The Lateral Order of Dipalmitoylphosphatidylcholine Model Membranes in the Presence of N-alkyl-N,N,N-trimethylammonium Ions as Studied by Raman Spectroscopy*

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Abstract. Effects of N-alkyl-N,N,N-trimethylammonium ions with different alkyl substituents (hexyl, nonyl, dodecyl, and octadecyl) on the lateral packing of lipids in dipalmitoylphosphatidylcholine (DPPC) dispersions in H_2O was investigated by Raman spectroscopy in a spectral region of 2800—3100 cm^{-1} at temperatures between 22—70 °C. The lateral order parameter S_{lat} calculated by empirical equation reveals that the addition of the ions decreases the lateral ordering of lipid hydrocarbon chains in the gel phase, while in the liquid crystalline state the lateral ordering is increased. In addition, this observation is supported by decomposition of the spectra into component bands using a computer fitting program. This enabled to follow changes in individual band parameters (position, amplitude, and height) in dependence on temperature and/or additives. The results suggest that N-alkyl-N,N,N-trimethylammonium ions have a condensing effect on DPPC bilayer in the liquid crystalline state, the effect increasing with the increasing length of the alkyl substituent.

Key words: Dipalmitoylphosphatidylcholine bilayers — N-alkyl-N,N,N-trimethylammonium ions — Raman spectroscopy—Lateral order

Introduction

The effect of N-alkyl-N,N,N-trimethylammonium ions (C_nTMA) comprises a broad spectrum of biological aspects. The ions act on muscle and nerve cells as

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well as on cells of microorganisms and plants; they also have a local anaesthetic effect. The biological activities of C_nTMA depend, to a significant degree, on the length of the alkyl substituent. Several expressive examples can be picked out. Experiments with squid axon have shown that C_nTMA block the outward potassium current with the maximum effect for $n = 9$ derivates; C_2TMA — C_8TMA ions have a cholinolytic effect on muscles, while $C_{10}TMA$ — $C_{20}TMA$ ions are cholinomimetics; the alkyl ammonium ions with $n < 10$ accelerate the drug resistance transfer between bacterial cells, whereas those with $n > 10$ have an inhibitory effect (see Balgavý et al. 1984 and references therein).

Considerable attention has been paid to biological effects of C_nTMA diming at detecting the site of their action. The understanding of the action of these compounds on model membranes, of their structure and dynamics, may present a suitable starting point in this respect.

On a macroscopic level, the effects of C_nTMA ions on model membranes has been studied using differential scanning calorimetry (Frischleder and Gleichmann 1977; Jain and Wu 1977). C_nTMA with shorter alkyl substituents (C_6, C_9, C_{12}) have been reported to decrease the gel-to-liquid crystal phase transition temperature of the lyotropic mesophase of dipalmitoylphosphatidylcholine bilayer with the maximum effect for C_9TMA and $C_{12}TMA$.

In our previous work (Balgavý et al. 1984), the addition of C_6TMA or C_9TMA to egg yolk phosphatidylcholine (EYPC) dispersion in water was found to induce the formation of nonlamellar phases at EYPC : C_nTMA molar ratios higher than 0.7. No similar effects could be observed in phospholipid-water dispersions mixed with the ammonium derivates with $n = 3$, 12, 16, 18 alkyl chains. In the lamellar liquid crystalline mesophase, the addition of C_nTMA ions to EYPC model membrane resulted in changes in the phospholipid conformation as manifested by changes in chemical shift anisotropy and in $^{31}P—^{1}H$ dipolar coupling constants in proton decoupled and undecoupled ^{31}P-NMR spectra, respectively.

The incorporation of C_nTMA ions into a lamellar mesophase should also result in changes in the lateral packing of lipid molecules constituting a membrane. The aim of this work was to investigate by Raman spectroscopy lateral packing in phospholipid dispersions and its changes upon the addition of alkyl ammonium ions $C_nH_{2n+1}N^+(CH_3)_3 . I^-$ ($n = 6$, 9, 12, 18).

