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Formation of non-bilayer structures induced by M13 coat protein depends on the conformation of the protein

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A comparison is made of the interaction of the coat protein of bacteriophage M13 in a predominant α-helix conformation and in a predominant β-sheet conformation. To perform a systematic study of the interaction between the protein in these two different forms and the surrounding lipid matrix, NMR spectra of ^{2}H-nuclei of specific labelled phospholipid systems are measured. In addition ^{31}P-NMR is employed to provide information about the morphological structure adopted by the reconstituted lipid/protein systems. From the ^{2}H-NMR studies on specific headgroup and chain deuterium labelled phospholipids it is found that the protein in the predominant β-sheet conformation causes a fraction of lipids to be trapped. By combining the results from the headgroup and acyl chains of the phospholipids, it is concluded that the trapped lipids are arranged in a non-bilayer structure, probably caused by a misfitting of the hydrophobic core of the protein and the membrane bilayer. The protein in the predominant α-helix conformation perfectly fits in the lipid bilayer and has only minor influences on the surrounding lipid matrix. A new model is proposed to explain the presence of the trapped lipids in the lipid/protein systems.

Introduction

Recently it has been reported that the coat protein of bacteriophage M13 adopts two different conformations, when reconstituted into lipid systems. Also the aggregation behaviour of these two M13 coat protein conformations differs considerably. The protein in a predominant β-sheet conformation forms large irreversible aggregates [1–8]. This form of M13 coat protein has been called the β-polymeric form [9]. M13 coat protein in a predominant α-helix conformation results in reversible small aggregates; this form has been called the α-oligomeric form [9]. The presence of either one of these M13 coat protein forms depends on the protein purification, lipid type and salt concentration [9–11].

Previous experiments conducted on phospholipids reconstituted with the M13 coat protein in the β-polymeric form showed a fraction of lipids, which could not exchange with the bulk lipids. This fraction of lipids was thought to be trapped by aggregates of the coat protein in the β-polymeric form [4,5]. In the present paper further investigations are presented to obtain information about the nature of these trapped lipids. In addition we investigate the interaction between the lipid matrix and the M13 coat protein in the α-oligomeric form.

To perform a systematic study of the interaction between the protein in its two different forms and the surrounding lipid matrix, NMR spectra of ^{2}H-nuclei of specific labelled phospholipids are measured. DOPC was specifically deuterated either in both chains at the 11-position (1,2-[11,11-$^2H_2$]DOPC) or at the trimethyl moiety in the headgroup (DOPC-d_9). DMPC was deuterated at the methyl positions of the *sn*-2 chain (2-[14,14,14-$^2H_3$]-DMPC). ^{31}P-NMR is employed to provide information about the morphological structure adopted by the reconstituted lipid/protein systems. A new model is proposed to explain the presence of the trapped lipids in the lipid/protein systems.

Materials and Methods

Lipids. DOPC-d_9 deuterated in the trimethyl groups of the choline moiety, was synthesized from DOPE as described by Eibl [12]. 1,2-[11,11-$^2H_2$]DOPC was a

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Abbreviations: DOPC, dioleoylphosphatidylcholine; DMPC dimyristoylphosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; *L/P*, lipid to protein molar ratio; NMR, nuclear magnetic resonance; T_{1e}, spin lattice relaxation time; T_{2e}, deuterium spin-spin relaxation time; Tris, tris(hydroxymethyl)aminomethane hydrochloride.

kind gift from B. de Kruijff (University of Utrecht). 2-[14,14,14-$^2H_3$]DMPC, labelled in the terminal methyl of the *sn*-2 chain, was synthesised from DMPC (Sigma, St. Louis, USA) using phospholipase A_2 for preparing lyso-phosphatidylcholine with subsequent esterification with [14,14,14-$^2H_3$]tetradecanoic acid (Larodan AB, Malmö, Sweden) as described by Boss [13]. After purification, the lipids appeared as one spot on silica thin-layer chromatography (solvent $CHCl_3$/MeOH/NH_4OH, 55:30:3, v/v/v) and were stored at −20°C.

