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ESR of spin-labeled bacteriophage M13 coat protein in mixed phospholipid bilayers

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Bacteriophage M13 major coat protein was spin-labeled with a nitroxide derivative of iodoacetamide, preferentially at the single methionine that is located in the hydrophobic region of the protein. The spin-labeled protein was incorporated at different lipid-to-protein ratios in phospholipid bilayers composed of dimyristoylphosphatidylglycerol (DMPG), dimyristoylphosphatidylcholine (DMPC), or the 1:1 molar mixture of these lipids. Both conventional and saturation transfer (ST) ESR studies were performed to investigate the rotational motions of the protein over a large dynamic range. The conventional ESR spectra indicate that the mobility of the spin labelled protein in the lipid gel phase decreases in the order: DMPG > DMPC/DMPG (1:1) > DMPC. In the liquid crystalline phase, the largest mobility is found in DMPC/DMPG (1:1, mol/mol) mixtures, but the mobility is still greater in DMPG than in DMPC. The results are interpreted in terms of different degrees of protein aggregation in the different lipids. Segmental motion with rotational correlation times on the order of tens of nanoseconds, motional anisotropy, and spectral overlap complicate the analysis of the STESR spectra. An estimate of the size of the protein aggregates is found to be in the region of 85 monomer units. Removing the polar tails from the protein by proteolytic digestion results in an enhanced aggregation in the gel phase. In the liquid crystalline phase, the segmental wobbling mobility of the protein is increased relative to the native protein, whereas the overall rotational diffusion is not changed greatly.

Introduction

The filamentous bacteriophage M13 consists of a single stranded DNA molecule, which is surrounded by about 2700 units of tubular coat protein [1]. When an *Escherichia coli* cell is infected by the bacteriophage M13, only the DNA enters the cell, where it is replicated, leaving the coat protein in the cytoplasmic membrane [2]. It has been suggested that these proteins, a product of gene 8, behave like integral proteins [3,4], as indicated by the amino acid sequence. A hydrophobic region of 20 amino acid residues in the protein is enclosed by two polar regions: an acidic N-terminus consisting of 20 residues and a basic C-terminus having 10 amino acid residues. The function of this protein is two-fold: on infection the coat protein resides in the membrane, whereas both newly synthesized and parental proteins are then used for the membrane-bound assembly of new virus particles [2]. It is quite possible that both the tendency to aggregation of the coat protein, and the interactions with the surrounding lipids, play an important role in the infection and assembly processes.

ESR studies have been shown to be useful in investigating the interactions of spin-labeled lipids with integral membrane proteins (see, for example, Refs. 5–7). Datema et al. [3] used this technique to study M13 coat protein reconstituted with a DMPC/DMPG mixture of fixed composition (80:20, mol/mol). The presence of the negatively charged lipid was found to facilitate incorporation of the protein. More recently, it was found possible to extend these studies to various differ-

Abbreviations: DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; ESR, electron spin resonance; STESR, saturation transfer ESR; V_1, first harmonic absorption ESR spectrum detected in-phase with respect to the magnetic field modulation; V_2', second harmonic absorption ESR spectrum detected 90° out-of-phase with respect to the magnetic field modulation; L/P, lipid/protein molar ratio.

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ent DMPC/DMPG mixtures [8]. It was found that the behaviour of the protein differed considerably in the DMPC and DMPG host lipids.

In the present work, we have used spin-labeled M13 coat protein, preferentially labeled at the methionine group in the centre of the hydrophobic region of the protein, to examine the influence of different lipid systems and of the lipid phase transition on the molecular mobility of the coat protein. Both conventional and saturation transfer (ST) ESR were used to cover a wide dynamic range of molecular motions, with sensitivity to rotational correlation times from 10^{-10} to 10^{-3} s. Particular attention has been paid to the different effects of DMPG and DMPC on the protein conformation and rotational mobility.