Materials and Methods

L-β,γ-dipalmitoyl-α-phosphatidylcholine (DPPC) was purchased from Fluka (Switzerland). C_nTMA iodides prepared at Faculty of Pharmacy, Comenius University, Bratislava, were analytically pure. The samples for measurements were prepared by mixing chloroform-methanol solutions of DPPC and C_nTMA. The organic solvents were evaporated in a stream of pure nitrogen. The

samples were then evacuated in a vacuum chamber keeping the pressure at 10^{-3} Pa for 12 hours at room temperature. Then, the samples were hydrated with H_2O at a weight ratio of (DPPC + C_nTMA) : H_2O = 1 : 1 and homogenized. The obtained material was filled in glass capillaries with an inner diameter of 1 mm, the capillaries were sealed and stored at −35 °C. Before each measurement, the sample was briefly heated to 45 °C (above the main phase transition temperature of DPPC) with a subsequent equilibration at room temperature. Proton-decoupled ^{31}P-NMR spectra of DPPC : C_9TMA = 1 : 1 (mol : mol) dispersion in H_2O recorded over the temperature region of 20—50 °C showed an axially symmetric shape typical of liquid crystalline lamellar (bilayer) phase; an isotropic signal characteristic of non-lamellar phase appeared above 50 °C (Balgavý et al. 1989). In comparison with the results obtained with EYPC dispersions as described by Balgavý et al. (1984), the formation of nonlamellar phases in DPPC : C_nTMA dispersions is shifted to higher temperatures and lower DPPC : C_nTMA molar ratios. Therefore, all the samples of DPPC : C_nTMA dispersions were prepared at a molar ratio of 2 : 1 to be sure that the Raman spectra be recorded from dispersions in the lamellar phase (*s*).

Raman spectra were recorded using a Jeol JRS-S1 spectrophotometer with a 514 nm excitation laser beam at a medium power (approximately 150 mW at the sample position) using Ar^+ laser from K. Zeiss, Jena. The spectral bandwidth was held on 3—4 cm^{-1}. The sample capillaries were aligned perpendicularly both to the incident laser beam and the scattered light entering the spectrometer. The temperature of the samples was maintained by a stream of heated nitrogen and measured by a Cu-Ko thermocouple. The temperature dependent spectra were recorded at ascending temperature between 22—70 °C.

Results and Discussion

For semi-quantitative characterization of the lateral interaction in phospholipid — water dispersions, Gaber and Peticolas (1977) proposed a lateral order parameter

$$S_{lat} = (I_{as} : I_s - 0.7) : 1.5 \quad (1)$$

where I_s is the intensity of the Raman band due to the methylene symmetric C—H stretch at about 2850 cm^{-1}, and I_{as} that due to the methylene asymmetric C—H stretch near 2885 cm^{-1}. The constants in Eq. 1 originate from $I_{as} : I_s$ values for crystalline and liquid hexadecane.

Typical Raman spectra measured in the 2800—3100 cm^{-1} region are shown in Fig. 1. In this region, the spectral bands exhibited well defined intensity changes during the temperature induced phase transitions in DPPC membranes, and the lateral order parameter, S_{lat}, calculated from the spectra by Eq. 1 decreased slightly at the pre-transition ($L_\beta \rightarrow P_\beta$) and sharply at the main gel-liquid crystal ($P_\beta \rightarrow L_\alpha$) phase transition (see Fig. 2). In addition to the typical sharp change in DPPC dispersions at 40—42 °C, also a temperature induced change in the lateral order parameter was observed in DPPC—C_6TMA = 2 : 1 mixture within the temperature interval between 29 °C and 41 °C (Fig. 2). The other DPPC : C_nTMA mixtures studied (n = 9, 12, 18) did not show any temperature induced changes in the lateral order; the values of the