Sample preparation. Bacteriophage M13 was grown and purified as described previously [9]. After removing the chloroform from the desired amount of lipids with nitrogen gas, samples were lyophilized for at least 12 h and solubilized in buffer (α-oligomeric coat protein in buffer A: 50 mM cholate, 10 mM Tris, 0.2 mM EDTA, 140 mM NaCl, pH 8.0; β-polymeric coat protein in buffer B: 8.0 M urea, 5 mM Tris, 0.1 mM EDTA, 20 mM ammonium sulphate, 140 mM NaCl, pH 8.0). To this solution the desired amount of either the α-oligomeric protein in buffer A or the M13 coat protein in the β-polymeric form in buffer B was added followed by dialysis at room temperature against 100-fold excess buffer (10 mM Tris, 0.2 mM EDTA, 140 mM NaCl, pH 8.0) for a total of 48 h changing the buffer every 12 h. Directly after the dialysis procedure the reconstituted lipid-protein complexes were concentrated using an Amicon stirring cell and lyophilized for at least 12 h and resuspended in deuterium depleted water (Sigma). The aggregation state, conformation and *L/P* ratio of the reconstituted coat protein in the phospholipid systems was checked as described previously [9].

NMR experiments. All NMR spectra were recorded on a Bruker CXP 300 spectrometer as described previously [14]. Oriented spectra were obtained numerically from the experimental spectra by using an iterative depaking program [15]. The quadrupolar splittings given in the tables are all obtained from these oriented spectra.

Results

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All samples were checked for their homogeneity and for the conformation and aggregation state of M13 coat protein. Sucrose gradients of all samples showed only one band indicating that the samples were homogeneous. Previously the homogeneity of the samples was also confirmed with electron microscopy [5]. Circular dichroism spectroscopy and high performance liquid chromatography elution profiles indicated that the α-oligomeric form of the protein was in a predominant α-helix conformation with no indication of strong protein aggregation, whereas the β-polymeric form was in a predominant β-sheet conformation and strongly aggregated. This is in agreement with results reported previously [9].

TABLE I

Quadrupolar splittings (kHz) of 1,2-[11,11-$^2H_2$]DOPC with M13 coat protein in the two conformations at different temperatures

L/P ratio	Temperature (°C)		
	10	20	30
∞	7.1	6.2/6.5 [a]	5.5/5.8 [a]
35 (α-oligomeric)	6.2	5.5	4.9
30 (β-polymeric)	7.4	5.8	5.6

[a] Two splittings are observed in the depaked spectra.

^{2}H-NMR on 1,2-[11,11-$^2H_2$]DOPC

In the ^{2}H-NMR spectra of 1,2-[11,11-$^2H_2$]DOPC at higher temperatures two quadrupolar splittings are observed (Table I). The most likely explanation is that the two powder patterns originate from deuterons on the two different chains. It is known that the *sn*-2 chain is positioned somewhat higher in the membrane than the *sn*-1 chain. This gives rise to the observed differences in quadrupolar splitting, due to the difference in the order parameter [16]. The sharp central peak visible in the spectra with and without M13 coat protein (Fig. 1), is assigned to a small fraction (< 5%) of lipids in small vesicles that have a fast random reorientation on the NMR time scale.

In the spectra of 1,2-[11,11-$^2H_2$]DOPC with the α-oligomeric form of the M13 coat protein no indication of a second quadrupolar splitting can be found (Table I, Fig. 1). This can either be due to the lack of resolution arising from the increased line width, or due to the fact that the protein removes the differences

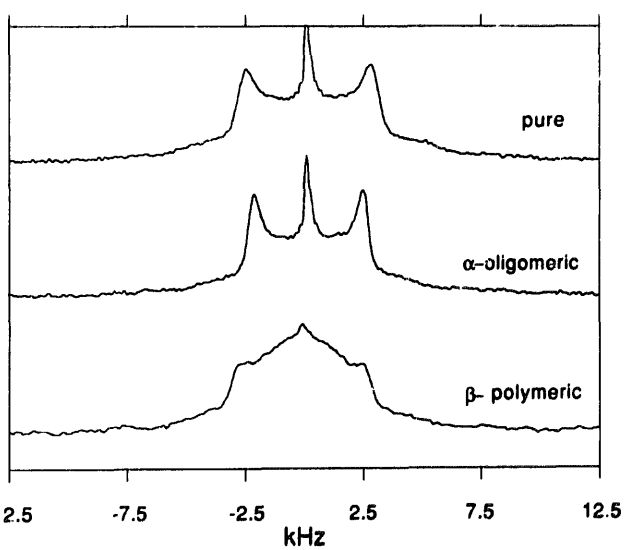

Fig. 1. 46.1 MHz ^{2}H-NMR spectra of 1,2-[11,11-$^2H_2$]DOPC at 30°C of pure and protein containing samples (both forms *L/P* 20). Number of scans 30000.