Materials and Methods

Incorporation of the M13 coat protein in DMPC/DMPG membranes. The gene 8 coat protein of the bacteriophage M13 was isolated and purified according to the method of Knippers and Hoffman-Berling [9]. 10 mg of total material (DMPC, DMPG, with M13 coat protein) were dissolved in 0.4 ml urea-cholate buffer (10 mM Tris, 2 mM EDTA, 20 mM $(NH_4)_2SO_4$, 2 wt% sodium deoxycholate, 8 M urea at pH 8.0 (HCl)) and dialyzed with six buffer changes against 75-fold excess Tris buffers. All buffers contained 10 mM Tris, 2 mM EDTA (pH 8.0) and all except the final one also contained 10% (v/v) methanol. In addition, the first two buffers contained 20 mM $(NH_4)_2SO_4$, the third 10 mM $(NH_4)_2SO_4$, the fourth 5 mM $(NH_4)_2SO_4$, and the last two contained no $(NH_4)_2SO_4$. After dialysis, the samples were freeze-dried under a vacuum of 0.1 Torr, suspended in a small volume of Tris buffer, centrifuged for 6 h at 183000 × *g*, and finally freeze-dried again. To obtain multilamellar vesicles, a small amount of water was added to hydrate the lipid system.

In some cases, the vesicles were checked for homogeneity with respect to protein content on a sucrose gradient (2–40 wt%), using centrifugation for 4 h at a maximum centrifugation force of 160000 × *g*. The lipid/protein molar ratios were determined using protein estimation according to Lowry et al. [10] and lipid phosphate determination according to Bartlett [11].

Covalent modification of the M13 coat protein. The single methionine residue at position 28, and the lysine groups at positions 8, 40, 43, 44 and 48, in the sequence of the M13 coat protein are sensitive to reaction with iodoacetamide under slightly acidic conditions [12]. 50 mg of the M13 coat protein and 12.5 mg of 4-(2-iodoacetamido)-2,2,6,6-tetramethylpiperidinooxyl were dissolved separately in 10 and 3.5 ml, respectively, of guanidine hydrochloride, acetate buffer (8 M guanidine hydrochloride, 10 mM sodium acetate (pH 6.0) adjusted with acetic acid) and then incubated together at 38°C for 3 h. The reaction was allowed to proceed further with dialysis for 18 h at 35°C and pH 4.0–4.5. Next, the sample was dialyzed at room temperature for 4 h against a 20-fold excess of urea-cholate buffer. Then the sample was dialyzed twice against a 50-fold excess of Tris-cholate buffer, and twice against a 50-fold excess of Tris buffer, in order to remove urea and cholate. The protein was then concentrated by centrifugation (2 h, 183000 × *g*) and freeze-dried.

Proteolytic digestion. For proteolytic cleavage of the M13 coat protein, trypsin was used at a concentration of 2.0 mg/ml in 0.1 M NaCl, 10 mM Tris, 1 mM EDTA (without Mg^{2+} or Ca^{2+}). The protein was incorporated in multilamellar vesicles of DMPC/DMPG (1 : 1, mol/mol) at a lipid/protein molar ratio of 10. The vesicles were suspended in Tris buffer (pH 8.5) at a concentration of 7.3 mg/ml. Then three 0.6-ml aliquots of the trypsin solution were added at intervals of 1 h, at a temperature of 38°C. The system was then dialyzed twice against 500 ml Tris buffer (pH 8.0), concentrated by centrifugation (3.5 h, 183000 × *g*), and freeze-dried.

Conventional ESR. ESR measurements were performed on a Varian E-12 Century Line ESR-spectrometer equipped with nitrogen gas flow temperature regulation (±0.2 deg). The ESR spectrometer settings were: modulation amplitude of 1.25 Gauss, field centre at 3244 Gauss (locked on the centre zero) with a scan range of 100 Gauss and a microwave frequency of 9.113 GHz. The Zeeman field modulation was 100 kHz for the V_1 spectra, and the scan time was 4 min. The same microwave power was used as for the STESR measurements (in the range 9–14 mW). The in-phase, absorption spectra (V_1 display) were recorded digitally using a Digital Equipment Corp. LPS system and a dedicated PDP 11/10 computer with a VT11 display. The spectra were accumulated for four scans. The 10 μl samples were contained in 100 μl glass capillaries which were accommodated within standard 4 mm diameter quartz tubes containing light silicone oil for thermal stability.

