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PULSE NMR STUDY OF PHASE TRANSITIONS IN DIPALMITOYL PHOSPHATIDYLCHOLINE MULTILAYER SYSTEMS

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Summary

Pretransition and main transition of aqueous dipalmitoyl phosphatidylcholine (DPPC) dispersions were investigated by pulse NMR. The second moment $M_{2\ \mathrm{inter}}$ of the proton absorption line shows significant changes at 42°C and about 35°C. Over the whole investigated temperature range between 25 and 50°C a superposition of at least two distinct second moments assigned to different molecular regions was observed.

Phosphatidylcholine membranes exhibit a number of properties which make them suitable as model systems for biological cell membranes. With a large excess of water they show various vesicle shapes [1--4] similar to those found in nature, e.g. red blood cells and myelin. Furthermore the molecular organization due to hydrophobic interactions in artificial bilayers is analogous to that of the lipid region of biological cell membranes. For dipalmitoyl phosphatidylcholine (DPPC)-water dispersions three phases (gel, intermediate and fluid) in the temperature range from 25 to 50°C are distinguished [5]. In this work we investigate by NMR changes of the molecular structure of DPPC at the two phase transitions.

The second moment of an NMR absorption line [6]

$$M_2 = -\frac{1/\hbar^2}{\mathrm{tr}\,\mathbb{I}_x^2} \cdot \mathrm{tr}\left\{\left[\mathbb{H}_d^0, \mathbb{I}_x\right]^2\right\} \qquad (1)$$

is determined by the dipolar interaction between all nuclei having a net magnetic moment. Boden and co-workers [7,8] showed that for a compound containing well defined spin –½ pairs such as saturated hydrocarbon chains M_2

can be split into two parts $M_{2\ \mathrm{intra}}$ and $M_{2\ \mathrm{inter}}$, which correspond to the dipolar interaction between nuclei of the same pair and between different pairs, respectively. In terms of microscopic structure parameters $M_{2\ \mathrm{inter}}$ is given by [9]

$$M_{2\ \mathrm{inter}} = \frac{9}{8}\gamma^4\hbar^2\frac{1}{N}\sum_{j>i}^{N}\left\langle\frac{3\cos^2\nu_{ij}-1}{r_{ij}^3}\right\rangle^2 \tag{2}$$

where γ is the magnetogyric ratio of the observed nuclei, r_{ij} is the distance of two spins, and ν_{ij} is the angle between the distance vector and the applied field. The double summation is carried out over the number N of nuclei in the sample with the restriction that i and j do not belong to the same pair. The influence of molecular motion is taken into account by averaging over the observation time indicated by the brackets in the sum. Thus the magnitude of $M_{2\ \mathrm{inter}}$ is determined by the molecular motional behaviour and the spatial arrangement of the molecules.

The treatment of the spatial arrangement is simplified for the case of static nuclear distance vectors. In a randomly oriented system spatial averaging results in

$$M_{2\ \mathrm{inter}} = \frac{9}{40}\gamma^4\hbar^2\frac{1}{N}\sum_{j>i}^{N}(r_{ik})^{-6} \tag{3}$$

which is now proportional to the square of the packing density of the spins represented by the sum over r_{ik}^{-6}. Eqn. 3 is a good approximation for DPPC-water systems in the gel phase where a two-dimensional lattice of the phosphatidylcholine molecules in the bilayer was found by X-ray studies [10]. For the fluid phase it is well known that lateral diffusion of the phosphatidylcholine molecules and rotational diffusion around a symmetry axis normal to the bilayer influence the magnitude of $M_{2\ \mathrm{inter}}$ in different ways due to different averaging in eqn. 2. As in our experiments we cannot separate the intermolecular contribution to $M_{2\ \mathrm{inter}}$ which is sensitive to both kinds of diffusion from the intramolecular part which is only sensitive to rotational diffusion we regard eqn. 2 as a general expression for the fluid phase.

$M_{2\ \mathrm{inter}}$ can be determined by measuring the spin-pair-dipolar-echo (SPDE) amplitude following an RF-pulse sequence $90°\text{-}\tau\text{-}90°_{xy}$, which for a homogeneous spin system is given by the relation [7]

$$E(\tau) = E(0)\,\mathrm{e}^{-\frac{M_{2\,\mathrm{inter}}}{2}\tau^2} \tag{4}$$

The decay constant represents the second moment $M_{2\ \mathrm{inter}}$ whereas $E(0)$ is proportional to the number of spins involved.

