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Deuterium nuclear magnetic resonance investigation of bacteriophage M13 coat protein in dimyristoylphosphatidylcholine liposomes using palmitic acid as a probe

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The effect of incorporation of various amounts of M13 bacteriophage coat protein on the bilayer order and acyl chain motion in dimyristoylphosphatidylcholine (DMPC) liposomes has been investigated using deuterium NMR of specifically deuterated palmitic acid as a bilayer probe, phosphorus NMR and additional spin-label electron spin resonance (ESR). The secondary structure of the M13 coat protein in these bilayers was determined from circular dichroism spectra. Phosphorus NMR spectra of the mixed liposomes are characteristic for DMPC organized in bilayers, also after incorporation of various levels of M13 protein. Circular dichroism spectra of the coat protein indicate that the protein conformation is predominantly a β-structure (more than 75%). Various incorporation levels of M13 coat protein do not affect the order of the deuterium-labelled positions along the acyl chain at the carbon-2, 9 and 16 positions. In contrast, the spin-spin relaxation times decrease at higher protein levels, especially at the carbon-16 position. The spin-label ESR spectra of the same system using 14-doxylstearic acid as a label show a second, motionally restricted component, that is not observed by deuterium NMR. The NMR and ESR results are consistent with a model in which the fatty acid molecules are in a fast two-site exchange (at a rate of approx. 10^7 Hz) between the sites in the bulk of the lipid bilayer and the motionally restricted sites on the coat protein.

Introduction

In model membranes of well-defined mixed lipids, proteins have been reconstituted to relate lipid–protein interactions to protein functioning, thereby obtaining an insight in the properties of integral membrane proteins. In magnetic resonance studies, the order and motions of the fatty acyl chains of phospholipids have been characterized extensively in presence of a variety of proteins. The results have been summarized and evaluated in a number of recent reviews [1–6].

The objective of our work is to study the infection mechanism of non-enveloped viruses, like

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Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; 14-SASL, 14-(4,4-dimethyloxazolidine-*N*-oxyl)-stearic acid; L/P ratio, phosphatidylcholine to coat protein molar ratio; T_{2e}, spin-spin relaxation time determined from the decay of the quadrupolar echo; T_{2f}, spin-spin relaxation time of free palmitic acid in the bulk of the lipid bilayer; T_{2b}, spin-spin relaxation time of motionally restricted palmitic acid; $\Delta\nu_q$ quadrupolar splitting; τ_2, delay between 90° pulses of quadrupolar echo sequence; ESR, electron spin resonance; CD, circular dichroism.

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bacteriophage M13 and plant viruses, on a molecular level [7]. In comparison with plant viruses, of which the coat proteins do not behave as integral membrane proteins during infection [8], a more suitable system to study the interaction of viral coat proteins with membranes is the M13-*Escherichia coli* system. As an initial step towards a model for M13 penetration into the host cell, we have reconstituted its major coat protein (i.e., the gene-8 product) in model membranes [9–12]. In vivo M13 enters the *E. coli* host cell by leaving the coat proteins in the cytoplasmic membrane [13]. The M13 coat protein (M_r 5240) consists of 50 amino acid residues: a basic C-terminus, a hydrophobic central core of 19 amino acid residues and an acidic N-terminus [14,15]. It has been shown that, apart from the parental coat proteins, also newly synthesized progeny coat proteins are stored in the membrane [16].

Recently, the effect of palmitic acid on membrane structure has been investigated by ^{2}H-NMR, showing that palmitic acid is an accurate reporter molecule in membranes [17]. Previous spin-label electron spin resonance (ESR) work on M13 coat protein incorporated into mixed lipid bilayers indicated a high specificity of the coat protein for free fatty acid molecules [11]. For this reason, in the present study a mixed lipid system, consisting of neutral dimyristoylphosphatidylcholine (DMPC) and perdeuterated (unspecific: U) ([U-$^2H_{31}$]-) or specifically deuterated ([2,2-$^2H_2$]-, [9,9-$^2H_2$]- or [16,16,16-$^2H_3$]-) palmitic acid has been used with various incorporation levels of M13 coat protein. The effect of the coat protein on membrane structure and motion is investigated with ^{2}H-NMR of three labels and additional spin-label ESR of stearic acid labelled at the carbon-14 position of the acyl chain and ^{31}P-NMR. Circular dichroism (CD) spectra were taken of the M13 coat protein in these bilayers to determine the secondary structure.

Preliminary results of this work were presented at the meeting Biological Membranes and Other Liquid Crystals, Southampton, U.K. in September 1985.

