Deuterium Relaxation in Specifically Labeled Lecithin-Bilayers

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Deuterium magnetic relaxation measurements are reported for two samples of specifically labeled dipalmitoyl lecithin bilayers in the temperature range 0° to 60 °C. While the liquid-crystalline phase shows a "dynamic gradient" along the alkane chains, which is already known from ^{13}C-investigations, there is no such gradient in the gel phase at least between the second and tenth methylene group. Moreover there is a common T_1-minimum compatible with that observed in studies of the (non-specific) proton relaxation.

Deuterium signals from the gel phase could only be detected by a combined spin-locking / echo method allowing to substract spurious signals arising from magneto-acoustic ringing typical for low-frequency / high-field pulse experiments in iron magnets.

In a previous publication [1] we have treated several models basing on the diffusion of structural defects in the alkane phase of lipid bilayers. As defects one can envisage e. g. kinks or torsions. It has been shown that a strong dependence of the nuclear magnetic relaxation behaviour can be expected concerning the type of motion, the stability of the defects and the profile of the defect jump rate across the lamellae.

It turned out that there are two crucial experiments for a decision between the diverse model situations:

a) T_1-dispersion measurements are desirable in a *broad* frequency range in order to detect the special type of fluctuation caused by defect diffusion and furthermore to distinguish the diffusion of defects which have a mean lifetime smaller than T_1 from those which are stable.

b) Selective measurements of *local* relaxation times are needed in order to detect any profile of the parameters determining the local diffusion coefficient of the defects.

The experiment a) has already been performed on aqueous dispersions of dipalmitoyl lecithin and has been published elsewhere [2]. The conclusion was that step-wise [1] or continuous [3] diffusion of stable defects limited by two "reflecting walls" are suitable to explain the T_1-dispersion data in the frequency range 10^4 to 10^8 Hz.

Informations of the type b) can be obtained from local ^{13}C-relaxation in the liquid crystalline phase of dipalmitoyl lecithin bilayers [4]. The finding was a "dynamic gradient" along the alkane chains. The conclusion concerning defect diffusion is that the activation energy or the preexponential factor of the defect diffusion coefficient varies in the sense of increasing defect mobility towards the inner jointface of the bilayers [1].

Unfortunately this type of investigation fails in the gel phase because no resolved ^{13}C-lines can be observed any more. Therefore we have performed a deuterium relaxation experiment with two selectively deuterated dipalmitoyl lecithin samples. As the only deuterium signal in these samples arises from the labeled positions, informations concerning any dynamic gradient can principally be obtained even in the solid state. The results are discussed in the following.

Materials and Apparatus

2,2-^{2}H-Palmitic acid was prepared by repeated H-D exchange in D_2SO_4, following the method of van Heyningen et al. [5].

9,10-^{2}H-Palmitic acid was produced by double-bond-deuteration of palmitoleic acid in methanol solution, using perdeuterated hydrazine-hydrate, $N_2D_4 \cdot D_2O$, as a hydrogenation agent. This method was used instead of catalytic hydrogenation, since it is known that under the influence of metal catalysts double bond migration occurs, leading to a distribution of deuteration sites. A detailed description of these methods will be given elsewhere.

Dipalmitoyl-lecithin (DPL) was prepared by the method of Cubero-Robles and van den Berg [6]. The purity of the DPL was checked by thin-layer-chromatography.

50% DPL/H_2O dispersions were prepared as described previously [1], yielding stable and homogeneous multi-bilayer systems.

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A Bruker SXPC 4 – 100 spectrometer equipped with a B-E-38 magnet was used for the measurements. The field was stabilized at $2.114\,T$ by a Bruker B-SN 15 external NMR field stabilizer, the deuteron resonance frequency thus being 13.816 MHz. The signals were recorded by means of a Bruker B-C 104 transient-recorder with a dwell-time of 100 nsec, and processed in a Nicolet 1180 computer.