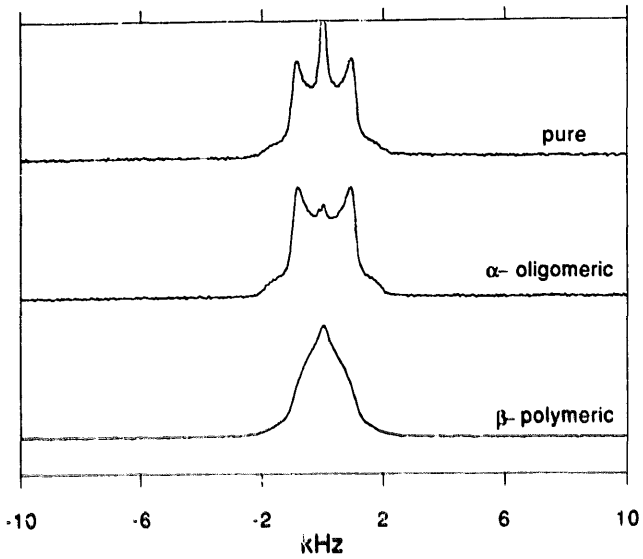

Fig. 2. 46.1 MHz ^{2}H-NMR spectra DOPC-d_9 at 30°C of pure and protein containing samples (both forms L/P 20). Number of scans 10000.

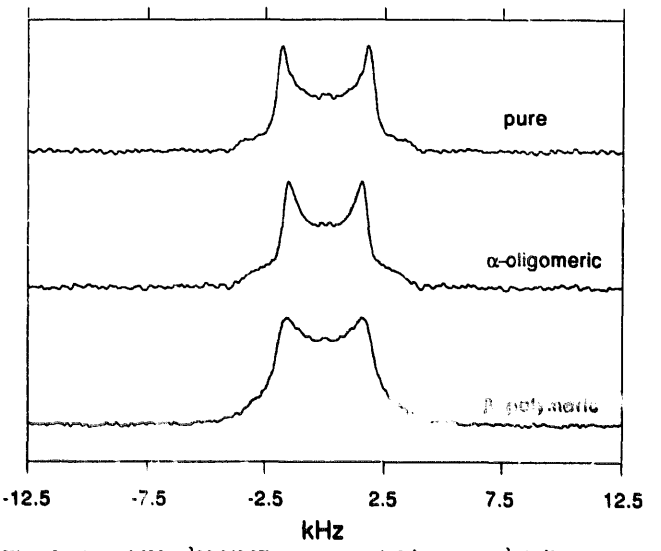

Fig. 3. 46.1 MHz ^{2}H-NMR spectra of 2-[14,14,14-$^2H_3$]DMPC at 30°C of pure and protein containing samples (both forms L/P 20). Number of scans 2000.

between the *sn*-1 and *sn*-2 chain. In addition the quadrupolar splitting has decreased upon addition of M13 coat protein in the α-oligomeric form (Table I), which indicates that the order in the hydrophobic part of the bilayer as determined from the spectra has decreased. This order is determined only for fast motions on the ^{2}H-NMR time scale. The T_{2e} slightly decreases with the content of the α-oligomeric form of the M13 coat protein (Table III).

Introduction of the M13 coat protein in the β-polymeric form causes the deuterium spectra to change strongly. In addition to a component, which behaves the same as the normal bilayer component, a broad protein-dependent component, which has an isotropic appearance, can be observed. In addition to these changes in the spectra the T_{2e} relaxation time decreases more considerable as compared to the changes in T_{2e} induced by the M13 coat protein in the α-oligomeric form (Table III). The T_{1z} relaxation time of 1,2-[11,11-$^2H_2$]DOPC is independent of the L/P ratio for both protein conformations (Table III).