Material and Methods

Materials. DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, 99% purity) and palmitic acid (99% purity) were obtained from Sigma and used without further purification. [U-$^2H_{31}$]- (99.1 atom% ^{2}H), [2,2-$^2H_2$]- (98.8 atom% ^{2}H), [9,9-$^2H_2$]- (99.3 atom% ^{2}H), and [16,16,16-$^2H_3$]palmitic acid (99 atom% ^{2}H) were obtained from MSD isotopes, Montreal. 14-Doxylstearic acid (14-SASL) was prepared as described [18].

M13 bacteriophage was purified as described [19]. The major (gene-8 product) coat protein of M13 was isolated by the method of Knippers and Hoffmann-Berling [20].

Sample preparation. Samples for CD measurements were prepared by cholate dialysis, as described [21], with a few modifications. For each DMPC/palmitic acid ratio, 1 mg M13 coat protein and a total amount of 10 mg DMPC and palmitic acid were suspended in 1.0 ml of 8.0 M urea/5.0 mM Tris-HCl/2% (w/w) sodium cholate/0.1 mM EDTA/20 mM ammonium sulphate buffer (pH 8.0). A clear, homogeneous suspension was obtained by vortexing and heating the sample to 55°C. Subsequently, the suspension was dialysed at 4°C against a 50-fold excess of 10 mM Tris-HCl/0.2 mM EDTA/10% (v/v) methanol buffer (pH 8.0) for a total of 48 h with changes at 12, 24 and 36 h. In the last step, no methanol was added to the buffer. This results in dispersions of unilamellar vesicles [21].

Samples for ESR were prepared as above, with small modifications. Typically, 32 mg DMPC, 8 mg palmitic acid and 0.36 mg 14-SASL was suspended in urea/cholate buffer (pH 8.0). Thus, the spin label amounted to 1.8 mol% of the total lipid. Coat protein was added and dissolved by vortexing and incubation at 55°C until a clear solution was obtained. Incorporation of the coat protein and sample homogeneity were checked by sucrose gradient centrifugation. Samples were concentrated by freeze-drying and redissolved in 0.15 ml H_2O. To obtain homogeneous samples of multilamellar bilayers, the samples were vortexed and heated through the phase transition several times. Before the ESR measurements, aliquots of the samples were taken to determine the protein [22] and phosphatidylcholine content [23], yielding the phosphatidylcholine to coat protein molar ratio (L/P ratio) of the samples. Before measurement, the samples were transferred into 0.05 ml glass capillaries.

Samples for ^{2}H- and ^{31}P-NMR were prepared as described above, with the following modifications. Typically, 177.2 mg DMPC, 50 mg deuterated palmitic acid and 0, 22.7, 49.9 or 127.8 mg M13 coat protein were suspended in 10, 20 or 40 ml urea/cholate buffer (pH 8.0), respectively. After dialysis and removal of the solvent by freeze-drying, 10 mM of Tris-HCl buffer (pH 8.0) prepared from deuterium oxide-depleted water was added to the solid mixture at a ratio of 2:1 (w/w). Before measurement, the L/P ratio and homogeneity of the samples were determined as described above.

Determination of DMPC *to palmitic acid ratio.* The DMPC to palmitic acid ratio was determined by gas chromatography. After ^{2}H-NMR measurement, the samples were freeze-dryed. Typically, 15 mg of the powder was dissolved in 0.5 ml of 2 M sodium methanolate/methanol to re-esterify the myristic acid chains of DMPC. Next, 1 ml of 2 M sulphuric acid/methanol was added to the solution to also esterify the palmitic acid. The esters were extracted into hexane for gas chromatography. After separation of hexane from the aqueous fraction, sodium sulphate (anhydrous) was added to remove residual water in the hexane solvent.

Spectroscopy. CD spectra were recorded at room temperature on a Jobin-Ivon Auto-Dichrograph Mark V in the wavelength range 250–190 nm. A sample cell of 1-mm path length was used. Spectra are average of four scans taken from the same sample. To determine the secondary structure, the CD spectra were fitted to reference spectra of Greenfield and Fasman [24] by a least-squares fit procedure using spectral points in the 250–190 nm range with 5-nm steps.

^{31}P-NMR spectra of DMPC/palmitic acid systems were obtained with a Bruker CXP300 Fourier Transform spectrometer at a frequency of 121.48 MHz. For the spectra, an exponential multiplication equivalent to a Lorentzian line broadening of 50 Hz in the frequency domain was applied. All spectra were recorded in the presence of broad-band proton decoupling (20 W/12 dB) using a 16 μs 45° pulse with a repetition rate of 1 s and a spectral width of 50000 Hz.