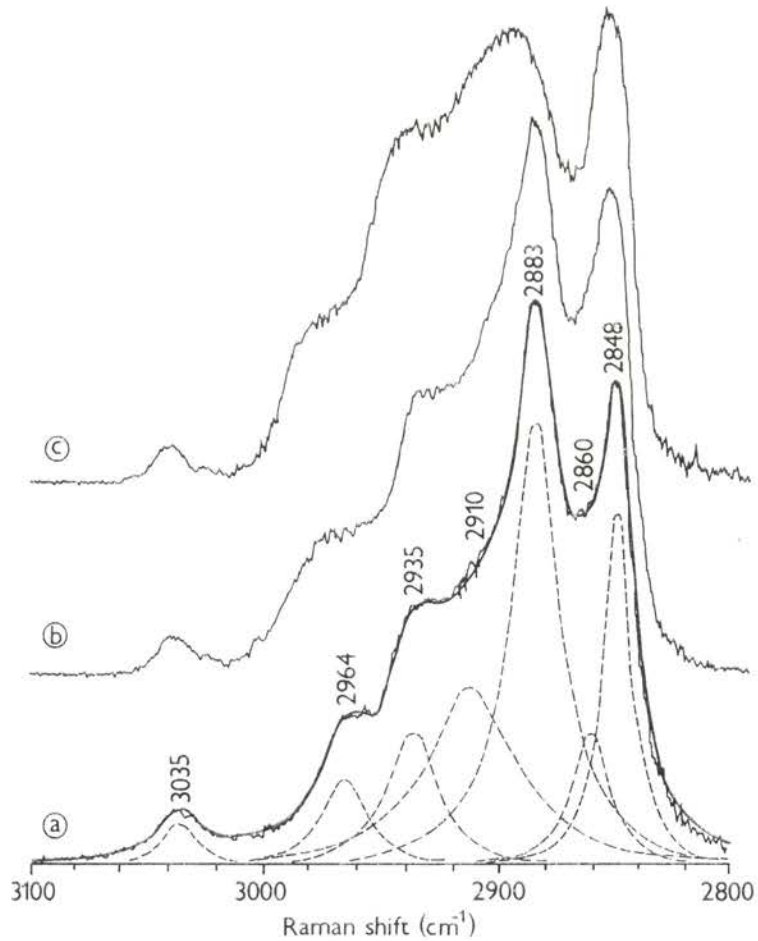

Fig. 1. Raman spectra of DPPC dispersions in H_2O (1 : 1 weight ratio), in the 2800—3100 cm^{-1} region at 25 °C (*a*), 35 °C (*b*), and 44 °C (*c*). The spectrum recorded at 25 °C is shown together with computer simulated component bands. The assignment of the bands is listed in Table 1.

parameter were typical of lipid mesophases in the liquid crystalline state (0.2—0.3). These results suggest that all the C_nTMA ions studied decrease the gel-liquid crystal phase transition. For C_nTMA ions with longer alkyl substituents (n = 9, 12, 18), phase transitions of their mixtures with DPPC probably occur below the lowest temperature used in our experiments (22 °C).

The value of the lateral order parameter, S_{lat}, in the gel phase of DPPC is larger than that in the gel phase of DPPC: C_6TMA = 2 : 1 mixture, as clearly seen from Fig. 2. This indicates that the incorporation of C_6TMA ion into the