^{2}H-NMR on DOPC-d_9

The quadrupolar splitting of DOPC-d_9 does not change upon addition of M13 coat protein in the α-oligomeric form and is 1.0 kHz at 30°C. The spectrum of DOPC-d_9 with the M13 coat protein in the β-polymeric form shows a broad component, which has an isotropic appearance (Fig. 2). Similar as in Fig. 1, the sharp central peak in the spectra is assigned to lipids in small vesicles. The T_{2e} relaxation time of DOPC-d_9 slightly decreases from 2.4 ms at $L/P = \infty$ to 2.1 ms at L/P 20. The T_{1z} relaxation time of DOPC-d_9 is independent of the L/P ratio for both protein forms and is 35 ms at 30°C.

^{2}H-NMR on 2-[14,14,14-$^2H_3$]DMPC

The effect of the M13 coat protein on the spectra of 2-[14,14,14-$^2H_3$]DMPC is displayed in Fig. 3. The typical powder pattern observed for the pure phospholipid is not changed upon adding the α-oligomeric form of the M13 coat protein, whereas upon adding M13 coat protein in the β-polymeric form an ideal powder pattern can no longer be observed. If the temperature is increased to 45°C the shape of the spectrum of 2-[14,14,14-$^2H_3$]DMPC with the M13 coat protein in the β-polymeric form becomes more isotropic. This effect is fully reproducible (results not shown). As can be seen from Table II, the quadrupolar splitting decreases upon addition of M13 coat protein independent of its forms. The T_{1z} relaxation time is hardly affected, but the T_{2e} relaxation time decreases substantially on adding M13 coat protein (Table III). The effect on the T_{2e} is larger for systems with the M13 coat protein in the β-polymeric form as compared to the systems with the α-oligomeric form of the M13 coat protein.

^{31}P-NMR on DOPC and DMPC

The ^{31}P-NMR spectra of the DOPC (Fig. 4) and DMPC (Fig. 5) are typical for those obtained from phospholipid bilayer systems with no indication of any

TABLE II

Quadrupolar splittings (kHz) of 2-[14,14,14-$^2H_3$]DMPC with M13 coat protein in the two conformations at 30°C

Protein	L/P ratio		
	∞	50	20
α-Oligomeric	3.9	3.8	3.3
β-Polymeric	4.1	4.1	3.7

TABLE III

Relaxation times T_{1z} and T_{2c} for phospholipids deuterated on different positions in the pure lipid systems and in lipid systems with M13 coat protein in the two conformations

The measuring temperatures are 10°C for 1,2-[11,11-$^2H_2$]DOPC and 30°C for 2-[14,14,14-$^2H_3$]DMPC.

Label	T_{1z} (ms)		T_{2c} (ms)	
	pure	+ M13	pure	+ M13
1,2-[11,11-$^2H_2$]DOPC [a]	11.7 ± 1.0	11.2 ± 1.0	0.66 ± 0.05	0.58 ± 0.05
1,2-[11,11-$^2H_2$]DOPC [b]	11.7 ± 1.0	11.1 ± 1.0	0.66 ± 0.05	0.40 ± 0.05
2-[14,14,14-$^2H_3$]DMPC [a]	3.0 ± 0.1	3.0 ± 0.1	1.21 ± 0.05	0.91 ± 0.05
2-[14,14,14-$^2H_3$]DMPC [b]	3.0 ± 0.1	3.0 ± 0.1	1.37 ± 0.05	0.71 ± 0.05

[a] Sample containing α-oligomeric protein.
[b] Sample containing β-polymeric protein.

