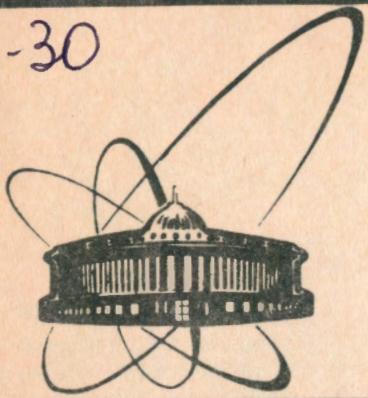

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1-PALMITOYL-2-OLEOYL-rac-GLYCERO-3-PHOSPHOCHOLINE AND 1-O-HEXADECYL-2-OLEOYL-rac-GLYCERO-3-PHOSPHOCHOLINE MEMBRANES. STRUCTURAL STUDIES USING NEUTRON DIFFRACTION

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Исследование структуры липидных мембран
из 1-пальмитоил-2-олеил-рац-глицеро-3-фосфохолина
и 1-0-гаксадецил-2-олеил-рац-глицеро-3-фосфохолина
методом дифракции нейтронов

Методом дифракции нейтронов была изучена структура мембран из 1-пальмитоил-2-олеил-рац-глицеро-3-фосфохолина (POPC) 1-0-гексадецил-2-олеил-рац-глицеро-3-фосфохолина (HOPC) при относительной влажности паров воды $\psi = 60\%$ и температуре $T = 28^\circ$ C (образцы находились в L_α-фазе). С точностью до экспериментальных ошибок ($\sim 0{,}5$ Å) вычисленные структурные параметры мембран оказались одинаковыми. Периоды повторяемости мембран из POPC и HOPC равны 51.2 ± 0.2 Å и 50.9 ± 0.2 Å соответственно. Температура фазового перехода гель-жидкий кристалл в HOPC мембранах равнялась $18 \pm 1^\circ$ C при $\psi = 60\%$, на несколько градусов выше таковой для мембран из POPC.

Работа выполнена в Лаборатории нейтронной физики ОИЯИ.

Сообщение Объединенного института ядерных исследований. Дубна 1992

Anikin M.V. et al.

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1-Palmitoyl-2-Oleoyl-rac-Glycero-3-Phosphocholine
and 1-0-Hexadecyl-2-Oleoyl-rac-Glycero-3-Phosphocholine Membranes.
Structural Studies Using Neutron Diffraction

The structures of oriented multilayer membranes constituted of 1-palmitoyl-2-oleoyl-rac-glycero-3-phosphocholine (POPC) and 1-0-hexadecyl-2-oleoyl-rac-glycero-3-phosphocholine (HOPC) were studied by means of neutron diffraction. The relative humidity ψ was 60%, the temperature was 28° C (L_α-phase). Within the experimental error (~ 0.5 Å), the structural parameters calculated for both types of membranes were actually quite similar. The repeat distances for POPC and HOPC membranes were 51.2 ± 0.2 Å and 50.9 ± 0.2 Å, respectively. The temperature of the gel-to-liquid crystalline phase transition in HOPC membranes at $\psi = 60\%$ was $18 \pm 1^\circ$ C, being several degrees higher than the transition temperature for POPC.

The investigation has been performed at the Laboratory of Neutron Physics, JINR.

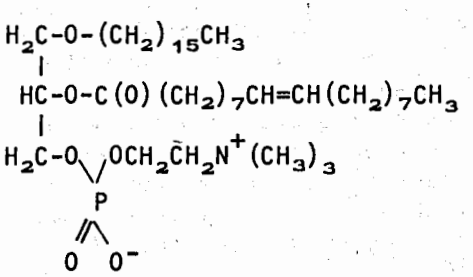
Communication of the Joint Institute for Nuclear Research. Dubna 1992

1. INTRODUCTION

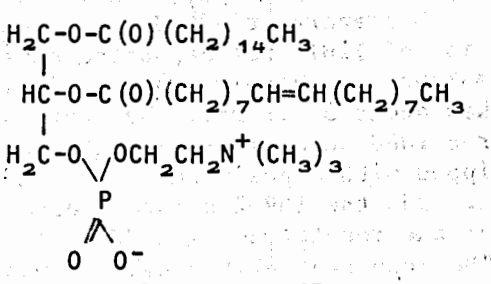
Alkylacylphospholipids belong to the class of ether lipids frequently met in membranes of certain cells where they exist together with the usual diacylphospholipid components. However, while the acyllipids and their role in membranes have been investigated with great zeal [1,2,3], the function of ether lipils, their influence on a membrane structure and properties are much less understood. Lately dialkyllipids have been shown to have some very peculiar structure-forming properties quite different from those of diacyllipids [4,5,6].

