^{13}C-NUCLEAR MAGNETIC RESONANCE RELAXATION MEASUREMENTS of α-LECITHIN–PEPTIDE INTERACTION IN MODEL MEMBRANES

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1. Introduction

Extensive investigations of phospholipid systems during the last years have resulted in a fairly good understanding of the physical states assumed by the hydrocarbon chain [1,2]. On the contrary, the interactions of the phospholipid polar groups are still poorly understood and they may be of major significance for the structure of biomembranes.

Recently, Nuclear Magnetic Resonance (NMR) has been used to study the interactions of the polar groups with water molecules [3–5]. Either the relaxation rate of added water protons in nonaqueous solutions of lecithins or proton chemical shifts in the lecithin molecule upon addition of water were measured, but no ^{13}C-NMR-relaxation measurements were performed with the purpose of studying the interactions involving the polar groups of lecithin with various small molecules. The present letter reports the results of such measurements of synthetic α-lecithin molecules in D_2O and in the presence of Alanyl-glycine (AlaGly) and Alanyl-alanine (AlaAla).

2. Experimental

Dipalmitoyllecithin (DPL) was obtained from Koch-Light. The samples (0.15 M) were sonicated at 50°C in deoxygenated D_2O buffer (45 mM NaCl–30 mM sodium acetate–5 mM sodium phosphate, pD 7.4) in glass vials (10 mm diameter) under argon until the sample was translucent. The vials were sealed under argon.

Alanyl glycine and Alanylalanine were obtained from Schwarz–Mann and were dissolved in the sonicated DPL suspensions to yield equimolecular mixtures. These samples were deoxygenated as previously. In order to check the possible influence of Na^+ on the T_1 values of DPL test measurements were performed on buffered and nonbuffered D_2O suspensions of DPL, in the pD range 6.4–7.4. In this range, the monovalent cations do not induce any change in conformation [6], a fact which could be confirmed by means of the ^{13}C-NMR measurements.

The T_1 relaxation measurements were made on proton decoupled ^{13}C-spectra by the Fourier transform technique on a Brucker WA 90 spectrometer locked on solvent deuterium, and employing the progressive saturation technique of Freeman et al. [7]. With this method the steady state response of the spin to a repetitive sequence of 90° pulses is measured and the relaxation times derived from the ratio of the intensities of a given line in the Fourier transformed spectra, as a function of the pulse rate. We selected time sequences varying from 8 to 0.2 sec. For each measurement 6000 accumulations of the spin decoupled spectra were made. The sample temperature was 65°C throughout this investigation.

3. Results

The T_1 values for the $\overset{+}{N}(CH_3)_3$, $(CH_2)_n$ and CH_3 groups of the DPL, DPL–AlaGly and DPL–AlaAla are presented in table 1.

With both the DPL and DPL–AlaAla suspensions

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Table 1
T_1 relaxation times of ^{13}C-lipid nuclei at 65°C in various systems

Systems	T_1 (sec)		
	$\overset{+}{N}(CH_3)_3$	$(CH_2)_n$	CH_3
DPL	1.0 ± 0.1	0.5 ± 0.05	3
DPL–AlaGly	0.45 ± 0.1	0.4 ± 0.05	3
DPL–AlaAla	1.0 ± 0.1	0.5 ± 0.05	3

plots of peak amplitude decrease vs. time delay between the pulses were consistent with a single T_1 relaxation time for each of the resonances. In the DPL-AlaGly suspensions the $-CH_3$ and $-(CH_2)_n$-resonances show a single relaxation while the $\overset{+}{N}(CH_3)_3$ shows a complex pattern, i.e. more than one T_1 value. Comparison of the T_1 values from the mixtures with those of the pure lipid suspension shows no significant differences with one notable exception: the T_1 value of the $\overset{+}{N}(CH_3)_3$ resonance is in the system DPL–AlaGly about 50% shorter than in the DPL and DPL–AlaAla systems. This is considerably more than any effect observed on addition of polyvalent cations to lecithin vesicles and cannot be attributed only to electrostatic interaction of the head group with the AlaGly molecule [8].

This is further supported by the upfield shifts of the CH_3 group and of the carbonyls of AlaGly (+ 3.7 ppm and + 2.2 ppm respectively) as compared to the same concentration of AlaGly in D_2O.