ESR spectra were recorded as described [11].

^{2}H-NMR spectra were recorded at 46.06 MHz with a Bruker CXP300 Fourier Transform spectrometer. All spectra were recorded with the center of the spectrum on resonance using the quadrupolar echo sequence [25]. For measurements of the [16,16,16-$^2H_3$]palmitic acid samples, two 180° pulses were inserted at $t = \tau_2/2$ and $3\tau_2/2$ to also refocus possible dephasing from dipolar interactions [26]. Quadrature detection was used and both signals from the in-phase and out-of-phase channel were taken for Fourier transformation. The temperature of the sample was regulated by a Bruker variable temperature unit B-VT 1000. For temperatures below room temperature, nitrogen gas, evaporated from a Dewar containing liquid nitrogen, was used for temperature regulation. Measurements were started at low temperature, and after each temperature increase, the samples were allowed to equilibrate for at least 0.5 h.

Spin-spin relaxation times, T_{2e}, were determined by varying the delay time, τ_2, between the 90° pulses of the quadrupolar echo. Typically, a range of 40–1000 μs was covered by 20 measurements, with the exception of the [16,16,16-$^2H_3$]palmitic acid samples, for which a range of 40–5000 μs was taken. Each measurement was the result of 2500–8000 free induction decays. The top of the quadrupolar echo signal was measured at each τ_2 value and plotted as a function of $2\tau_2$.

Results

Characterization of the lipid-protein system

M13 coat protein incorporates completely in a mixed DMPC/palmitic acid system containing 20% (w/w) initially added palmitic acid. Stable, unaggregated vesicles are formed after cholate dialysis, and they are homogeneous, as determined from sucrose gradient centrifugation.

The initially added amounts of coat protein and DMPC do not provide an accurate number for the L/P ratio in the vesicles after cholate dialysis. Therefore, after dialysis, DMPC samples were taken to determine the final L/P ratio. The DMPC and coat protein initially added had L/P ratios of 60, 30 and 15. Dialysis decreases the ratios to 39, 20 and 9, respectively. These values are reproducible within 10%.

By gas chromatography, the myristic to palmitic acid molar ratio in the ^{2}H-NMR samples was found to be 2.1 ± 0.2 for all samples in the range

from 0 to 36% (w/w) coat protein. This indicates that the initial weight percentage (20% (w/w)) of palmitic acid has increased to 27% (w/w).

Analysis of CD spectra of M13 coat protein incorporated in stable DMPC/palmitic acid (73/27 (w/w)) small unilamellar vesicles in solution yielded the following secondary structure: no α-helix, 92% β-structure, 8% other structure at an L/P ratio of 56 and no α-helix, 75% β-structure, 25% other structure at an L/P ratio of 1.5. In between these L/P ratios, intermediate amounts of secondary structure were found.

^{31}P-NMR spectra in the gel and in the liquid-crystalline phase of the phosphatidylcholine molecules in the DMPC/palmitic acid (73/27 (w/w)) system with (L/P ratio, 9) and without coat protein are powder-like spectra with a low field shoulder and a high field peak, characteristic for DMPC organized in bilayers (data not shown). In the gel phase (0°C), the spectra are broad with a chemical shift anisotropy parameter of −66 ppm. In the liquid-crystalline phase (45°C), the chemical shift anisotropy parameter is −43 ppm. No difference in the chemical shift anisotropy of the sample with or without coat protein is observed. Also, in none of the spectra is any intensity seen (1) at the isotropic peak position that could arise from the presence of rapidly tumbling phosphatidylcholine molecules [27], or (2) from an inverted powder pattern caused by H_{II} phase lipids [28]. The phase transition, which is visible in a temperature-dependent series as a decrease in the chemical shift anisotropy parameter [1], ranges from 29 to 38°C in both samples.

ESR spectra of 14-SASL spin label in DMPC/palmitic acid (73/27 (w/w)) systems with M13 coat protein were recorded at various L/P ratios. The spectra were taken at 45°C, which is well (7C°) above the phase transition, as observed by ^{31}P-NMR and ^{2}H-NMR (Fig. 3, discussed below). Apart from a motionally averaged sharp three-line spectrum, typical for liquid-crystalline phase lipid, the samples with M13 coat protein has a second, broader component with strongly restricted motion on the ESR time scale. The intensities of these components, determined by a previously described analysis [11], were found to be 0, 10, 19 and 36% of the total spin-label intensity at L/P ratios of ∞, 39, 20 and 9, respectively.