Alkylacyllipids actually have not been investigated as membrane components though they take part in many important cell functions, such as the metabolism of the lipid platelet activating factor [7,8] and the arachidonate cascade [9]. The activity of enzymes of lipid metabolism towards the acyl and alkyl phospholipids is not the same [10]. Of course such selectivity might be due to the molecular structure of the substrates; however when the substrate molecules are arranged in a supermolecular structure of a membrane, it seems possible, that the enzyme activity is regulated by the structural features of the alkylacyl phospholipid membranes or by membrane clusters enriched with the lipids in question.

Therefore it seemed reasonable to go further into the structure of membranes built with alkylacyl and diacyl phosphatidylcholines. For investigation we have taken 1-palmitoyl-2-oleoyl-*rac*-glycero-3-phosphocholine (POPC) and 1-O-hexadecyl-2-oleoyl-*rac*-glycero-3-phosphocholine (HOPC):



HOPC



POPC

These two lipids differ only in the way by which the hydrocarbon moiety is joined to the C-1 of the glycerol fragment: in the HOPC it is connected by an ether bond and in the POPC by an ester one. Thus in the primary hydrophonic chain of POPC there abides a polar carbonyl group (electrical dipole moment is 1,8 D) which is absent in HOPC. The presence or absence of carbonyl groups would considerably affect the conformation of polar fragments and the intermolecular interactions in a membrane, affecting consequently its hydration, structure parameters and phase behavior. The significance of the carbonyl group for bilayer architecture was clearly demonstrated by Kim et al. and by Laggner et al.[4,5,6]. They have shown that 1,2-di-O-hexadecylglycero-e-phosphocholine, a lipid with no carbonyl groups in both hydrophobic chains at high hydrations gave an interdigitated gel bilayer, whereas its diacyl analog under the same conditions did not form an interdigitated phase. However, it is still unclear how important is for the bilayer structure whether this is the primary or the secondary hydrophobic chain that carries the carbonyl group. In our study here we have attempted to understand how does a carbonyl group in a primary chain of phosphatidylcholine molecule contribute to the structure parameters of a liquid crystalline bilayer.

The investigations have been carried by the neutron diffraction method which suited very nicely to our structural aims[11].

2. MATERIALS AND METHODS

POPC and HOPC were synthesized by phosphorylation of the corresponding 1,2-disubstituted glycerols as described in ref.[12] with subsequent purification by column chromatography on silica gel. The lipid gave single spots on the thin layer chromatographic analysis.

To prepare oriented membranes 0.2 ml of 5% ethanolic solution of lipid was deposited on a 25×25 ml quarz plate and the solvent was slowly evaporated. The mosaic spread of the sample was about 3° (FWHM). The neutron scattering measurements were recorded on a time-of-flight DN-2 neutron diffractometer equipped with a position-sensitive detector[13]. The neutron source was the IBR-2 pulsed reactor (Dubna)[14], the average power of the reactor being 2 NWt with the frequency equal to 5 Hz. The mean scattering angle 2θ was 16°.

The time required for registration of diffraction reflections with a statistical accuracy not worse than 3% was 2 hrs. For the determination of the phase-structure factors the measurements were carried out under H_2O; H_2O:D_2O-1:1; and D_2O relative humidity ψ = 60% and T = 28°C[11].

The experimental spectra were treated as described in[15]. The neutron scattering length density $\rho(x)$ along an axis normal to the membrane plane (the Fourier profile) was calculated according to the equation:

$$\rho(x) = \sum_{h=1}^{h_{max}} F(h) \cos\frac{2\pi hx}{d}, \qquad (1)$$

where h is the number of the diffraction order, h_{max} is the maximum diffraction order observed in the experiment, d is the repeat distance, F(h) are the structure factors equal to:

$$F(h) = h^2\sqrt{I(h)}, \qquad (2)$$

where I(h) are the integral intensities of the corresponding diffractions[15]. The Fourier profiles make it possible to calculate some membrane structure parameters including water distribution in the membrane:

$$\rho_\omega(x) = \sum_{h=1}^{h_{max}} [F_{D_2O}(h) - F_{H_2O}(h)] \cos\frac{2\pi hx}{d}, \qquad (3)$$

where $F_{D_2O}(h)$ and $F_{H_2O}(h)$ are the structure factors of the samples measured in D_2O and H_2O vapour respectively.