Saturation transfer ESR. Because of inhomogeneities of the microwave and modulation field in the cavity, samples smaller than 5 mm in length were used. These were obtained by centrifuging the lipid-protein dispersions within the ESR capillary and removing the excess buffer. STESR spectra were recorded in the second harmonic, 90° out-of-phase, absorption mode (V_2' display). Phase setting was achieved using the 'self-null' method [13,14]. The modulation amplitude was 5 Gauss and the modulation frequency was 50 kHz. The microwave power was adjusted for each sample at each temperature to obtain a root mean square microwave field over the sample of 0.25 G, as suggested in the standardized protocol given in Refs. [15,16]. To obtain effective rotational correlation times from the diagnostic lineheight ratios, reference spectra were measured under the same experimental conditions as described

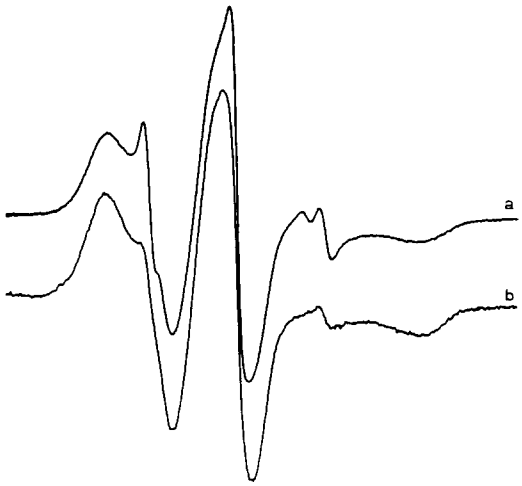

Fig. 1. Conventional ESR spectra of spin-labeled M13 coat protein in DMPC/DMPG (1:1, mol/mol) bilayers ($L/P = 10$ mol/mol). (a) Before, and (b) after, proteolytic digestion. Total scan width = 100 gauss; $T = 16°C$.

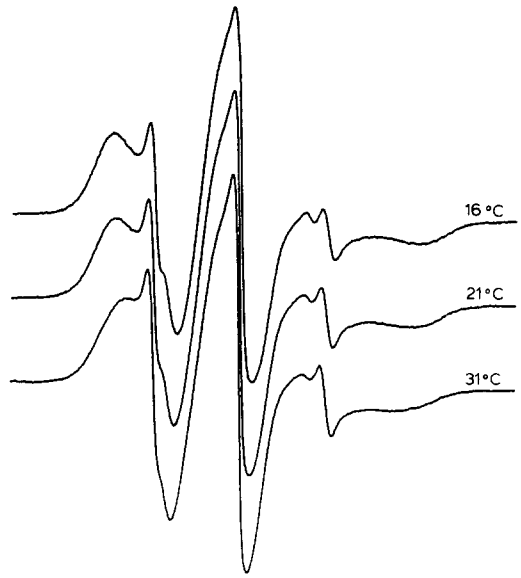

Fig 2. Temperature dependence of the conventional ESR spectra of spin-labeled M13 coat protein in DMPC/DMPG (1:1, mol/mol) bilayers ($L/P = 10$ mol/mol). Total scan width = 100 gauss.

above, using maleimide spin-labeled hemoglobin in glycerol-water solutions [17].

Results

Conventional ESR

In Fig. 1a, the conventional, in-phase, absorption ESR spectrum (V_1) is presented of iodoacetamide spin labeled M13 coat protein incorporated in DMPC/DMPG (1:1, mol/mol) vesicles at a lipid/protein ratio of 10 mol/mol. Fig. 1b shows the spectrum of the proteolytically digested spin-labeled M13 coat protein in the same lipid system. In these spectra, two components can be distinguished: the broader (and intense) component arising from the slow motion of the labeled methionine group located in the apolar region of the bilayer, and a smaller sharp, three-line component arising from the more mobile lysine groups. This latter component is strongly reduced after removing the C- and N-terminal regions of the protein, in which all the lysines are situated. Digital subtraction between the spectra of Figs. 1a and 1b indicates that only 5% of the total spin label intensity is incorporated into the mobile (lysine-associated) spectral component.

In Fig. 2, conventional ESR spectra are shown of spin-labeled M13 coat protein incorporated in DMPC/DMPG (1:1, mol/mol) bilayers ($L/P = 10$ mol/mol) for three different temperatures. The sharp resonances, mainly arising from spin-labeled lysine groups, are not very sensitive to temperature, whereas the outer hyperfine splitting in the spectrum of the spin-labeled methionine group clearly decreases with increasing temperature.

