pipette P. The angle between HS and P was about 45° as suggested in [18], and the flow of nitrogen gas was kept as low as possible but strong enough to secure efficient atomization. The beam of atomized solution then passed through a 3mm × 6mm rectangular orifice in the copper plate CP1. The atomized particles were deposited as a spot at the center of the glass/quartz plate PL laying on the copper plate CP2. The glass/quartz plate with the deposited lipid film was inserted into a cylindrical glass tube GT (external diameter 5 mm) opened at both ends. A short tube filled with P_2O_5 was connected to the lower end of the glas tube GT by a stick tape.

The sample was then evacuated at about 10^{-3} Pa for several hours at room temperature. After evacuation the glas/quartz plate was fixed in the centre of the glas tube GT by a teflon plug TP and the free end of glass tube GT was sealed tightly with Parafilm R. For hydration, P_2O_5 under the sample was replaced with a saturated aqueous solution of the ionorganic salt with defined relative water vapour pressure. The sample tube GT was then positioned in a N° 24 GX goniometer (Radiopan, Poland), which allows precise positioning of the sample within the microwave cavity of ESR spectrometer, so that the precise angle Θ of the PL plate normal in reference to the applied magnetic field could be achieved.

ESR spectra of spin probes in the oriented samples were measured by an ERS 230 X-band ESR spectrometer (ZWG AdW DDR, Berlin, Germany) using the 100 kHz modulation technique. Typical instrumental settings were: 5 mW microwave power, modulation amplitude less than one half of the peak-peak width of the central line in the spectra, and the rate of magnetic field sweep 0.25 G/s at 0.5 s time constant. All measurements were made at temperature t=20 ± 1° C.

3. Theory

The spin Hamiltonian required to describe unpaired electron of the oriented nitroxyl spin probe in the external magnetic field $\vec{B}$ is

$$\mathcal{H} = \beta_e \vec{B} \mathbf{g} \mathcal{S} + \mathcal{S} \mathbf{A} \mathcal{I} \qquad (1)$$

where β_e is the Bohr magneton, $\mathcal{S}$ is the electron spin operator, $\mathcal{I}$ is the nuclear spin operator, $\mathbf{g}$ is the g-value tensor and $\mathbf{A}$ the electron-nuclear hyperfine interaction tensor.

Several other interactions have been ignored, including nuclear Zeeman interaction, electron-proton hyperfine interactions, interactions of the nitrogen nuclear quadrupole moment with electric field gradients, and exchange and dipolar interactions among rarely occuring neighboring pairs of spin probe molecules (magnetically diluted sample).

The representation of each tensor in eq. (1) depends on the choice of coordinate system. In the nitroxyl spin probes, the unpaired electron occupies an almost pure (80–90%) $2p\pi$ molecular orbital on the nitrogen. This orbital interacts with the ^{14}N nuclear spin and gives rise to a large anisotropic hyperfine interaction which is almost symmetrical about the axis of this orbital. The spin-orbital interaction induces anisotropy in the g tensor. We can ascribe a cartesian coordinate system x, y, z to the nitroxyl fragment such that the x axis is parallel to the N→O bond direction, and the z axis is parallel to the nitrogen $2p\pi$ orbital. Within this molecular coordinate system, the g and **A** tensors are diagonalized. In the x, y, z coordinate system, the z axis is parallel also to the long axis of the spin labeled palmitoyl chain of the m-DPPC molecule, which in the first approximation was found to be parallel to the bilayer director. It is supposed that the palmitoyl chains of the m-DPPC molecule rotate fast (on the ESR time scale) around their long axes with the correlation time of $\tau << 10^{-8}$s, while the motion in the perpendicular directions is much slower. Due to the trans-gauche isomerization, the nitroxyl fragment fluctuates fast around the long molecular axis.