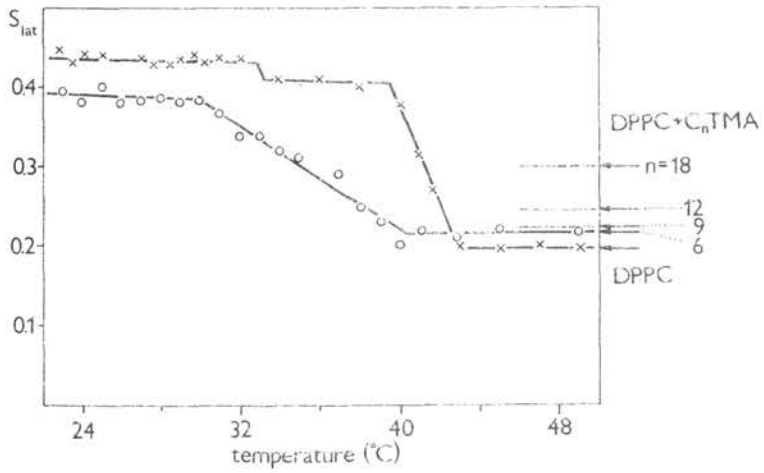

Fig. 2. Effect of temperature on the lateral order parameter, S_{lat}, of DPPC (×) and DPPC : C_6TMA = 2 : 1 (○) dispersions in H_2O. S_{lat} was calculated by Eq. 1. The values of S_{lat} for DPPC and DPPC : C_nTMA = 2 : 1 (n = 6, 9, 12, 18) dispersions in H_2O at 44 °C are indicated by arrows.

tightly packed DPPC bilayer disturbs the lateral order of lipid molecules. On the other hand, we observed an increase in the lateral order of DPPC : C_nTMA = 2 : 1 mixtures in the liquid crystalline state, with the values of S_{lat} increasing with the length of the alkyl substituent in C_nTMA. This is seen in Fig. 2, and more clearly in Fig. 3, where the values of lateral order parameters of DPPC : C_nTMA mixtures normalized with respect to the order parameter for liquid crystalline DPPC mesophase are plotted as a function of the length of C_nTMA alkyl substituents.

The Raman spectra of DPPC in the 2800—3100 cm^{-1} region consist of several overlapping bands with significant contributions from the methylene stretching vibrational modes (Spiker and Levin 1975; Gaber and Peticolas 1977; Gaber et al. 1978; Okabayashi and Kitagawa 1978). Consequently, the above changes in the lateral order parameter might be due not only to changes in the lipid packing, but also to relative shifts in the positions of bands and/or their intensities owing to different numbers of methylene groups in various DPPC : C_nTMA mixtures. Therefore, we decided to decompose the Raman spectra into constituent bands (Table 1) by iterative computer fitting. This approach proved successful in evaluating the Raman spectra in the region of 1000—1200 cm^{-1} (Cirák and Horváth 1985) and in that of 2800—3200 cm^{-1} (Cirák and Horváth 1988). Band profile analyses were performed as described previously (Cirák and Horváth 1985 1988). Briefly, the 2800—3100 cm^{-1} region

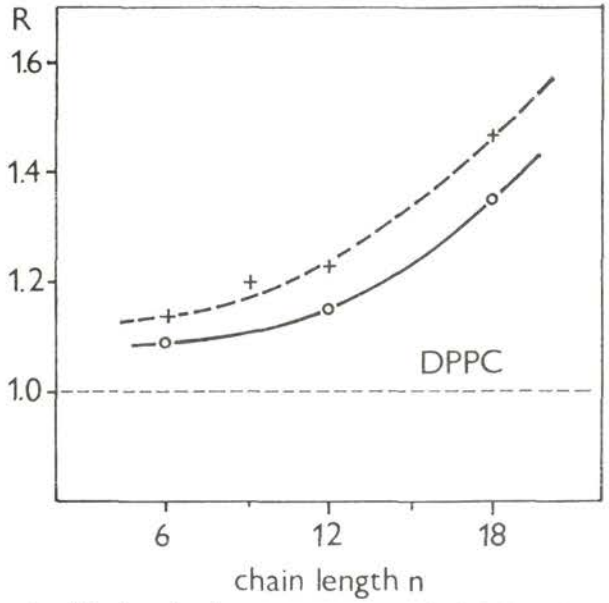

Fig. 3 Relative values, R, of the lateral order parameter (+) and of the intensity of the 2886 cm^{-1} band (○) for DPPC : C_n TMA = 2 : 1 dispersions in H_2O at 44 °C in dependence on the length, n, of the C_nTMA alkyl substituent. The data were normalized with respect to the lateral order (+) or the intensity ($\pi I/W$) of the 2886 cm^{-1} band (○) of DPPC dispersion in H_2O at 44 °C.