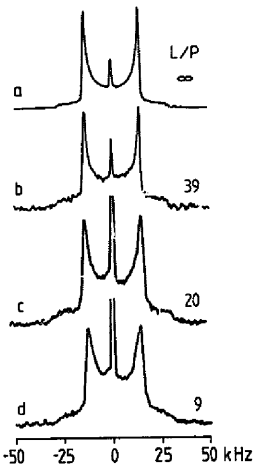

Fig. 1. 46.06 MHz ^{2}H-NMR spectra of a DMPC/[9,9-$^{2}H_2$]palmitic acid system (73/27 (w/w)) at 45°C without (a) and with coat protein at an L/P ratio of 39 (b), 20 (c) and 9 (d). The number of scans was 40000 (a) or 16000 (b–d) with a relaxation delay of 333 ms, a τ_2 of 40 μs, a 90° pulse length of 5.8 μs and an artificial line broadening of 100 Hz.

^{2}H-NMR spectra

^{2}H-NMR spectra of [9,9-$^{2}H_2$]palmitic acid/DMPC (27/73 (w/w)) in the liquid-crystalline phase (45°C) with M13 coat protein at various L/P ratios are shown in Fig. 1. Typical powder line shapes [3,29] are observed with quadrupolar splittings, $\Delta\nu_q$, of 28, 28, 28 and 26 kHz for L/P ratios of ∞, 39, 20 and 9, respectively (Fig. 1). For samples with a low coat protein content (L/P ratio ≥ 20), $\Delta\nu_q$ is the same as for the reference sample. However, due to the coat protein, the spectra are broadened. The phase transition is visible in a temperature-dependent series as double component spectra from 29–38°C for the reference sample, which is not changed by the presence of coat protein in the range of L/P ratios examined. The ^{2}H-NMR spectrum of the reference sample has an isotropic peak of small intensity (Fig. 1a) that increases with increasing coat protein content (3, 5 and 20% of total intensity in Fig. 1b,c,d, respectively). The same effect is also observed in the spectra of palmitic acid labelled at the other positions (Fig. 2).

^{2}H-NMR spectra of [U-$^{2}H_{31}$]-, [2,2-$^{2}H_2$]-, [9,9-$^{2}H_2$]- and [16,16,16-$^{2}H_3$]-palmitic acid in

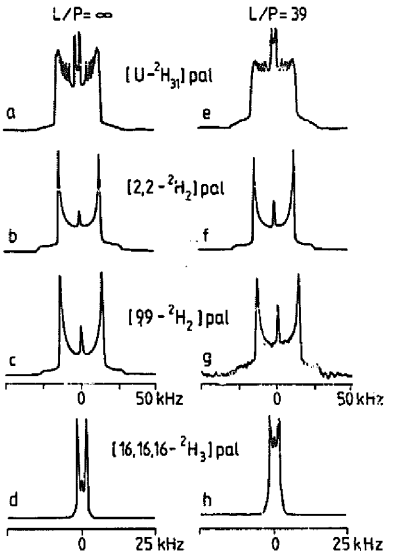

Fig. 2. 46.06 MHz ^{2}H-NMR spectra of a [U-$^2H_{31}$]- (a,e), [2,2-$^2H_2$]- (b,f), [9,9$^2H_2$]- (c,g) and [16,16,16-$^2H_3$]- (d,h) palmitic acid/DMPC (27/73 (w/w)) system without (a–d) and with (e–h) M13 coat protein at an L/P ratio of 39 in the liquid-crystalline phase. The number of scans and the temperature were 2500 and 41°C (a), 25000 and 45°C (b), 40000 and 45°C (c), 20000 and 41°C (d), 2000 and 41°C (e), 25000 and 45°C (f), 15000 and 45°C (g) and 20000 and 41°C (h). In all cases, a relaxation delay of 333 ms, a τ_2 of 40 μs, a 90° pulse length of 5.8 μs and a line broadening of 100 Hz was applied.

DMPC/palmitic acid (73/27 (w/w)) systems with (L/P ratio, 39) and without coat protein in the liquid-crystalline phase are shown in Fig. 2. The spectrum of perdeuterated palmitic acid (Fig. 2a) consists of several overlapping powder patterns of nine resolved quadrupolar splittings of 29, 27, 25, 24, 22, 19, 15 and 11 kHz of deuterated sites along the acyl chain and 2.7 kHz of the terminal C^2H_3 site. The sample with M13 coat protein gives identical values (Fig. 2e). However, all peaks in this spectrum are broadened due to the presence of M13 coat protein.