To determine the distance between the middle line in the bilayer and the headgroup-hydrocarbon boundary (ℓ_b) we have built up a strip-function model by a procedure described by King and White[16]. The parameters for the strip-function model were calculated by R-factor minimization[16]:

$$R = \frac{\sum_h ||F_{strip}(h)| - K'|F_{obs}(h)||}{\sum_h K'|F_{obs}(h)|}, \qquad (4)$$

where K′ is a scaling factor equal to:

$$K' = \left[\frac{\sum_h |F_{strip}(h)|^2}{\sum_h |F_{obs}(h)|^2}\right]^{1/2}, \qquad (5)$$

where $F_{strip}(h)$ are the structure factors for the neutron scattering length density distributions in the model.

3. RESULTS AND DISCUSSION

Structure factors for POPC and HOPC membranes are given in Table 1. Figure 1 shows the water distribution across the membrane along the normal axis to the bilayer plane. The water distribution was calculated according to eq.(3) and was consequently normalized. One can see that actually water distribution as well as repeat distance ($d_{POPC} = 51.2 \pm 0.2$ Å ; $d_{HOPC} = 50.9 \pm 0.2$ Å) in both types of membranes are very similar. The repeat distance for POPC membranes is similar to that for egg phosphatidylcholine bilayers (d = 51.0 Å at $\psi = 60\%$ [15]). Actually this is a result of the egg phosphatidylcholine having POPC as its main component[17].

Figures 2 and 3 show the Fourier profiles for membrane water and a lipid bilaeyr in POPC and HOPC membranes. The corresponding structure parameters calculated from these profiles (Table 2) are practically identical except the ℓ_b parameters.

Table 1. Structure factors for POPC and HOPC membranes in the H_2O and D_2O vapour phase at relative humidity ψ = 60% and temperature T = 28°C

	Sample, ψ = 60%, T = 28°C			
	POPC		HOPC	
h	F_{H_2O}	F_{D_2O}	F_{H_2O}	F_{D_2O}
1	-20.6	-46.6	-24.3	-47.9
2	+0.9	+18.4	+3.2	+20.1
3	+5.7	-6.2	+1.2	-8.4
4	-9.4	-5.2	-7.7	-3.5
5	+1.2	0	+2.7	+1.4
6	+0.6	+2.5	-1.5	+0.6
7	+1.3	-1.4	+1.2	-2.6

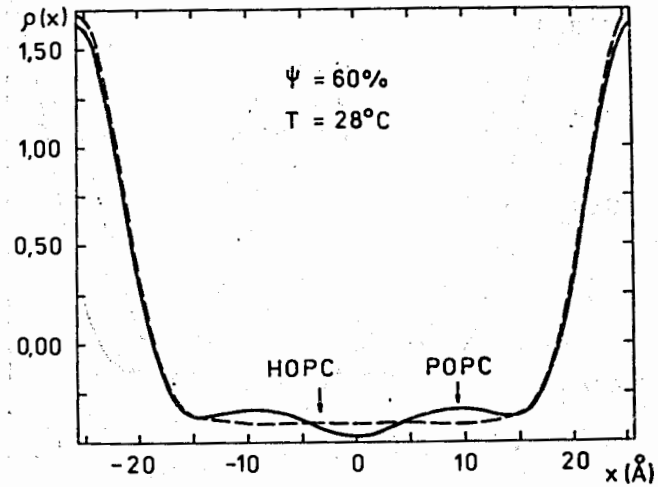


Fig.1. Water distribution in POPC and HOPC membranes at ψ=60% and T= 28°C

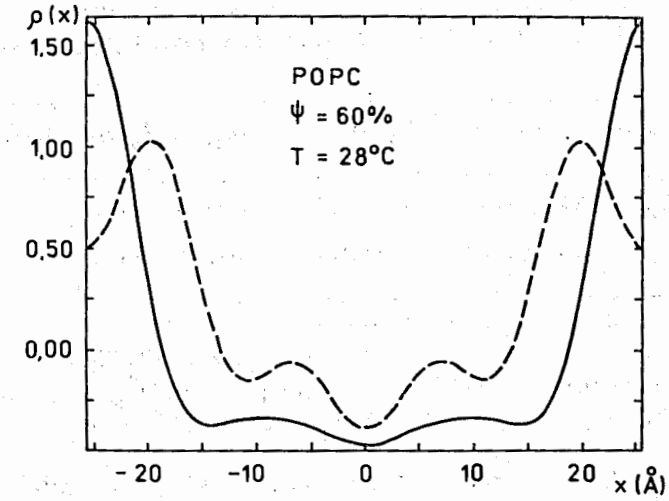


Fig.2. Neutron scattering length density distribution for membrane water and lipid bulk in the POPC membrane at ψ = 60% and T=28°C