The second coordinate system X, Y, Z is related to the macroscopic sample. Here the Z axis coincides with the director of the oriented bilayers in the lamellar liquid crystal phase. Transforming **g** and **A** into the X, Y, Z system and taking into account the molecular motion, the averaged tensors $\mathbf{A}'$ and $\mathbf{g}'$ are obtained which are invariant against rotation around the Z axis ($A'_{XX} = A'_{YY} = A_\perp$, $A'_{ZZ} = A_\parallel$, $g'_{XX} = g'_{YY} = g'_\perp$, $g'_{ZZ} = g_\parallel$, $A'_{IJ} = g'_{IJ} = 0$ for $I \neq J$). The values of the averaged hyperfine splitting tensor's elements $A_\parallel$ ($A_\perp$) and that of the g'-tensor $g_\parallel$ ($g_\perp$) are the experimental values observed when the director is parallel (perpendicular) to the static magnetic field $\vec{B}$. In the case, when the angle between the director and the magnetic field $\vec{B}$ is equal to Θ, the following equation is obtained for the experimentally observed hyperfine splitting

$$A(\Theta) = (A_\parallel^2 \cos^2\Theta + A_\perp^2 \sin^2\Theta)^{1/2} \tag{2}$$

Similar equation is obtained for $g(\Theta)$

$$g(\Theta) = (g_\parallel^2 \cos^2\Theta + g_\perp^2 \sin^2\Theta)^{1/2} \tag{3}$$

From the invariance of tensor traces against coordinate transformations, it follows $2A_\perp + A_\parallel = 3a$ and $2g_\perp + g_\parallel = 3g$ where a and g are the isotropic hyperfine splitting constant and the g-factor value, respectively.

It is well known (see [26]) that the order parameters S_i are defined as

$$S_i = 3\langle\cos^2\Theta_i\rangle - 1 \tag{4}$$

where $i = x, y, z$. The expression $\langle\cos^2\Theta\rangle$ determines the mean square deviation of the particular molecular frame related axis from the director. Due to the orthogonality

properties of the cosines, it follows that

$$\sum_i S_i = 0 \tag{5}$$

For molecules with an axially symmetric shape, which is the case of fatty acids and phospholipids labeled at the chain, one order parameter is sufficient to describe the mean orientation. If the molecular z-axis is taken as the symmetry axis, it follows that

$$S_x = S_y = -S_z/2 \tag{6}$$

Usually, the order parameter S_z is determined using values $A_\parallel$ and $A_\perp$ whose experimental observation is easier compared to $g_\parallel$ and $g_\perp$. It can be shown that

$$S_z = \frac{A_\parallel - A_\perp}{A_{zz} - \frac{A_{xx}+A_{yy}}{2}} \tag{7}$$

If $g_\perp$ and $g_\parallel$ are available, an independent evaluation of S_z is possible using an analogical equation

$$S_z = \frac{g_\parallel - g_\perp}{g_{zz} - \frac{g_{xx}+g_{yy}}{2}} \tag{8}$$

Equations (7, 8) were derived under assumption of axialy symetrical motion of the spin probe molecule around the director. As a consequence, $S_x = S_y$ can be computed after (6).

In our paper we study the angular dependences of $A(\Theta)$ and $g(\Theta)$ (eqns. 2 and 3) to calculate isotropic hyperfine splitting constant a, g-factor value g and order parameters S_i, which, according to definition, are measures of the mean angular deviations of the spin probe molecular axes from the normal to oriented bilayers.

The principal values of **A** and g tensors depend on the unpaired electron density at the nitrogen nucleus, which is influenced by the polarity of the environment. The precise values of the A_{ii} and g_{ii} have been determined for several spin probes from monocrystal measurements or by comparing of the experimental and simulated ESR spectra. These values are usually used in equations (7, 8) after corrections for the polarity [27]. Correction factors are

$$f_a = \sum_i \frac{A_{ii}^c}{A_\parallel + 2A_\perp} \tag{9}$$

$$f_g = \sum_i \frac{g_{ii}^c}{g_\parallel + 2g_\perp} \tag{10}$$

where components A_{ii}^c and g_{ii}^c are those determined on monocrystals or using computer simulated ESR spectra. The order parameters corrected for polarity effects are then

$$S_z = f_a \frac{A_\parallel - A_\perp}{A_{zz}^c - \frac{A_{xx}^c + A_{yy}^c}{2}} \tag{11}$$

$$S_z = f_g \frac{g_{\parallel} - g_{\perp}}{g^c_{zz} - \frac{g^c_{xx}+g^c_{yy}}{2}}. \quad (12)$$