Method and Experimental Results

In the case of nuclei with low magnetogyric ratios, magneto acoustic ringing (MAR) can obscure the free induction decay following the RF-pulse [7, 8]. Often, MAR appears for several hundred microseconds after the pulse, due to an electromagnetic feedback caused by acoustic modes stimulated by the pulse in conducting materials surrounding the sample. When the amplitude and the decay time of the FID is small, this effect totally impedes the observation of a signal. To overcome this difficulty, the probehead was reconstructed of Poly (methyl methacrylate), and a RF-coil made of lead wires was used. Secondly, a novel method of measurement was applied, similar to the SLARC method described by van der Klink [9]. The method is illustrated in Figure 1. Phase and amplitude of the MAR are determined by phase and amplitude of the generating RF-pulse. Thus, ringing can be subtracted by phase alternating techniques. For this purpose the $90^0_{\pm x}$ detection-pulse P_1 is followed by a spin-locking pulse P_2 in $+y$-direction in the rotating frame of reference. The duration of P_2 must be sufficiently long, to ensure that all MAR contributions of P_1 have decayed to zero, but short with respect to $T_{1\varrho}$ of the sample. At the end of P_2 a FID appears whose phase depends on that of P_1. By alternating the phase of P_1 between $+x$ and $-x$ and alternative addition and subtraction of the signals in the computer memory, the phase-coherent MAR-signals are wiped out, the FID, however, is enhanced. This method does still not lead to satisfactory results, particularly if one has to deal with rapidly decaying FID's, as in the case of the DPL gel-phase, where the signal is completely obscured by the receiver dead time. As demonstrated in Ref. [10 to 12], for example, a $90_x{}^\circ$-t-$90_y{}^\circ$ pulse-sequence will produce so-called quadrupolar echoes in solids at certain intervals determined by the spin I of the observed nuclei. For $I = 1$, a single echo occurs at a time $2\,t$ after the first pulse. Now we can treat the signal following P_2 as if it were created by a single $90^0_{\pm x}$ pulse. If we fire another $90^0_{\pm y}$ pulse P_3 after an interval t, the quadrupolar echo will be detectable after $2\,t$, the amplitude of which will be proportional to the longitudinal magnetization at the time when P_1 was applied. Thus we can measure T_1 with the classical 90°-τ-90° pulse-sequence by simply replacing the detection-pulse by the pulse sequence described above and evaluating the echo-amplitude in dependence on τ.

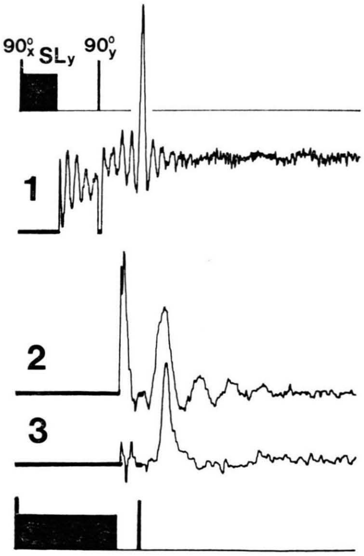

Fig. 1. Complete signal train obtained by the quadrupolar echo sequence with ringing compensation. (1) Perdeuterated palmitic acid at 300 K, total scan length 2 ms, number of scans 200. (2) 9,10 deuterated DPL in a 50% H_2O dispersion at 320 K, total scan length 800 μs, number of scans 10 000. (3) dto. at 300 K.

In all cases, a purely exponential behaviour has been observed. An additional test of the echo method has been applied in the DPL liquid-crystalline phase, where the FID can partially be detected directly after the spin-locking pulse P_2 (see Figure 1). It has been found that the relaxation behaviour of the FID was completely identical to that of the echo.

Typical values of the experimental parameters were:
Pulse time for a 90°-RF-pulse: 3.6 μs.
Spin locking field P_2: $7 \cdot 10^{-3}$ T; duration of P_2: 250 μsec.
Delay of the echo-pulse P_3: 35 ... 55 μsec.
Number of acquisitions: 5000 ... 20 000 for each pulse delay.
Number of evaluated pulse delays: 10 ... 20.