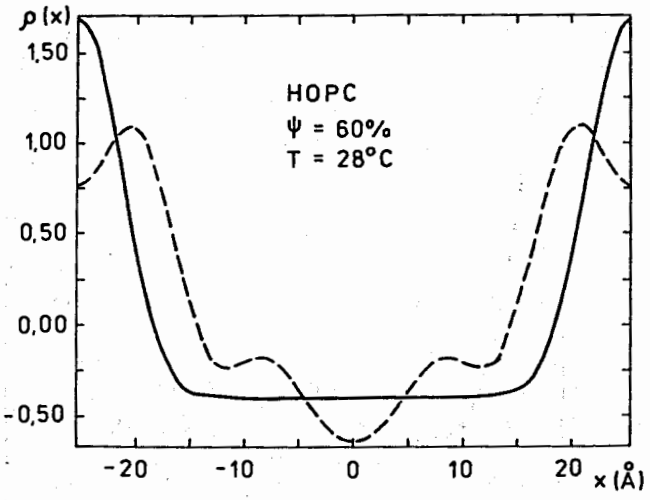


Fig.3. Neutron scattering length density distribution for membrane water and lipid bulk in the HOPC membrane at $\psi = 60\%$ $T = 28°C$

Table 2. Structure parameters for POPC and HOPC membranes calculated from the Fourier profiles ($\psi = 60\%$ and $T = 28°C$)

Lipid	Phase	d(Å)	ℓ_ω(Å)	n_ω	ℓ_b(Å)	R-factor (%)
POPC	L_α	51.2±0.2	16.7±0.4	6.2±0.3	14.6	1.5
HOPC	L_α	50.9±0.2	16.7±0.4	5.8±0.3	16.7	2.0

where ℓ_ω is the depth of water penetration into the membrane; n_ω, the number of water molecules per a lipid molecule; ℓ_b, the hydrophobic boundary location; ℓ_w and ℓ_b were determined with the bilayer middle line as a starting point.

However, the ℓ_b parameters thus found really are additional evidence of structural similarities of the membranes in question. In fact, since HOPC has no polar carbonyl group in the primary side chain, the density increase of the neutron scattering length would be located near the etheric oxygen atom. Because of that the hydrophobic region boundary in the HOPC membrane would be situated farther from the bilayer middle

line than in the POPC membrane. This difference will be approximately equal to the C-O bond length, that is 1.4 Å[1]. This value is consistent with the 2.1 Å value we have found for the ℓ_b difference for both membranes.

Thus we see that POPC and HOPC membrane parameters calculated on the basis of neutron diffraction data are quite similar.

Meanwhile the phase behaviour of the two types of membranes is specific for each type of membrane. The temperature for the gel-to-liquid crystalline phase transition for HOPC membranes at $\psi = 60\%$ is $18 \pm 1°C$, a little higher than the corresponding transition temperature for the POPC membrane. This might mean that hydrocarbon chains in HOPC interact much stronger than do the chains in the POPC membranes. This is in conformity with ^{2}H-NMR data by Gusev et al.[18], showing slower molecular motions in a fully hydrated HOPC liquid crystalline bilayer in comparison with an POPC membrane. At full hydration gel to liquid crystalline transtition the temperature for HOPC membranes is also higher than that for POPC ones, being correspondingly 0–3°C and -5 – -2°C [18]. These differences in the phase behaviour and in the molecular dynamics seem to show that POPC and HOPC are inclined to form structurally different membranes. On the other hand, neutron diffraction data indicate that the differences in the structure parameters for both lipids are never more than 0,5 Å, the amounts of membrane water (n_w) also not differing more than 0.5 molecule per a molecule of lipid.

Structural features of diacyl and alkylacyl phosphytidylcholines should be more noticeable with saturated lipids. The loss in the hydration due to the absence of a polar carbonyl group would practically be masked by the effect of the unsaturatedness in the chain[1] that enhances the hydration of the membrane.

The above discussed data and the molecular dynamics described in[18] show that HOPC membranes are less prone to hydration than POPC membranes.

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