The principal values of the **A** and **g** tensors of m-DPPC spin probes used in the present paper were taken from the literature [28]: $g^c_{xx} = 2.0088$, $g^c_{yy} = 2.0061$, $g^c_{zz} = 2.0027$, $A^c_{xx} = 0.65$ mT, $A^c_{yy} = 0.58$ mT, $A^c_{zz} = 3.35$ mT. The S_z values determined with two different position isomers of m-DPPC enable to extract information about the probability of gauche conformations P_g and about effective energy difference E_g between trans and gauche conformations in the liquid-crystalline phase of the lipid. Supposing that the rotations around a single C-C bonds are interdependent and the first segment is immovable, Seelig [9,29] derived for the order parameter of the n-th segment $(S_z)_n$

$$(S_z)_n = S_\sigma^{n-1} S_0 \quad (13)$$

where n is the number of C-C single bonds between the doxyl and carbonyl group of the m-DPPC spin probe, S_σ is the order parameter for a single C-C bond and S_0 the order parameter of the labeled acyl chain of the m-DPPC molecule as a whole. Supposing that S_σ is approximately constant between C_{12} and C_{16} carbons, it can be calculated from the S_z values obtained with 12-DPPC and 16-DPPC spin probes. Provided that intramolecular, resp. intermolecular interactions disfavoure combinations $g^{\pm}g^{\mp}$ [29], resp. $g^{\pm}g^{\pm}$ [30], Marsh [30] derived the following equation for the temperature dependence of S_σ

$$1 - S_\sigma^2 = 9\sigma/(1 + 8\sigma + \sqrt{1+8\sigma}) \quad (14)$$

together with the equation for $P_g = P_{g+} + P_{g-}$

$$P_g = \frac{1}{2}(1 - (1+8\sigma)^{-1/2}) \quad (15)$$

The effective energy difference E_g between gauche and trans conformation at the temperature T is then given by

$$\sigma = \exp[-E_g/RT] \quad (16)$$

where R is the molar gas constant.

4. Results and discussion

The example of the hyperfine splitting dependence on the director orientation relative to the magnetic field $\vec{B}$ is depicted on the Fig.2 and is typical for all samples used in this work. The linear course of the $A(\Theta) = f(\sin^2\Theta)$ dependence is an evidence of well oriented samples.

EYPC multilayers hydrated in the atmosphere of relative vapour pressure $p/p_0 = 0.81$ were investigated using Mn^{2+} standard which enables the determination of $g_{\parallel}$ and $g_{\perp}$ besides of $A_{\parallel}$ and $A_{\perp}$. Results in Table I obtained by using the 12-DPPC spin probe show that the values of the order parameter S_z calculated according to equation (12) (using the **g**′ tensor components) are higher compared to the values of S_z when calculated according to equation (11) (using the **A**′ tensor components). Similar results

Table 1. The parameters $A_{\parallel}$, $A_{\perp}$, $g_{\parallel}$, $g_{\perp}$ and S_z for the 12-DPPC spin probe in EYPC bilayers at different time of hydration in the environment with relative vapour pressure $P/P_0 = 0.81$.

time of hydr.	5	20.3	24.5
$A_{\parallel}$ [mT]	1.932	1.932	1.928
$A_{\perp}$ [mT]	1.105	1.100	1.100
$g_{\parallel}$	2.0072	2.0073	2.0075
$g_{\perp}$	2.0091	2.0092	2.0095
S_z (eq.(11))	0.334	0.337	0.336
S_z (eq.(12))	0.389	0.404	0.418

Table 2. Nominal pressure P_0 and exponential decay factor ν determined using the order parameter S_z of 16-DPPC and 12-DPPC spin probes or using the sorption isotherms of Jendrasiak and Hasty [38] and Elworthy [39].

	P_0 [10^8Pa]	ν
16-DPPC	3.9 ± 0.6	2.6 ± 0.3
12-DPPC	4.3 ± 1.3	2.4 ± 0.6
Jendrasiak, Hasty [38]	3.6 ± 0.1	3.0 ± 0.1
Elworthy [39]	5.0 ± 0.1	2.56 ± 0.08

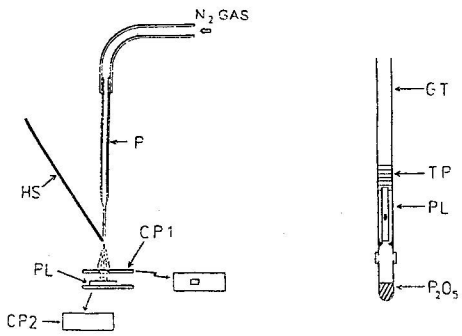

Fig.1: Schematic picture of the apparatus for the preparation of oriented lipid multilayers and the positioning of the oriented sample in the measuring glass tube. The abbreviations in the figure are described in the text.

were achieved with the 16-DPPC spin probe. Observed differences could be caused by at least three factors: 1. The motion of the spin probe is not axialy symmetric. 2. The principal axes of the **A** and **g** tensors do not coincide. 3. The polarity correction is not "correct". It is not possible to distinguish between these possibilities without computer simulation of the ESR spectra. According to most papers dealing with ESR order parameter we shall use the equation (11) for the S_z calculation. It follows from the results in Table I that a care must be taken by independent evaluation of the S_z (e.g. from **A**′ tensor) and S_x (e.g. from **g**′ tensor).