The CH_2OP groups, which have quite short T_1 values [8], show very weak signals and could not be followed precisely enough with the methods used in this investigation. It is noteworthy that AlaGly caused a constant though limited enhancement of the peak intensities – mainly of the glycerol carbons – as compared to samples of DPL vesicles of the same concentration in the absence of AlaGly.

Sonication of the DPL suspensions in the presence of AlaGly, under the same conditions as above yields a single T_1 value for the $\overset{+}{N}(CH_3)_3$ resonances.

4. Discussion

The detailed investigation of the ^{13}C-NMR spectra of phospholipid bilayers and of membrane [8,10], have shown a strong decrease of the T_1 value of the $\overset{+}{N}(CH_3)_3$ resonance in membrane material as compared to phospholipid vesicles. Robinson et al. [8] found at 31°C in sarcoplasmic reticulum membranes for this group a T_1 of 0.36 ± 0.03 s as compared to 0.55 ± 0.06 for lipid vesicles, while Millett et al. [10] reported for T_1 in the Bovine Retinal Rod outer segment membrane a value of 0.4 ± 0.2 s at 31°C. At 65°C Levine et al. [9] measured for the $\overset{+}{N}(CH_3)_3$ resonance of 1.15 ± 0.07 s in DPL vesicles. At the same temperature we find 1.0 ± 0.1 s in our DPL vesicles and a decrease to 0.45 ± 0.1 s when AlaGly is added. No change of the T_1 value was observed with AlaAla.

The change observed in this value with added AlaGly is quite similar to that observed in the membranes as compared to the DPL vesicles. It should be mentioned that there are no significant differences between the T_1 values for the CH_3 and $(CH)_n$ resonances of membranes and of phospholipid vesicles [8] just as in our case. These similarities seem to suggest the possible involvement of the $\overset{+}{N}(CH_3)_3$ group, when the phospholipid is mixed with AlaGly in an interaction similar to that existing between lipids and proteins in plasma membranes. The lack of changes observed when AlaAla is added to the phospholipid suggests that geometrical requirements have also to be met in order that this interaction takes place. An interesting point is the complexity of the relaxation processes undergone by the $\overset{+}{N}(CH_3)_3$ resonance in the DPL–AlaGly mixtures. While both $-CH_3$ and $(CH_2)_n$ show a single relaxation process, the choline group shows a complex relaxation. The value of 0.45 s is an average value since we could not, due to the low signal : noise ratio single out all the contributions, e.g. at very short t values. Bergelson et al. [11] added Mn^{2+} ions to a sonicated lipid system and used the line broadening effect of paramagnetic ions to distinguish between outer and inner $\overset{+}{N}(CH_3)_3$ groups. Sonication of the phospholipid together with the Mn^{2+} ion led to the complete disappearence of the ^{1}H NMR of $\overset{+}{N}(CH_3)_3$ groups, both inner and outer, while addition of Mn^{2+} to the already sonicated lipids caused the disappearance of the NMR signals yielded by the outer groups only.

Addition of AlaGly to DPL before sonication and subsequent sonication leads to a single T_1-relaxation

process which suggest that previously only outer $\dot{N}(CH_3)_3$ groups were interacting with the peptide. This could explain the complex relaxation pattern.

The upfield shifts of the CH_3 and CO groups in the mixture with DPL as compared to the same concentration of AlaGly in D_2O is further evidence of the interaction between these two species. Shifts of the carbonyl ^{13}C have been observed as a result of hydrogen-bond formation in variety of protic and aprotic solvent for ketones, carboylic acids, etc. [12, 13]. Hydrogen bonding can deshield carbonyl carbons and lead to shielding differences of 25 ppm, or even 40 ppm [12,13,14]. It seems that the major contributions to these shielding differences arise from polar and van der Waals interactions in aprotic solvents, while in the protic solvents the varying tendencies of these solvents to form hydrogen bonds are primarily responsible for the deshielding. Formation of hydrogen bonds involving the AlaGly carbonyls and the $\dot{N}(CH_3)_3$ groups of DPL are strongly suggested by our results. The CH_3 upfield shift suggests as possible cause for the deshielding of this group a hydrophobic interaction with the hydrocarbon chain of the phospholipid molecule.

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