The ^{2}H-NMR spectra of samples with palmitic acid selectively labelled at the C-2 or C-9 position result in sharp single powder pattern spectra with $\Delta\nu_q$ of 28 kHz (Fig. 2b,c). Again, a distinct line broadening is observed in samples with coat protein (Fig. 2f,g).

^{2}H-NMR spectra of samples with palmitic acid labelled at the C-16 display a powder spectrum with a $\Delta\nu_q$ of 12 kHz in the gel phase (0°C) and of 2.7 kHz in the liquid-crystalline phase (Fig. 2d). This is in agreement with the values found for the terminal group of perdeuterated palmitic acid. In Fig. 3a, the quadrupolar splitting from this sample is plotted as a function of temperature. Due to its high mobility, the terminal C^2H_3 site also gives sharp powder spectra in the gel phase. This property is used to determine the amount of gel phase lipid by means of the integrated intensities [17] as a function of temperature (Fig. 3b). The phase transition region is seen from 29 to 38°C as double component spectra. From 29 to 35°C, $\Delta\nu_q$ of the fluid component remains constant at 3.2 kHz and decreases above 35°C with increasing temperature for both samples, with and without coat protein (Fig. 3b).

Quadrupolar echo decay measurements

Quadrupolar echoes of samples with [2,2-$^2H_2$]-, [9,9-$^2H_2$]- and [16,16,16-$^2H_3$]-palmitic acid/

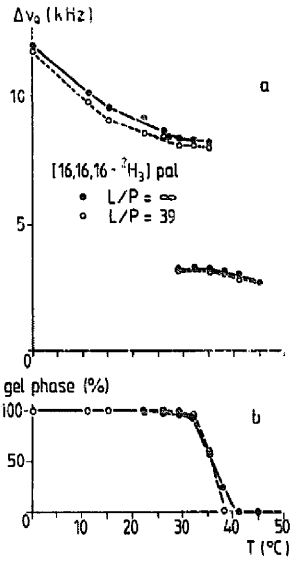

Fig. 3. (a) Quadrupolar splittings, $\Delta\nu_q$, as a function of temperature from ^{2}H-NMR spectra of a DMPC/[16,16,16-$^2H_3$]palmitic acid (73/27 (w/w)) system without (solid line) and with (dashed line) M13 coat protein (L/P ratio, 39). (b) The gel phase percentage, as determined from the intensity distribution in the double-component ^{2}H-NMR spectra.

TABLE I

SPIN-SPIN RELAXATION TIMES ALONG THE ACYL CHAIN IN MIXED BILAYERS WITH M13 COAT PROTEIN AT 45°C

The coat protein was incorporated at an L/P ratio of 39. Typical error in T_{2e} and T_{2f} is 5%.

Label position at the acyl chain	T_{2e} (M13) (μs)	T_{2f} (ref.) (μs)	T_{2b} [a] (calc.) (μs)
C-2	540	620	320
C-9	430	480	220
C-16	3500	5400	880

[a] For the calculation of T_{2b} from Eqn. 1, the specifically deuterated palmitic acid (27% (w/w) in DMPC bilayers) is assumed to undergo a fast two-site exchange between the sites in the bulk of the lipid bilayer and the motionally restricted sites (10% as seen by ESR).

DMPC (27/73 (w/w)) systems with (L/P ratio, 39) and without M13 coat protein were measured in the liquid-crystalline phase (45°C) as a function of twice the spacing between the 90° pulses of the quadrupolar echo sequence, $2\tau_2$. The decays are monoexponential [26], characterized by one spin-spin relaxation time, T_{2e}. The T_{2e} values obtained in this way are listed in Table I. For other L/P ratios, the T_{2e} measurement becomes inaccurate, because of the appearance of the isotropic peak in the spectra (see Fig. 1).

Discussion

M13 coat protein secondary structure

The dominant conformation of the M13 coat protein in the mixed DMPC/palmitic acid system is a β-structure (75–92% for L/P ratios from 1.5 to 56) as determined from the CD spectra. A β-structure for an integral membrane protein is remarkable, since many proteins are known to span the membrane by an α-helix [5,6]. However, a β-structure conformation of fd coat protein (which differs from M13 coat protein in only one amino acid residue) has been observed before in a model membrane [10,30–32], even though the M13 and fd coat protein are known to be entirely in an α-helix conformation in the virus [33,34]. This has been explained in terms of a major conformational change during the membrane-bound assembly of the bacteriophage [30]. From time-resolved tryptophan fluorescence anisotropy measurements [10] and deuterium NMR measurements of the exchangeable sites at the coat protein backbone [12] in similar mixed bilayers, protein aggregations was concluded. In analogy, in mixed DMPC/palmitic acid bilayers, the coat protein is probably also aggregated. Within the aggregate, the positively charged C-terminus and the negatively charged N-terminus [13,14] can very well cross-link to form antiparallel β-sheet structures, whereas the central core forms strong hydrophobic contacts. Also, a U-shaped conformation of the protein unit, which has been considered before [32], can be present in the protein aggregates. It is not clear whether the β-structure is the protein conformation in vivo in the *E. coli* membrane, although a model for M13 coat protein assembly has been proposed [35], which includes a U-shaped procoat, in which the mature coat protein domain could well form an antiparallel β-structure conformation with its N-terminal leader sequence.