was digitized and stored as 0.5 K data points (2 cm^{-1} per channel). In all calculations Lorentzian lineshapes were assumed

$$Y = I : [1 + (X - P)^2 : W^2]$$

where P is the position and $2W$ the half-height width of a line in cm^{-1} units, and I is the amplitude (height) in relative units. The input information for the computer included the analytical formula of the resolved bands, the number and the preassumed centers of these bands. After each iteration step the experimental and simulated spectra were compared and their squared deviations were minimized by a least — square optimization algorithm.

The experimental and the computer simulated spectra of DPPC are compared in Fig. 1. The three traces (a, b, c) correspond to 3 different lyotropic mesophases (L_β, P_β, and L_α, respectively).

In order to evaluate the influence of alkylammonium ions on DPPC bilayer in liquid crystalline state L_α, the spectral parameters obtained by the computer fitting procedure at 44 °C for both DPPC and DPPC + C_nTMA dispersions were compared. The results of the fitting are summarized in Table 2. The line

Table 1. Assignment of Raman modes in the C—C stretching region (2800—3100 cm^{-1}) of DPPC-water dispersions. Assignment and symmetry group symbols follow the recommended abbreviations (Schachtsschneider and Snyder 1963).

band №	band centre (cm^{-1})	assignment
1	2848	$(CH_2)C—H$ symmetric stretch d_s^+
2	2860	(CH_2) C—H symmetric stretch (crystal mode) d_S^+ / cryst
3	2883	(CH_2) C—H asymmetric stretch d_a^- Fermi-resonance enhanced CH-bending overtones $d_s^+ + 2w_a(o)$
4	2913	(CH_2) C—H asymmetric stretch (gauche rotation) d_a^- (π)
5	2935	(CH_3) C—H symmetric stretch of the terminal methyl group r_s^+
6	2964	(CH_3) C—H asymmetric stretch of the terminal methyl group r_a^-
7	3035	(CH_3) C—H asymmetric stretch of the choline methyl group

Table 2. Spectral parameters of component lines in the 2800—3100 cm^{-1} region of the Raman spectra of DPPC : C_nTMA = 2 : 1 dispersions at 44 °C. For the band designation see Table 1, *P* — Raman shift (cm^{-1}), *W* — bandwidth (cm^{-1}), *I* — amplitude (lineheight) in relative units.

band №:		1	2	3	4	5	6	7
DPPC	*I*	69.1	19.4	68.6	50.4	30.5	17.6	10.0
	W	8	11	15	25	14	12	11
	P	2852	2865	2886	2910	2933	2964	3025
DPPC + + C_6TMA	*I*	74.0	25.1	81.7	51.0	45.4	17.5	15.0
	W	8	10	15	22	15	14	12
	P	2851	2865	2885	2908	2936	2962	3035
DPPC + + C_{12}TMA	*I*	80.1	28.6	93.8	44.7	44.4	16.5	15.0
	W	8	12	15	23	15	11	12
	P	2851	2864	2884	2907	2932	2964	3036
DPPC + + C_{18}TMA	*I*	86.1	39.2	119.0	42.6	44.0	16.8	15.0
	W	8	10	14	23	14	13	11
	P	2852	2865	2884	2908	2936	2965	3036

amplitudes shown in this Table were normalized by accounting for the relative increase in the number of polar head methyl groups as compared with that of glycerol groups after the addition of a defined amount of alkyl ammonium ions to DPPC. Upon the addition the amplitude of the line at 3035 cm^{-1} which represents choline C—H asymmetric stretching vibrational modes should grow