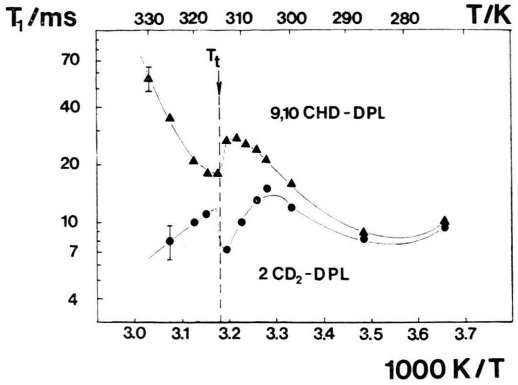

Fig. 2. Longitudinal deuterium relaxation times versus reciprocal temperature measured at 13.8 MHz in aqueous dispersions of 9,10 CHD-DPL and 2 CD_2-DPL. The dashed line indicates the transition temperature from the gel to the liquid crystalline state.

In order to prevent the sample from being overheated by the long P_2 pulse, long trigger periods were chosen (250 . . . 500 msec). Thus a single T_1 evaluation required a measuring time of approximately 24 hours.

In this way, the longitudinal deuteron relaxation times of 50% DPL/H_2O dispersions were evaluated for DPL samples deuterated in the 2,2 position and in the 9,10 position of both chains in a temperature range from 0 °C to 60 °C. The results are plotted in Figure 2.

Discussion and Conclusion

It is well known that deuterium relaxation is totally dominated by quadrupolar interactions with local electric field gradients, so that any dipolar contributions have not to be discussed. The results can then be outlined as follows.

In the liquid crystalline phase, we find that T_1 strongly depends on the position of the reference segment in the fatty acid residue. In the 9,10-position, the T_1-values increase with temperature, while those of the 2,2-position decrease. This behaviour is consistent with the findings of ^{13}C-relaxation measurements [4]. It clearly indicates a "dynamic gradient" along the chains, which should thoroughly be distinguished from a gradient of "motional freedom" which can potentially be observed with deuterium or spin label order parameters [13].

For an explanation of the dynamic gradient we refer to the free volume theory of Cohen and Turnbull [14]. A diffusion step of a defect requires a certain amount of free volume, allowing the formation of a gap large enough for the reorientation of the involved segments. The rate with which gaps of a sufficient size occur, is now dependent on the local mean displacement of the segment out of the average chain orientation. This mean displacement depends on the distance from the fixed ends of the alkane chains. It is therefore plausible that defects near the inner jointface diffuse more rapidly than those near the polar headgroups, producing thus the observed relaxation behaviour of the liquid crystalline phase. This qualitative explanation also includes the fact that the relaxation near the polar headgroups behaves as the low-temperature wing of the T_1-minimum, while at the 9,10-position we have to deal with a high-temperature flank.

In the gel-phase below the temperature of the so-called pretransition the relaxation times at both positions converge, clearly indicating that between these segments no gradient of the diffusion parameters exists. This fits quite well with the T_1-dispersion results [2], and any model with a dynamic gradient along the outer part of the chains is ruled out. As a consequence one must conclude that the rate of gap formation is independent of the segment position. This conclusion is supported by the T_1-minima apparently appearing at the same temperature for both positions (in clear contrast to the behaviour in the liquid crystalline state).

These results are compatible with those obtained for perdeuterated soaps by Davis et al. [15]. In that paper a relatively weak position dependence of the relaxation times has been found (except for the end groups).

To summarize, we can state that the major part of gel-phase lamellae shows a constant profile of the local segment dynamics, while in the liquid crystalline phase the segment motion is slowed down from the inner jointface of the bilayers towards the polar headgroups. This means in terms of the defect diffusion model proposed in Ref. [1] that we have a constant jump rate of the defects in the gel-phase at almost all chain positions as assumed in Ref. [2]. In the liquid crystalline state, however, defect steps are the more hindered the more they approach the polar head groups.

A quantitative interpretation cannot be given unambiguously with the deuterium data alone. The combination of all available data from ^{1}H, ^{2}H and

^{13}C-relaxation allows however to establish a well founded quantitative picture [16]. This extended discussion is beyond the scope of this note and will be published in a separate paper on defect diffusion in lipid bilayers.

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