Lipid organization

The lipid organization in DMPC/palmitic acid (73/27 (w/w)) systems with and without M13 coat protein can be summarized as follows:

(1) Reference lipid system (L/P = ∞). The spectra obtained in the liquid-crystalline phase by ^{31}P-NMR from the phospholipids (data not shown) and by ^{2}-NMR from the labelled palmitic acid (Figs. 1a, 2a–d), show powder patterns that are characteristic for phospholipid and palmitic acid organized in a bilayer in the liquid-crystalline phase. The narrow isotropic peak is assigned to small amounts of palmitic acid that rotate freely in solution. Another indication for a bilayer structure is the phase behaviour of the system. The phase transition region, observed by ^{31}P-NMR (data not shown) and ^{2}H-NMR (Fig. 3), for the mixed reference bilayers is broadened to a range of 9°C and shifted upward as compared to the phase transition of pure DMPC (24°C). Both effects are caused by palmitic acid (27% (w/w)), as reported before for phospholipid bilayers containing free fatty acids [36]. Free fatty acids are assumed to fill the voids that exist between the phospholipids. The voids arise from crowding of the headgroups, that have a larger excluded area

in the bilayer plane than the lipid acyl chains [37–39]. As a consequence, the phase transition is shifted upwards, consistent with previous results [17].

(2) DMPC/palmitic acid mixtures with a high amount of coat protein ($L/P \leq 9$). The ^{2}H-NMR spectrum of Fig. 1d displays a broad isotopic peak contributing 20% to the total spectral intensity. This shows that the major part (80%), but not all palmitic acid, is organized in a bilayer-type organization. Clearly, at this protein content and higher, part of the palmitic acid (20%) no longer reflects the acyl chain behaviour of the phosphatidylcholine molecules in the bilayer.

A similar ^{2}H-NMR spectrum has been reported for a system consisting of a very high amount (67% (w/w)) of f1 coat protein in DMPC labelled at the terminal C-14 atom position of the *sn*-2 chain [40]. Since f1 coat protein has an amino acid sequence almost identical to M13 coat protein, it may be concluded from these results that at extremely high protein contents, some of the coat protein molecules are no longer incorporated in stable vesicles in solution, but appear in precipitated phospholipids in a non-bilayer type of aggregation.

(3) DMPC/palmitic acid systems with intermediate levels of coat protein ($L/P = 20$ and 39). ^{2}H-NMR spectra (Fig. 1b and c) show that more than 95% of the palmitic acid is in a bilayer structure.

Order and dynamics of the palmitic acid

Previous spin-label ESR experiments have shown that incorporation of M13 coat protein into phosphatidylcholine bilayers with negatively charged phospholipids results in a second, motionally restricted component [9,11]. Additional spin-label ESR spectra from DMPC/palmitic acid (73/27 (w/w)) systems probed with the 14-SASL spin label have been carried out here, enabling direct comparison with the ^{2}H-NMR spectra. These experiments show that the coat protein also interacts preferentially with 14-SASL in the DMPC/palmitic acid bilayers. The intensity of the motionally restricted component is proportional to the protein content, in agreement with the previous results [11]. The 14-SASL spin label results can be compared with the ^{2}H-NMR spectra of Figs. 1 and 2. Since in the ESR spectra two distinct sites, i.e., of a motionally restricted and of a fluid component, are seen, these sites are in slow exchange on the ESR time scale (slower than 10^{-9} s). The motionally restricted component is well-resolved from the fluid component, similar to previously reported observations for 14-doxyl-dimyristoylphosphatic acid spin label (14-PASL) in mixed DMPC/dimyristoylphosphatidylglycerol (80/20 (w/w)) bilayers [11]. Therefore, the fatty acid spin label in the mixed DMPC/palmitic acid is likely to exchange at approximately the same rate, i.e., 10^7 s^{-1} [11]. As a consequence, no second component is seen in the present ^{2}H-NMR spectra of deuterated palmitic acid, i.e., the two sites, seen in the spin-label ESR spectra, are in fast exchange with respect to the ^{2}H-NMR time scale (10^{-5} s).