up by a factor of 1.5. (The presence of C_nTMA ions is supposed not to affect choline group vibrational modes). Accordingly, if no effect is also expected on alkyl and acyl terminal methyl C—H stretching vibrations, the line amplitudes at 2935 and 2964 cm^{-1} should exhibit a relative increase of 5 : 4 as compared with pure DPPC dispersion. This assumption is roughly met for the sum of both amplitudes, although the addition of C_nTMA ions causes a significant increase in the symmetric mode (2935 cm^{-1}) "to the detriment" of the asymmetric one (2964 cm^{-1}). Thus, the band profile analysis reveals that in DPPC bilayers the environment of the terminal methyl groups of the hydrocarbon chains in liquid crystalline state partly differs from that in DPPC + C_nTMA mixtures. This phenomenon is independent on the length of the C_nTMA alkyl substituent.

In attempting to characterize the lateral order some investigators (Larsson 1973; Larsson and Rand 1973; Gaber and Peticolas 1977) have made use of the relative intensity of the 2886 cm^{-1} line (methylene C—H vibrations), the nature of this line is predominantly determined by Fermi resonance between the totally symmetric CH_2 stretching modes and overtones of CH_2 scissoring modes (Okabayashi and Kitagawa 1978). As shown elsewhere (Gaber et al. 1978; Okabayashi and Kitagawa 1978), the intensity enhancement of this line by Fermi resonance (resulting in an alteration of the relative intensity at 2886 cm^{-1}) is sensitive to changes in molecular conformation induced by temperature or additives. The amplitude of this line, as shown in Table 2, must be normalized with respect to the number of CH_2 groups if the number of glycerol groups is taken as reference (e.g. for DPPC + $C_{12}TMA$, the relative increase in the number of methylenes is 66 : 56). The dependence of the relative normalized intensity of the 2886 cm^{-1} band on C_nTMA alkyl chain length is illustrated in Fig. 3. Obviously, results obtained using the computer fitted data and those of empirical analysis using Eq. 1 show the same tendency: the lateral packing of lipid molecules is tighter in DPPC : C_nTMA mixtures than in pure DPPC mesophase in liquid crystalline state, and it increases with the increasing C_nTMA alkyl length.

Similar information about the condensing effect of C_nTMA ions on DPPC membranes in liquid crystalline state can be derived from the amplitude of the 2910 cm^{-1} band (methylene C—H stretching vibrations of gauche segments). The normalized intensity levels down to 0.92, 0.75, and 0.66 for $n = 6$, 12, 18, respectively.

As shown by Gaber et al. (1978), the Raman line at 2865 cm^{-1} can be assigned to splitting of the CH_2 symmetric stretch caused by crystalline interactions characteristic for a triclinic lattice. A significant increase in the amplitude of this line was observed in our experiments upon the addition of C_nTMA ions. C_6TMA causes a relative increase by a factor of 1.18; the respective figures for

C_{12}TMA are 1.24, and for C_{18}TMA 1.61. The latter value is at the gel phase DPPC level below pretransition.

In summary, the results of both the empirical and the computer assisted evaluation of the Raman spectra of DPPC: C_nTMA mixtures in the 2800—3100 cm^{-1} region show that C_nTMA ions have a condensing effect on DPPC bilayer in liquid crystalline state. *n*-Alcohol molecules intercalated into DPPC bilayers were observed to have similar effects (Horváth et al. 1980), with the lateral order parameter increasing with the alcohol length up to tetradecanol — hexadecanol; a decrease in the parameter was observed with longer chains. The difference in effect between C_nTMA ions and alcohols suggests that for the optimal lateral packing of long chain amphiphilic molecules there is an optimal length which depends on the structure of the polar moiety of the molecules. The type of counterions might influence this optimal packing. It is well known that interactions between charged aggregates of amphiphiles (including bilayers) depend a great deal on counterions (Evans and Ninham 1986). If observed experimentally, counterion effects on the lateral packing in DPPC + C_nTMA systems might provide a link between the interactions within a bilayer and those between bilayers. This is an interesting problem worth of detailed investigation.

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