The influence of EYPC multilayer hydration on the order parameter was investigated using the 16-DPPC spin probe. The sample was dried at first and than exposed successively to the suroundings with increasing humidity. It is seen from the Fig.3 that the rise in relative vapour pressure p/p_0 i.e. the rise in hydration of the EYPC bilayers is accompanied by the decrease of S_z. Qualitatively similar results were reported by other authors. Jost et al. [20] observed a decrease in order parameter S_z for four position

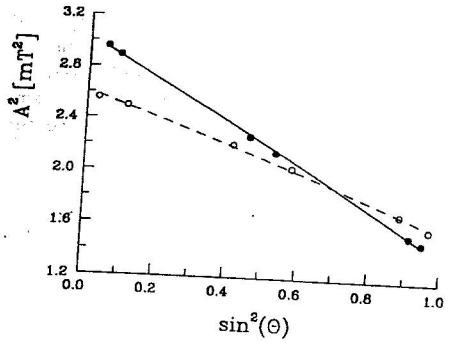

Fig.2: The dependence of $A^2 = f(\sin^2\Theta$ for the 16-DPPC spin probe in EYPC bilayers. Relative vapour pressures $P/P_0 = 0.31$ (full symbols) and 0.81 (open symbols).

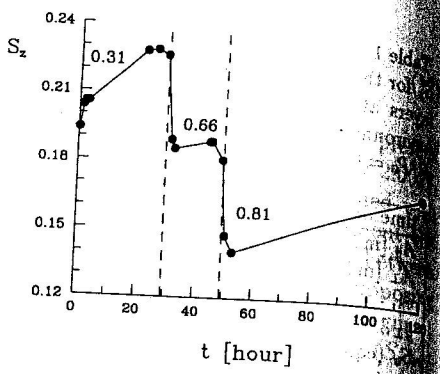

Fig.3: The dependence of the order parameter S_z on the time of hydration at different relative vapour pressure for the 16-DPPC spin probe in EYPC multilayers. The experimental error is ±0.004.

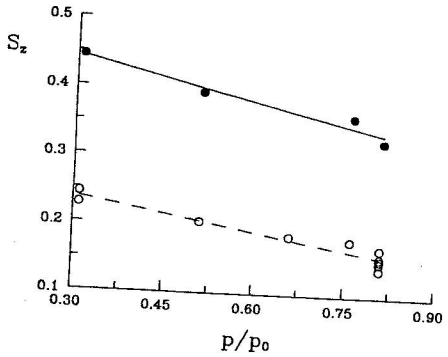

Fig.4: The dependence of the order parameter S_z on the relative vapour pressure P/P_0 for the 12-DPPC (full symbols) and 16-DPPC (open symbols) spin probes. The experimental error is ±0.004.

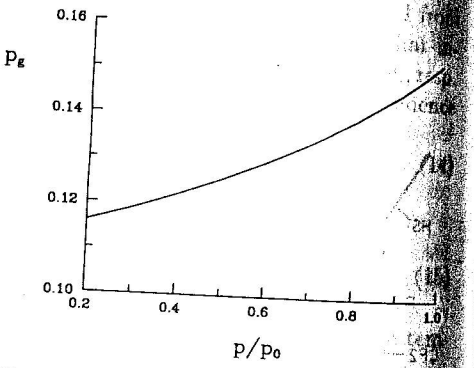

Fig.5: The dependence of the probability P_g of gauche conformers between the 12-th and 16-th carbons of the m-DPPC spin probe on the relative vapour pressure P/P_0 in oriented EYPC bilayers. The interpolated values of S_z (see Fig.4) were used in the calculation of P_g (eq.(15)).