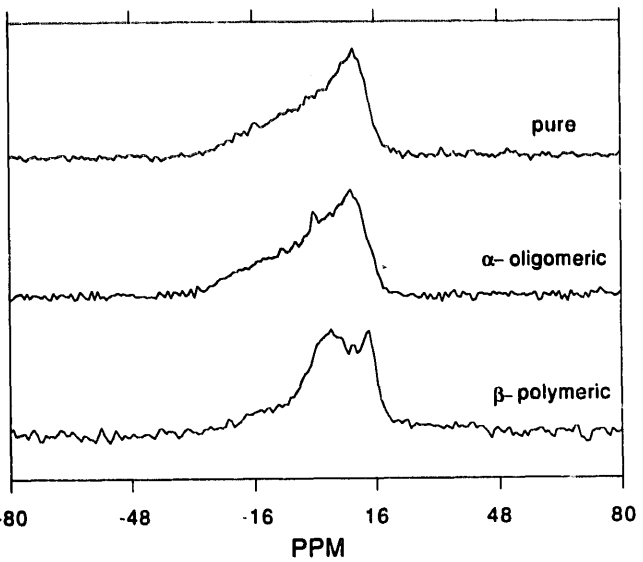

Fig. 4. 121.4 MHz ^{31}P-NMR spectra of DOPC at 30°C of pure and protein containing samples (both forms L/P 20). Number of scans 3600.

additional component in the case of pure bilayers and systems with the α-oligomeric form of the M13 coat protein. However, in samples with the M13 coat protein in the β-polymeric form, a distorted powder pattern is observed (Fig. 5). Increasing the temperature causes the line shape to become more isotropic, subsequent cooling completely reverses this effect. Since the vesicles formed were homogeneous in L/P ratio on a sucrose gradient, the intensity of the central peak in the phospholipid spectra with the M13 coat protein in the β-polymeric form is suggested to arise from protein related lipids.

Discussion

M13 coat protein adopts two forms in model membranes, the α-oligomeric and the β-polymeric form. M13 coat protein in the β-polymeric form is predominantly in a β-sheet conformation and strongly aggregated, whereas the α-oligomeric form of the M13 coat protein is in a predominant α-helix conformation and forms reversible small aggregates [9]. The unique opportunity provided by this viral coat protein to adopt two different conformations in membranes has enabled us to study the effect of these different secondary structures of the protein on the surrounding lipid matrix and visa versa.

The quadrupolar splitting in the ^{2}H-NMR spectra of DOPC-d_9 and the chemical shift anisotropy in the ^{31}P-NMR spectra of DOPC do not change with increasing α-oligomeric protein content (Figs. 2 and 4), indicating that the headgroups of the phospholipids are not strongly affected by the M13 coat protein in the α-oligomeric form and that on the ^{2}H-NMR time scale the boundary and bulk lipids are in fast exchange. This is in agreement with a previous NMR study on various headgroup-labelled phospholipids, were it was shown

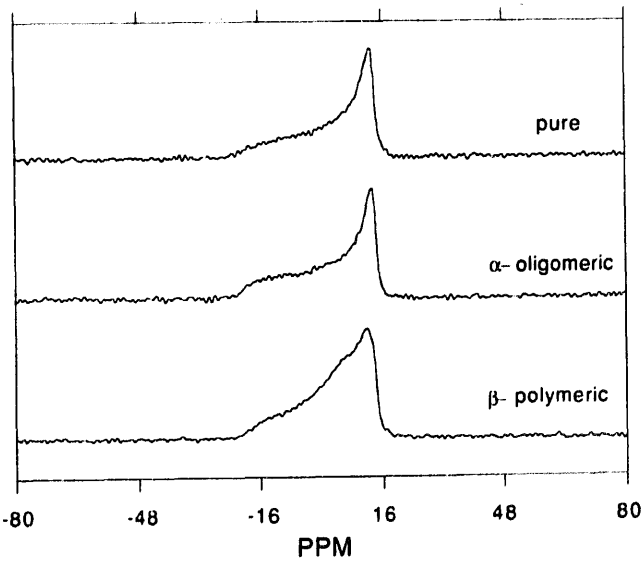

Fig. 5. 121.4 MHz ^{31}P-NMR spectra of DMPC at 30°C of pure and protein containing samples (both forms L/P 20). Number of scans 3600.