The spectra of the spin-labeled M13 coat protein incorporated in lipid systems containing different relative amounts of DMPC and DMPG (with total $L/P =$ 10 mol/mol) are shown in Fig. 3, for temperatures both below (16°C) and above (31°C) the gel-to-fluid phase transition temperature, T_t. The ESR signal from the lysine groups is very little affected, either by the lipid composition or by the temperature. In contrast, the ESR signal from the spin-labeled methionine group is sensitive both to the lipid composition and to the phase

TABLE I

Outer hyperfine splitting, $2A_{max}$, and half-widths at half-height of the low-field, ΔH_l, and high-field, ΔH_h, resonance lines in the conventional ESR spectra (V_l) of iodoacetamide spin-labeled M13 coat protein in different lipid hosts

Estimated errors, based on duplicate samples, are 1–2%, i.e. maximally ±0.9 G in $2A_{max}$ and ±0.1 G in $\Delta H_{l,h}$.

Lipid	L/P (mol/mol)	Temp. (°C)	$2A_{max}$ (gauss)	ΔH_l (gauss)	ΔH_h (gauss)
DMPG	10	16	60.2	4.2	6.8
		31	57.8	4.6	6.7
	40	16	61.2	4.0	5.3
		31	56.8	4.2	5.9
DMPG/DMPC	10	16	61.0	3.8	6.4
(1:1, mol/mol)		21	59.9	4.0	6.0
		31	56.8	4.1	6.8
	40	16	61.4	3.9	5.3
		21	59.5	4.3	7.0
		31	55.9	4.6	5.8
(after proteolytic	10	16	61.7	4.0	6.1
digestion)		21	58.7	4.6	8.2
		31	54.6	4.7	9.7
DMPC	10	16	63.2	3.6	5.9
		31	60.1	4.3	5.4
	40	16	63.0	4.2	5.7
		31	60.3	4.1	6.2

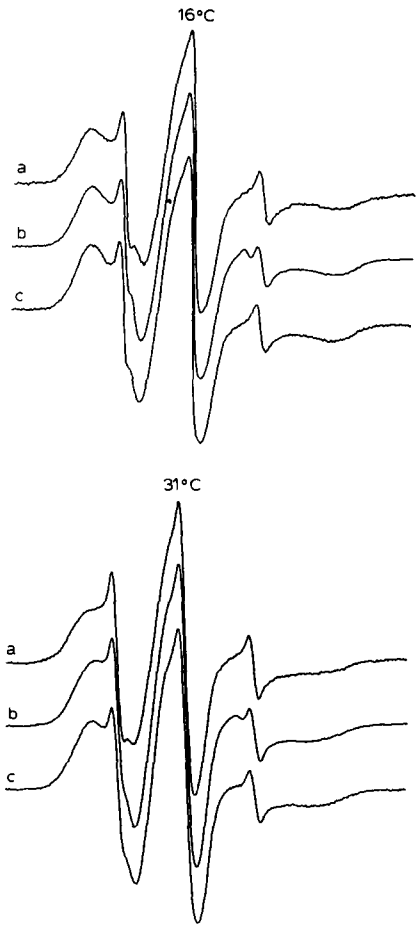

Fig. 3. Conventional ESR spectra of iodoacetamide-labeled M13 coat protein at a L/P ratio of 10 mol/mol in three different lipid systems, recorded at 16°C (upper set) and 31°C (lower set). (a) In DMPC, (b) in DMPC/DMPG (1:1, mol/mol), and (c) in DMPG. Total scan width = 100 gauss.

state of the lipid. The outer hyperfine splitting, and the linewidths of both the low- and high-field resonance lines, for the methionine signal are presented in Table I. In the gel phase (16°C), the outer hyperfine splitting of the spin-labeled methionine group increases on changing the lipid host from DMPG to DMPC. Correspondingly, the observed linewidths decrease for both the low- and high-field resonance lines. This indicates a decreased rotational mobility in the slow motion region of conventional ESR for the M13 coat protein incorporated in DMPC as opposed to DMPG (see, for example, Refs. 5, 22, 23). In the liquid crystalline phase (31°C), the smallest outer hyperfine splitting is now observed for the 1:1(mol/mol) DMPG/DMPC mixture; the outer hyperfine splitting is still larger in DMPC, however, than in DMPG.