isomers of spin labeled stearic acid in oriented EYPC multilayers by increasing the relative vapour pressure (p/p_0=0, 0.31, 0.81). However, the S_z values determined in their paper are considerable lower compared to our results. For example, the values of S_z obtained by Jost et al. [20] using stearic acid labeled at the 12-th carbon are comparable with our results obtained using 16-DPPC. Kornstanje et al. [21] studied the oriented multilayers of dimyristoylphosphatidylcholine, palmitoyloleoylphosphatidylcholine and dioleoylphosphatidylcholine using cholestane spin probe. They found that an increase

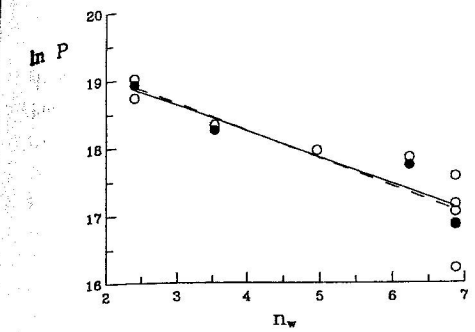

Fig.6: The dependence of the hydration pressure P between EYPC bilayers on the number of water molecules n_W. Full symbols - determined from the order parameter S_z of 12-DPPC, open symbols - determined from the order parameter S_z of 16-DPPC spin probes.

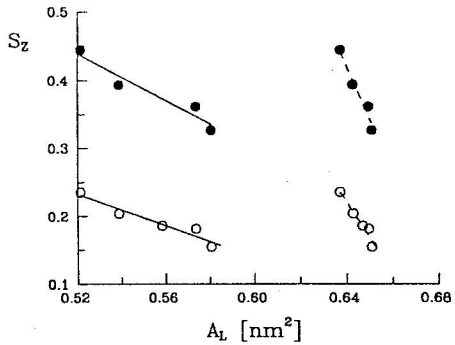

Fig.7: The correlation between the order parameter S_z of 12-DPPC (full symbols) and 16-DPPC (open symbols) spin probes and the area per one lipid molecule A_L determined by Uhríková [34] (full lines) and by White and King [33] (dashed lines).

in the water content induces a decrease in the ordering and increases the mobility of the spin probe. It is evident from the Fig.3 that the value of S_z falls suddenly (during one hour) after the increase of the humidity and a small increase in S_z can be observed with prolonged time of hydration. While the sudden change might be related to the diffusion of water vapour within a sample holder and to consequent diffusion of water molecules through numerous lipid bilayers within the sample itself, the slow process reflects most probably reordering effects within the sample after full hydration, e.g. an elimination of structural defects during such an annealing. According to our experience the rate of the EYPC bilayer hydration may depend on the history of the sample.

The dependences of the S_z on the degree of hydration for the both spin probes are shown in the Fig.4. As in the Fig.3, the samples were dried at first and than exposed successively to the influence of increasing humidity for 20-30 hours. The decrease in the order parameter is observed for both spin probes. The values of the 12-DPPC order parameter are always higher than for the 16-DPPC spin probe which is in accordance with the well known "flexibility gradient" [9, 10, 16, 29].

It was not possible to investigate the dry samples. The motion of the spin probes was probably too slow and the spectra resembled a solid-state powder spectrum. Moreover, a broad single peak was observed in the spectrum of 12-DPPC spin probe. We suppose that the tightly packed acyl chains exclude the 12-DPPC spin probe molecules with bulky doxyl group into less ordered lateral defects within the bilayer where the local high spin probe concentration leads to Heisenberg and dipolar spin-spin interaction and results in a typical broad single-line spectrum. Because the 16-DPPC spin probe has its doxyl group located in less ordered center of the bilayer, similar effect was not observed with this probe.

The dependences $S_z = f(p/p_0)$ for both 12-DPPC and 16-DPPC (Fig.4) were in-

terpolated using a linear least squares method. These interpolated values of S_z were used in calculation of the probability P_g of gauche conformers between the 12-th and 16-th carbons of the m-DPPC molecule and of the effective energy difference E_g between trans and gauche conformations. An increase in P_g (Fig.5) and a decrease in E_g (results not shown) are observed with increasing the relative vapour pressure.

It follows from these results that the increasing amount of water molecules incorporated in the interlamelar region changes not only the properties of the lipid polar head groups but influences also the hydrophobic part of the lipid bilayer, even its central region. Small [31], Torbet and Wilkins [32], White and King [33] and Uhríková [34] have shown that the increase in hydration causes the increase in the area per one lipid molecule on the lipid-water interface. Cevc and Marsh [35] suppose that the rise in the area per one lipid molecule leads to a weakening of inter- as well as intramolecular restrictions in the acyl chain motion. This is in accordance with our results.