The present ^{2}H-NMR experiments indicate that M13 coat protein does not affect the order at the C-2, C-9 and C-16 position in the liquid-crystalline phase, as reflected by the quadrupolar splitting (Fig. 2). However, the presence of coat protein increases the line width of the spectra (Figs. 1 and 2). In the liquid-crystalline phase, the bilayer order decreases gradually along the acyl chain [3], which is clearly seen in the ^{2}H-NMR spectra of perdeuterated palmitic acid as a series of decreasing quadrupolar splittings. The smallest originates from the terminal C^2H_3 site. The order along the acyl chain, seen as individual splittings in the spectra from the perdeuterated sample, is not affected by M13 coat protein (Fig. 2a and e). This agrees with the result obtained from samples with C-2-, C-9- and C-16-labelled acyl chains (Fig. 2b–h).

From Table I, it can be seen that in the liquid-crystalline phase, the coat protein decreases T_{2e} at all three sites along the fatty acid acyl chain. To analyse these data, a model is assumed in which the free fatty acid molecules are in fast two-site exchange (at a rate of 10^7 s^{-1}, as concluded above) between the sites in the bulk of the lipid bilayer and the motionally restricted sites (see also Refs. 41–43). The observed T_{2e} is then the weighted average of the spin-spin relaxation times of free palmitic acid in the bulk of the lipid bilayer (T_{2f}) and at the motionally restricted sites (T_{2b}).

$$1/T_{2e} = f/T_{2b} + (1-f)/T_{2f} \quad (1)$$

where f is the fraction of motionally restricted fatty acid, as seen by ESR (which is 0.10 at an L/P ratio of 39). In Eqn. 1 line broadening due to exchange of the probe lipid between the bulk and the coat protein-associated site is neglected, since the exchange is at a rate of approx. 10^7 Hz (see also the appendix of Ref. 41). The calculated values for T_{2b} are given in Table I. For the C-2 and C-9 positions, the T_{2b} values are a factor of 2 smaller than T_{2f} of the reference sample. At the C-16 position, the difference is a factor of 6 (Table I).

Assuming that the contribution of the fast motions in such multilamellar vesicle bilayers dominates the T_{2e} of the deuterons along the acyl chain, the limit of fast motion [3,44] applies and the observed decrease of T_{2e} values corresponds directly to a decrease in mobility. From this, it follows that the motion of the palmitic acid is restricted by the presence of the coat protein in the bilayer at all positions along the acyl chain, but selectively more at the terminal position.