A similar behaviour is observed for samples with L/P ratio of 40 mol/mol. In the gel phase, the outer hyperfine splittings are comparable to those found for the samples with $L/P = 10$ mol/mol. Again, the largest change on going to the fluid phase is observed for the 1:1 (mol/mol) DMPG/DMPC mixture. In DMPG, the change in outer splitting is almost twice that found for the samples with $L/P = 10$ mol/mol. This is not the case in DMPC, where the decrease in A_{max} observed on going to the fluid phase is almost the same as that for the $L/P = 10$ mol/mol sample. This gives an indication that in pure DMPG lipid samples the motions of the protein are most sensitive to the protein content. Results consistent with the above were also obtained at higher lipid/protein ratios and at different temperatures (data not shown).

For the proteolytically digested M13 coat protein, an increased outer hyperfine splitting of the spin-labeled methionine group is observed in the gel phase of a DMPC/DMPG (1:1, mol/mol) system with respect to that for the native protein. A large decrease in the splitting is observed when going to the liquid crystalline phase. Also the increase of the linewidths of both the low- and the high-field resonance lines in the fluid phase are larger for the cleaved protein than for the native protein.

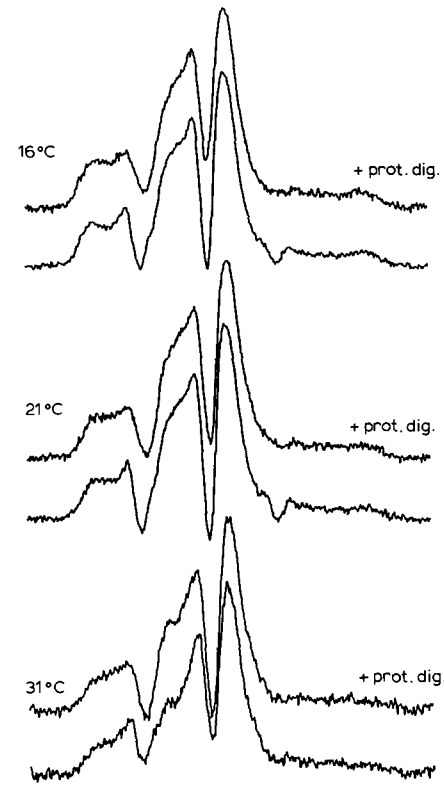

Fig. 4. Saturation transfer ESR spectra of spin-labeled M13 coat protein in DMPC/DMPG (1:1, mol/mol) bilayers ($L/P = 10$ mol/mol), before (lower spectrum of each pair) and after (upper spectrum of each pair) proteolytic digestion. Total scan width = 100 gauss. Temperatures at which the spectra were recorded are indicated on the figure.

TABLE II

Effective rotational correlation times, τ_R^{eff}, determined from the normalized integral, I_{ST}, and the low-field, L''/L, central, C'/C, and high-field, H''/H, diagnostic lineheight ratios in the STESR spectra (V_2') of iodoacetamide spin-labeled M13 coat protein in different lipid hosts

Values from I_{ST} and H''/H not shown correspond to points which were below the lower, or upper limit of the haemoglobin calibration spectra, respectively. Estimated errors in the primary parameters, based on duplicate samples, are: ±5% in I_{ST}, L''/L and C'/C, and ±10–25% in H''/H.

Lipid	L/P (mol/mol)	Temp. (°C)	τ_R^{eff} (μs)			
			I_{ST}	L''/L	C'/C	H''/H
DMPG	10	16	4.8	60	15	65
		31	0.2	17	8	125
	40	16	2.7	32	29	30
		31	–	19	7.5	20
DMPG/DMPC (1 : 1, mol/mol)	10	16	6.2	56	7.1	60
		21	2.7	30	4.3	90
		31	–	21	20	–
	40	16	2.1	95	5.4	45
		21	1.5	85	3.7	60
		31	0.2	30	1.4	15
(after proteolytic digestion)	10	16	7.2	85	22	65
		21	4.3	38	9.0	85
		31	0.3	31	6.3	20
DMPC	10	16	10.8	42	10	105
		31	4.6	28	2.1	65
	40	16	8.3	31	4.4	80
		31	7.0	13	1.3	70