Ulrich and Watts [36, 37] studied the influence of dioleoylphosphatidylcholine (DOPC) hydration on the quadrupolar splitting $\Delta\nu_Q$ and the relaxation time T_1 of the specifically deuterated headgroup segments [-O-CD_2-CD_2-N(CD_3)]. They found that the generalized ^{2}H NMR parameter $f(n_W)$ represented by $1/T_1$ or $\Delta\nu_Q$ depends linearly on the water activity a_W for all three deuterated segments. Mathematically, this linear relationship is conveniently expressed by the generalized equation

$$f(n_w) = f_0 + (f_s - f_0)a_W \qquad (17)$$

where f_s and f_0 represents the extrapolated intercepts with $a_W = 1$ and 0, respectively, and corresponds to the hypothetical NMR values at zero and full hydration. The water activity a_W, which is equal to the relative vapour pressure (p/p_0), was related to the number of water molecules n_W per one lipid molecule using published sorption isotherms for DOPC. The equation (17) enables the calculation of effective repulsive pressure P between the lipid bilayers according to the equation

$$P = [-RT/V_W]\ln a_W \qquad (18)$$

where $V_W = 18 \times 10^{-6}\mathrm{m}^3$ is the molar volume of water. To express the hydration pressure P at any hydration level n_W in terms of the NMR parameter $f(n_W)$, Ulrich and Watts [37] substituted equation (17) into (18), giving

$$P = -\frac{RT}{V_W}\ln\frac{f(n_W) - f_0}{f_s - f_0} \qquad (19)$$

It is known that the hydration pressure decays exponentially with the number of water molecules n_W

$$P = P_0 \exp[-n_W/\nu] \qquad (20)$$

where ν is an exponential decay constant in units of number of molecules. Ulrich and Watts [37] found that the values of P_0 and ν determined from eq. (20) using NMR parameters $1/T_1$ and $\Delta\nu_Q$ for all three deuterated segments are in excellent agreement with the results derived from the sorption isotherm.

We applied the same procedure as Ulrich and Watts [37] where the generalized ^{2}H NMR parameter was replaced by ESR order parameter $S_z = f(p/p_0) = f(a_W)$. The EYPC sorption isotherm published by Jendrasiak and Hasty [38] was used for transformation to $S_z = f(n_W)$. f_s resp. f_0, the extrapolated intercepts with $a_W = 1$ and 0, respectively, were determined from the Fig.4, and were used for the hydration pressure P calculation according to eq. (19). The dependences $\ln P = f(n_W)$ in Fig.6 show a linear decay for both spin probes as could be expected from eq. (20). The values of nominal pressure P_o and exponential decay factor ν determined using ESR order parameter are presented in Table II together with P_0 and ν calculated from the sorption isotherms of Jendrasiak and Hasty [38] and Elworthy [39]. The agreement of these values is surprisingly good. These results lead to an important conclusion, that the dependence of ESR order parameter as a function of n_W represents in itself the sorption isotherm of the EYPC. This conclusion could be applied for both spin probes used in this work.

We studied also the correlation between the area A_L per one lipid molecule on the lipid-water interface and the order parameter S_z for both 12-DPPC and 16-DPPC spin probes (Fig.7). The linear course of the dependence $S_z = f(A_L)$ at the same n_W implicates that A_L characterizing the polar part of the lipid bilayer as well as S_z reporting about the hydrophobic region are determined by the degree of hydration. Two sources of the A_L values were used. Uhríková [34] has applied a procedure of Luzzati [40] by extracting the A_L values from the X-ray small angle diffraction data obtained on non-oriented EYPC samples, while in the paper of White and King [33] the raw data of Torbet and Wilkins [32] obtained with oriented EYPC bilayers were used. Both of these results show good correlation with the order parameter.

In conclusion, we have confirmed that the method of Kawano et al. [18] enables the preparation of well oriented lipid multilayers. The increasing hydration of these multilayers induces a decrease in the order parameter and an increase in the probability of gauche conformers as detected by spin labeled DPPC molecules. The dependences of S_z on the number of incorporated water molecules contain the information equivalent to sorption isotherm.

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