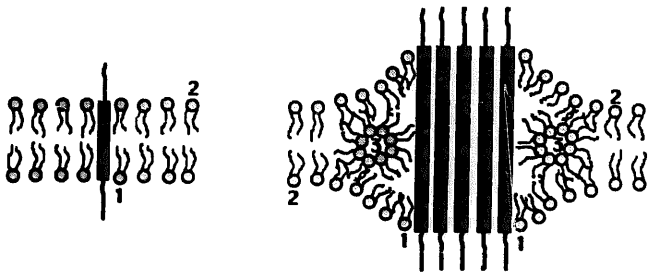

Fig. 6. A schematic and speculative cartoon of the model proposed of the interaction of the M13 coat protein in the α-oligomeric state (a) and in the β-polymeric state (b), with phospholipids. The protein is represented as a black bar. Bulk lipids (1) are in fast or intermediate exchange with boundary lipids (2). Lipids trapped in rodlike structures (3) are formed to match the hydrophobic part of the stretched β-polymeric protein in the lipid bilayer.

that the main effect of the coat protein in the α-oligomeric form was a change in the torsion angles in the headgroup as a result of the introduction of positive charges by the M13 coat protein [14]. In systems of 1,2-[11,11-$^2H_2$]DOPC and 2-[14,14,14-$^2H_3$]DMPC a decrease of the quadrupolar splitting is observed on addition of the coat protein in the α-oligomeric conformation, which indicates that the order in the hydrophobic part of the bilayer has decreased. A small decrease in order of the hydrophobic part of the bilayers induced by various proteins has been observed previously by other workers with cytochrome-*c* oxidase [17] and rhodopsin [18].

The constant value of T_{1z} and the decreasing value of T_{2c} on addition of M13 coat protein in the α-oligomeric form to the phospholipids show that the protein influences only slow molecular motions. Under exchange conditions the overall T_{2c} relaxation rate is the population average of the T_{2c} relaxation time of boundary and bulk lipids plus an extra term, which takes into account the exchange process [19]. The boundary and bulk lipids are schematically depicted in Fig. 6a. The exchange contribution as well as a different T_{2c} value of the boundary lipids, could result in a decrease of the overall T_{2c} values. The lower T_{2c} value for the boundary lipids could be the result of motions of the protein or reorientational motions of lipids while bound to the protein [20].

Previous experiments conducted on phospholipid systems with various amounts of the M13 coat protein in the β-polymeric form, suggested that the protein induces a fraction of lipids trapped within protein aggregates [4,5]. These trapped lipids manifested themselves as a second broad isotropic-like spectral component in ^{2}H-NMR spectra of DMPC-d_9 [5] and in a very slow exchanging boundary component in the EPR spectra obtained in various lipid systems [4]. For a reference, we have repeated some experiments to enable us to make a systematic comparison of the effect of the protein in the β-polymeric form on the headgroup and chains of lipids. Also in our ^{2}H-NMR and ^{31}P-NMR spectra of the headgroup-labeled DOPC-d_9 with various amounts of M13 coat protein in the β-polymeric form, a second protein-induced component is observed (Figs. 2 and 4), indicating the presence of trapped lipids, in agreement with results obtained by Van Gorkom et al. [5].

To investigate whether the interaction of the protein in the β-polymeric form also affects the other parts of the lipid molecule, we have studied by ^{2}H-NMR the influence of the protein in the β-polymeric form on chain-labelled phospholipids (1,2-[11,11-$^2H_2$]DOPC and 2-[14,14,14-$^2H_3$]DMPC). In the ^{2}H-NMR spectra of 1,2-[11,11-$^2H_2$]DOPC, in addition to a central broad isotropic-like component, of which the intensity is dependent on the amount of protein in the lipid system, a normal bilayer component can be observed, which indicates a slow exchange between a protein-influenced lipid component and bulk lipids. In the spectra of 2-[14,14,14-$^2H_3$]DMPC at 30°C only one component can be observed. At 40°C a decrease of the quadrupolar splitting is observed and a second central broad component appears. This suggests that similar effects can be observed for both lipid types, DOPC and DMPC, but at a different temperature.

The constant T_{1z} value at various amounts of M13 coat protein in the β-polymeric form indicates that M13 coat protein in this conformations causes no changes in the fast motions of the both lipid types in the membrane as detected from the constant T_{1z}. However, the slow motions are more drastically influenced by the M13 coat in the β-polymeric form as compared to the changes induced by the M13 coat protein in the α-oligomeric form, as is observed from T_{2c}. This larger decrease of the T_{2c} relaxation time suggests an additional influence on the motional behaviour of the lipids by the protein in the β-polymeric form.