We have measured the temperature dependence of $E(\tau)$ in DPPC dispersions with various water contents (30, 40, and 60% ^2H_2O by weight) in the temperature range from 25 to 50°C. Three echo decays each representative for one of the three phases are shown in Figs. 1a—c. In the gel phase (Fig. 1a) and in the intermediate phase (Fig. 1b) the experimental values can be fitted by a

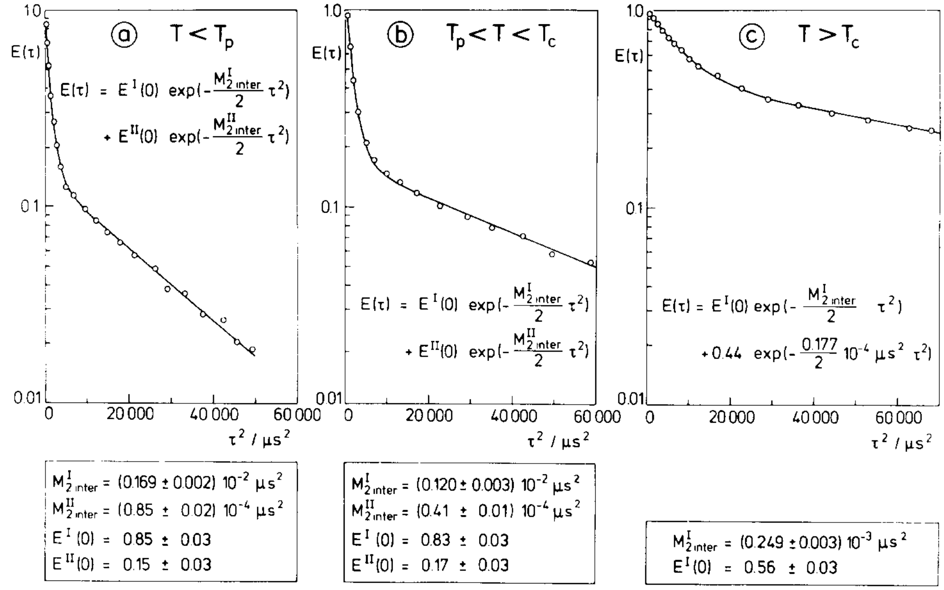

Fig. 1. Spin-pair-dipolar-echo (SPDE) decays representative for the gel (a), the intermediate (b) and the fluid phase (c). The echo amplitudes are normalized to the maximum amplitude $E(0) = 1$.

superposition of two decays with different parameters $E(0)$ and $M_{2\,\text{inter}}$:

$$E(\tau) = E^{\text{I}}(0) \exp\left[-\frac{M_{2\,\text{inter}}^{\text{I}}}{2}\tau^2\right] + E^{\text{II}}(0) \exp\left[-\frac{M_{2\,\text{inter}}^{\text{II}}}{2}\tau^2\right] \qquad (5)$$

with

$$E(0) = E^{\text{I}}(0) + E^{\text{II}}(0) = 1$$

Within experimental accuracy the relative number of spins involved in the two different decays given by $E^{\text{I}}(0) = 85 \pm 3\%$ and $E^{\text{II}}(0) = 15 \pm 3\%$ remains constant for both phases and for all investigated water concentrations. In the fluid phase (Fig. 1c) the fit by eqn. 5 is sufficient for echo amplitudes larger than 20% of $E(0)$ i.e. for $\tau < 200\ \mu$s. The further development is a superposition of more than two decays. Bloom et al. [11] showed that in time region $\tau \gg (M_2)^{-1/2} \approx 100\ \mu$s non-oriented soap water systems in the fluid phase exhibit non-gaussian free induction decays which could result in an echo decay different from eqn. 5. Thus we had to restrict our analysis to the fast decay given by $M_{2\,\text{inter}}^{\text{I}}$ and $E^{\text{I}}(0)$.

The temperature dependence of $M_{2\,\text{inter}}^{\text{I}}$ exhibits two significant changes. The step of about 30% between 32 and 37°C can be assigned to the pretransition detected earlier by various methods [12—15]. The change of about 60% at 42°C with 1 K width corresponds to the well known main transition.

The values of $M_{2\,\text{inter}}^{\text{II}}$ are one order of magnitude smaller than those of $M_{2\,\text{inter}}^{\text{I}}$ but their absolute experimental error is the same. Therefore, we feel that the observed changes of $M_{2\,\text{inter}}^{\text{II}}$ at the pretransition temperature are not very reliable.