Saturation transfer ESR

Typical 90° out-of-phase, second harmonic, absorption STESR spectra (V_2') of the iodoacetamide labeled M13 coat protein, in the native state and after proteolytic digestion, incorporated in DMPC/DMPG (1 : 1, mol/mol) vesicles with a L/P ratio of 10 mol/mol are shown in Fig. 4. These spectra reveal the presence of a mobile spin label component, indicated by the sharp second derivative-like peaks at the isotropic resonance positions. This component, which arises from spin-labeled lysine groups, complicates the analysis of the STESR spectra. In Table II, the effective rotational correlation times, τ_R^{eff}, of the different samples are given. These are obtained from the diagnostic lineheight ratios, L''/L, C'/C and H''/H, as defined by Thomas et al. [13], and from the normalized integral of the saturation transfer spectrum, I_{ST} [17].

Discussion

Modification of the M13 coat protein

The spin labeling of the M13 coat protein with iodoacetamide is shown to be highly preferential for the methionine group and only a slight reactivity to the lysine groups is observed (approx. 5% of the total label intensity in the latter component). This reactivity pattern was also established by Gundlach et al. [12] in model peptides. Using a maleimide spin label yields a less preferential labeling of the methionine group (unpublished results). According to Chamberlain et al. [18], treatment of the M13 coat protein with trypsin, removes the polar N- and C-terminus. The reaction is only partial, and it is expected that approx. 60% of the lysine groups at the 8th position and only approx. 2% of the other lysines will be cleaved off (results of Ref. 18 for M13 coat protein in sonicated DMPC/DMPG (80 : 20, mol/mol) vesicles. The ESR spectra of Fig. 1 show that the intensity of the sharp, more mobile component is strongly reduced after proteolytic digestion, indicating that the labeling occurs mainly at the lysine group at the 8th position and that the C-terminus of the protein is hardly available for covalent modification by iodoacetamide. Nor is it available for proteolytic digestion [18].

Conventional ESR

The ESR spectra in Figs. 1, 2 and 3 reveal that the motion of the spin labels at the lysine groups are not affected by lipid composition nor by temperature, including the phase transition of the lipids. This indicates that the polar end tails have no interaction with the hydrophobic part of the bilayer, as expected, but are directed outwards from the membrane. In Fig. 2, it is shown that the more immobile component is very sensitive to the phase properties of the lipids, indicating that it is situated at the methionine in the hydrophobic part of the protein. The spin label at the methionine group has a decreasing outer hyperfine splitting in the gel-phase if the DMPG/DMPC ratio is increased, indicative for an increased motion of the spin label (cf. Refs. 5, 22, 23). This is in good agreement with results of Wolfs et al. [8] who found that adding DMPG to the lipid system not only changes the packing of the protein aggregates within a larger protein complex, but also decreases the aggregate size (approx. 30 protein monomers in DMPG and approx. 400 monomers in DMPC). The tendency to form very large aggregates in DMPC is also shown here by the insensitivity of the spectra from the labeled protein to the L/P ratio, whereas the presence of DMPG increases the sensitivity to protein content.

In all cases, the largest change in mobility of the spin label caused by the phase transition of the lipids is found for the 1 : 1 (mol/mol) DMPC/DMPG mixtures. This could indicate that for this lipid composition it is energetically most favourable if the aggregate complexes are partially dissolved in the liquid crystalline phase. This change in the dynamic behaviour is also evidenced by the result that, if the polar ends of the protein are removed, an increased outer splitting is observed in the gel phase (see Table I), indicating an enhanced aggregation, which has previously been observed by Cham-

berlain et al. [18]. However, above the phase transition temperature, a decreased outer hyperfine splitting and increased linewidths are observed with respect to the native protein, showing that the enhanced aggregation state of the cleaved protein in the gel phase, becomes dissociated above T_t. If the differences in behaviour of the protein are interpreted completely in terms of aggregate size, this implies that in the liquid crystalline phase the smallest aggregates are found in the DMPC/DMPG (1 : 1) mixture. This correlates with the results of Wolfs et al. [8], who found that above T_t the specificity of a chain-labeled phosphatidylglycerol for the M13 coat protein was the largest for the 1 : 1 DMPC/DMPG mixture, suggesting an increased exposure of the intramembranous section of the protein. This reversible aggregation of a protein at the phase transition has been observed in other systems. Hasselbacher et al. [19] found that bacteriorhodopsin reconstituted in DMPC bilayers was present as a monomer in the liquid crystalline phase, and below the transition temperature the proteins formed aggregates. Fajer et al. [20] have also shown that cytochrome oxidase is dispersed in the fluid phase of DMPC, but becomes aggregated in the gel phase.