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References

1 Seelig, J. and Seelig, A. (1980) Quart. Rev. Biophys. 13, 19–61.
2 Jacobs, R.E. and Oldfield, E. (1981) Prog. Nucl. Magn. Reson. Spectrosc. 14, 113–136.
3 Davis, J.H. (1983) Biochim. Biophys. Acta 737, 117–171.
4 Devaux, P.F. (1983) in Biological Magnetic Resonance (Berliner, L.J. and Reuben, J., eds.), Vol. 5, pp. 183–299, Plenum Press, New York.
5 Bloom, M. and Smith, I.C.P. (1985) in Progress in Protein-Lipid Interactions (Watts, A. and De Pont, J.J.H.H.M., eds.), Ch. 2, pp. 61–88, Elsevier, New York.
6 Marsh, D. (1985) in Progress in Protein-Lipid Interactions (Watts, A. and De Pont, J.J.H.H.M., eds.), Ch. 4, pp. 143–172, Elsevier, New York.
7 Hemminga, M.A., Datema, K.P., Ten Kortenaar, P.W.B., Krüse, J., Vriend, G., Verduin, B.J.M. and Koole, P. (1985) in Magnetic Resonance in Biology and Medicine (Govil, G., Khetrapal, C.L. and Saran, A., eds.), pp. 53–75, Tata McGraw-Hill, New Delhi.
8 Datema, K.P., Spruijt, R.B., Verduin, B.J.M. and Hemminga, M.A. (1987) Biochemistry 26, 6217–6223.
9 Hemminga, M.A. (1987) J. Chem. Soc., Faraday Trans. 1, 83, 203–210.
10 Datema, K.P., Visser, A.J.W.G., Van Hoek, A., Wolfs, C.J.A.M., Spruijt, R.B. and Hemminga, M.A. (1987) Biochemistry 26, 6145–6152.
11 Datema, K.P., Wolfs, C.J.A.M., Marsh, D., Watts, A. and Hemminga, M.A. (1987) Biochemistry 26, 7571–7574.
12 Datema, K.P., Van Boxtel, B.J.H. and Hemminga, M.A. (1988) J. Magn. Reson. 77, 372–376.
13 Marvin, D.A. and Wachtel, E.J. (1975) Nature 253, 19–23.
14 Van Asbeck, F., Beyreuther, K., Koehler, E., Von Wettstein, G. and Braunitzer, G. (1969) Hoppe-Seyler's Z. Physiol. Chem. 350, 1047–1066.
15 Nakashima, Y. and Konigsberg, W. (1974) J. Mol. Biol. 88, 598–600.
16 Wickner, W. (1976) Proc. Natl. Acad. Sci USA 73, 1159–1163.
17 Pauls, K.P., MacKay, A.L. and Bloom, M. (1983) Biochemistry 22, 6101–6109.
18 Marsh, D. and Watts, A. (1982) in Lipid-Protein Interactions (Jost, P.C. and Griffith, O.H., eds.), Vol. 2, pp. 53–126, Wiley Interscience, New York.
19 Garssen, G.J., Hilbers, C.W., Schoenmaker, J.G.G. and Van Boom, J.H. (1977) Eur. J. Biochem. 81, 453–463.
20 Knippers, R. and Hoffmann-Berling, H. (1966) J. Mol. Biol. 21, 281–292.
21 Hagen, D.S.,, Weiner, J.H. and Sykes, B.D. (1978) Biochemistry 17, 5860–5966.
22 Peterson, G.L. (1977) Anal. Biochem. 83, 346–56.
23 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.
24 Greenfield, N. and Fasman, G.D. (1969) Biochemistry 8, 4108–4116.
25 Davis, J.H., Jeffrey, K.R., Bloom, M., Valic, M.I. and Higgs, T.P. (1976) Chem. Phys. Lett. 42, 390–394.
26 Datema, K.P., De Jager, P.A. and Hemminga, M.A. (1988) J. Magn. Reson., in press.
27 Burnell, E.E., Cullis, P.R. and De Kruijff, B. (1980) Biochim. Biophys. Acta 603, 63–69.
28 Van Echteld, C.J.A., De Kruijff, B., Verkleij, A.J., Leunissen-Bijvelt, J. and De Gier, J. (1982) Biochim. Biophys. Acta 692, 126–138.
29 Seelig, J. and Seelig, A. (1974) Biochemistry 13, 4839–4845.
30 Nozaki, Y., Chamberlain, B.K., Webster, R.E. and Tanford, C. (1976) Nature 259, 335–337.
31 Nozaki, Y., Reynolds, J.A. and Tanford, C. (1978) Biochemistry 17, 1239–1246.
32 Chamberlain, B.K., Nozaki, Y., Tanford, C. and Webster, R.E. (1978) Biochim. Biophys. Acta 510, 18–37.
33 Valentine, K.G., Schneider, D.M., Leo, G.L., Colnago, L.A. and Opella, S.J. (1986) Biophys. J. 49, 36–38.

34 Opella, S.J., Stewart, P.L. and Valentine, K.G. (1987) Quart. Rev. Biophys. 19, 7–49.
35 Kuhn, A., Wickner, W. and Kreil, G. (1986) Nature 322, 335–339.
36 Kantor, H.L. and Prestegard, J.H. (1978) Biochemistry 17, 3592–3597.
37 Mabrey, S. and Sturtevant, J.M. (1977) Biochim. Biophys. Acta 486, 444–450.
38 McIntosh, T.J. (1980) Biophys. J. 29, 237–246.
39 Büldt, G., Gally, H.U., Seelig, A. and Seelig, J. (1978) Nature 271, 182–184.
40 Oldfield, E., Gilmore, R., Claser, M., Gutowski, H.S., Hshung, J.C., Kang, S.Y., King, T.E., Meadows, M. and Rice, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4657–4660.
41 Paddy, M.R., Dahlquist, F.W., Davis, J.N. and Bloom, M. (1981) Biochemistry 20, 3152–3162.
42 Deese, A.J., Dratz, E.A., Dahlquist, F.W. and Paddy, M.R. (1981) Biochemistry 20, 6420–6427.
43 Bienvenue, A., Bloom, M., Davis, J.H. and Deveaux, P.F. (1982) J. Biol. Chem. 257, 3032–3038.
44 Brown, M.F. and Williams, G.D. (1985) J. Biochem. Biophys. Methods 11, 71–81.
45 Bloom, M. and Sternin, E. (1987) Biochemistry 26, 2101–2105.