To interpret these results emphasis is placed on the following questions: Which part of the bilayer is represented by the contributions $E^{I}(0)$ and $E^{II}(0)$ below T_c and what kind of changes occur at the two phase transitions? The suspicion, that inhomogeneities of the sample (e.g. phase separation) are responsible for the occurrence of two or more decay constants is disproved by the result that $E^{I}(0)$ is unchanged when varying the water concentration. Therefore it seems reasonable to assign the observed decays I and II to different molecular regions:

(1) Taking into account the higher mobility of the methyl protons the second moment of the head group is estimated to be one order of magnitude smaller than that of the chain region.

(2) According to the relative number of spins the head group contribution to the decay is expected to be 16%.

Considering these two arguments we can identify the molecular regions in question as the hydrophobic chain part (region I) and the polar head group (region II).

Thus the SPDE technique allows the distinction of molecular parts with significantly different second moments $M_{2\ \mathrm{inter}}$. No such separation would be possible by continuous wave techniques where additional superposition of $M_{2\ \mathrm{intra}}$ complicates the analysis.

For this interpretation we have to regard region I and II as two homogeneous spin reservoirs developing each its own spin temperature. This assumption is supported by two arguments. The two significantly different dipolar interactions act as a diffusion barrier between the two reservoirs. On the other hand the diffusion length during the decay time $(\frac{1}{2} M_{2\ \mathrm{inter}})^{-\frac{1}{2}}$ is estimated to be much less than the dimensions of each molecular region in question. Therefore spin diffusion occurs within each reservoir and low probability exists for a cross-relaxation process induced by dipolar contact between spins belonging to different reservoirs.

The decrease of $E^{I}(0)$ from 85 to 56% during the main transition indicates that one third of the chain protons are no longer part of the homogeneous spin system of region I. This can be explained by a discontinuity of mobility along the hydrocarbon chain provided there is no appreciable cross-relaxation between a more fluid and a more rigid part, which seems reasonable because spin diffusion is suppressed by motion. This view seems supported by experiments on soaps [16] where two strongly different values of $M_{2\ \mathrm{inter}}$ were observed in the fluid phase.

Region I belonging to the chain proton reservoir exhibits changes in $M_{2\ \mathrm{inter}}^{I}$ (Fig. 2) at the phase transitions due to either packing density or motional effects. Earlier results of Jacobs et al. [5] show that in phosphatidylcholine the packing density of molecules increases only at the main transition by about 2.6% with increasing temperature. This would correspond to a decrease of the second moment of 5.2% which is much less than the detected changes of 30% at the pretransition and 60% at the main transition. From these results one can assume that changes in $M_{2\ \mathrm{inter}}^{I}$ for both pretransition and main transition are predominantly caused by motional effects. It was found by X-ray studies [10] that the phosphatidylcholine molecules at the pretransition from the gel into the intermediate phase remain in a two-dimensional lat-

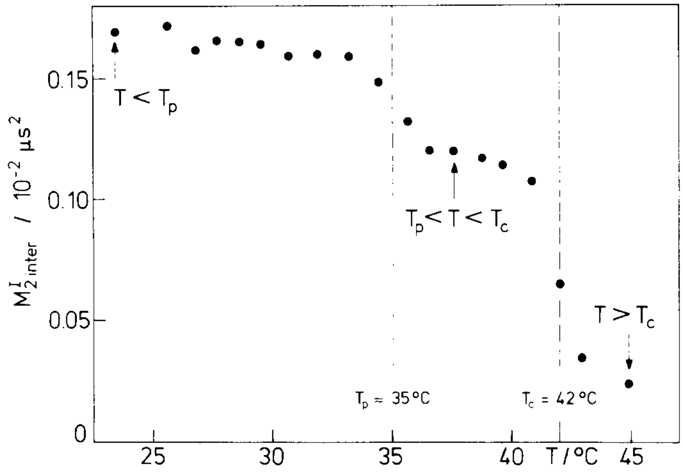

Fig. 2. Temperature dependence of the second moment $M^I_{2\,\mathrm{inter}}$ of region I. The step of 30% at about 35° C can be assigned to the pretransition. The change of 60% at 42° C corresponds to the well known main transition. The experimental errors of $M^I_{2\,\mathrm{inter}}$ are about 10%.

tice. Therefore, the spectral changes at the pretransition are likely to be caused by some intramolecular motion rather than by changes in translational molecular diffusion. A conceivable process is melting of the chain ends, a mechanism not in conflict with the Raman results of Gaber and Peticolas [17] It might be associated with a "going out of register" of the monolayer lattices [18].

The present results show that the SPDE technique is a suitable method to study the molecular structure of phosphatidylcholine membranes. Further information about the chain positions, which are involved in the phase transitions, and the behaviour of the head group could be obtained from experiments on partially deuterated phosphatidylcholines.

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