It has been found experimentally that lipid systems adopt different morphological structures [21,22] or show membrane curvature effects [23] on adding proteins of which the hydrophobic part does not match the hydrophobic part of the membrane. Also Bloom [20] argued, that it was possible that proteins induce different lipid structures, with a distinct temperature behaviour. On basis of the fact that the whole lipid molecule is affected by β-polymeric protein (see Figs. 1–5), it is suggested, as a possible and tentative model to explain the NMR results presented in this paper, that the protein induces an additional lipid structure, which consists of rods of inversed lipids in the membrane close to the protein aggregates (see Fig. 6b). These lipids will show up as a central isotropic-like component due to the additional fast averaging that is taking place in these rodlike structures. Previously similar effects were observed on the introduction of cytochrome *c* into lipid systems [22].

To understand why M13 coat protein in the β-polymeric form is capable of forming a different lipid structure, one has to take into account the shape of the protein. Tanford and Reynolds [24] suggested two main possibilities for β-sheet structures in lipid membranes. Proteins with a transmembrane β-sheet can either have turns and form intra-peptide hydrogen bonds or are in an extended conformation with inter-peptide hydrogen bonds. If the transmembrane part of the M13 coat protein would be in an extended β-sheet conformation, which has a rise of about 0.34 nm per residue, the about 20 hydrophobic amino acid residues of the M13 coat protein would result in a hydrophobic thickness of about 6.5 nm. This is about twice the thickness of the membrane core (3.2 nm) and would cause a severe mismatch of the hydrophobic amino acid part of the protein with the hydrophobic part of the membrane. By forming rodlike structures in the planar bilayer, the membrane will increase its thickness close to the protein resulting in an optimal hydrophobic matching. This would not be the case if M13 coat protein is considered to be in a folded β-conformation, with a turn in the hydrophobic part, forming strong intra-molecular hydrogen bonds that stabilize this structure in lipid bilayers [24]. However, it has been observed that when the protein is incorporated in a lipid bilayer, it is able to convert from the α-oligomeric form to the β-oligomeric form [25]. A turn in the β-sheet conformation, therefore, would imply the translocation of one of the hydrophilic termini through the lipid membrane upon this conversion. This is not very likely to occur. In addition, from molecular dynamics simulations of the β-U-shaped conformation it is found that the variation of the rms fluctuations of the C_α-atoms along the polypeptide chain shows a pronounced maximum at the reverse turn, suggesting that such a turn is unstable [26]. For these reasons the possibility of a folded β-conformation is rejected.

Lipids trapped in the protein induced rodlike lipid structure (lipid molecule 3, Fig. 6b) can not exchange with the bulk lipids (lipid molecule 1, Fig. 6b), because the flip-flop rates are slow. For this system it is argued that the formation of non-bilayer structures is observed with lipids, which can undergo the liquid-crystalline phase to hexagonal phase transition. It is known that this transition is more likely to occur for DOPC as compared to DMPC [22]. This explains that in the DOPC system at 30°C this second component originating from the lipids in the rodlike structures is already clearly resolved, whereas the second component for the DMPC system is only visible at 45°C. It should be stressed that because such a component is observed with phospholipids with a phosphatidylcholine headgroup, which have a very strong tendency to form bilayers, the forces on the lipid bilayer induced by the protein to match the hydrophobic areas must be strong.

In addition to lipids in the inversed rod structures (lipid molecule 3, Fig. 6b), as proposed in the possible model, there is a fraction of lipids in contact with the protein, but not present in the inversed rod structures. This fraction of lipids will behave as boundary lipids (lipid molecule 2, Fig. 6b), which can exchange with the bulk lipids (lipid molecule 1, Fig. 6b). This exchange process explains the decrease of the quadrupolar splitting with increasing amounts of protein in the lipid bilayer (Tables I and II). Such a decrease of the quadrupolar splitting is observed also with the M13 coat protein in the α-oligomeric form (see Tables I and II) and with various other integral membrane proteins [20]. The fact that this decrease in quadrupolar splitting is less upon the incorporation of the M13 coat protein in the β-polymeric form as compared to the α-oligomeric form (Tables I and II), can be related to the high aggregation state of the β-polymeric form of the M13 coat protein, which causes less lipids at a given lipid to protein ratio to be in a boundary state in comparison with the protein in the α-oligomeric form.

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