Saturation transfer ESR

The interpretation of the STESR spectra reveals some problems. These are due to spectral overlap of the methionine and lysine groups, possible anisotropy of the spin label motion, and segmental mobility of the spin label. Table II shows that there is a large difference between the effective rotational correlation times deduced from the three different diagnostic lineheight ratios. The high-field ratio, H''/H, is often outside the hemoglobin calibration range. This is probably due to distortion of the lineshape by the faster component of the spin label motion which is evident in the conventional spectra. The correlation times deduced from the L''/L ratio, which is more sensitive to off-axial rotation, are much longer than those deduced from C'/C. However, the faster motional component will also somewhat affect the L''/L and C'/C lineheight ratios, and the spectral overlap from the lysine groups complicates the interpretation of the spectral parameters, especially in the central region. The integral method as described by Horváth and Marsh [17] is able to correct for this mobile second component, but will directly reflect the faster motional component of the broad spectrum.

Presence of segmental motion

From Table II, it is clear that the effective rotational correlation times deduced from the STESR lineheight ratios are larger than those obtained by the integral method. This can indicate the presence of large scale segmental motions of the spin label, in addition to rotational diffusion of the protein as a whole (cf. Ref. 21). The presence of segmental motion is also revealed by comparing the STESR lineheight ratios (for example of the (1 : 1) mixture) at the different temperatures with the low- and high-field linewidths, ΔH_l and ΔH_h, and the outer hyperfine splitting, $2A_{max}$, in the conventional spectra of the methionine group. In general, the temperature dependence reflects that of the STESR parameters and indicates that, with increasing temperature, an increased rotational mobility is found in the slow-motional regime of conventional ESR. By simulation of conventional ESR spectra, the effective rotational correlation times can be found from the outer splitting and linewidths, which have been calibrated for these parameters by Freed [22]. The values obtained from these calibrations indicate that the iodoacetamide spin label undergoes some segmental motion with correlation times on the order of tens of nanoseconds. The extent of these motions is not clear from the slow-motion analysis.

Rotational diffusion and aggregation state

An estimate of the rotational diffusion coefficient of the M13 monomer, based on an approximately cylindrical conformation, can be given by hydrodynamic theory [23]:

$$D_{R\parallel} = (kT/4\pi\eta a^2 h) \quad (1)$$

where $2a \approx 10$ Å is the cylinder diameter [2], $h \approx 35$ Å is the height of the membrane spanning region [24], $\eta \approx 3.7$ P is the effective viscosity within the membrane [25], k is Boltzmann's constant, and T the absolute temperature. For M13 coat protein in the monomeric state, one then finds values of $D_{R\parallel} \approx 1.0 \cdot 10^6$ s^{-1}. A relation between the effective rotational correlation times determined from the STESR spectra, τ_R^{eff}, (Table II) and the rotational diffusion coefficient has been given by Robinson and Dalton [26]:

$$\tau_R^{eff} = 1/(3D_{R\parallel} \sin^2\Theta) \quad (2)$$

where Θ is the angle between the nitroxide z-axis and the principal rotation axis. Whereas the STESR lineheight ratios are distorted by the motional narrowing observed in the conventional spectrum, the STESR integrals will reflect directly both the segmental motion and the overall rotational diffusion of the protein. Therefore, the low-field STESR lineheight ratio is likely to give an upper estimate of the correlation time for rotation of the entire protein complex, and the STESR integral will give a lower limit. Using the L''/L lineheight ratios of the STESR spectra, an approximate value of $D_{R\parallel} \geq 1.2 \cdot 10^4$ s^{-1} is deduced from Table II for the protein incorporated in DMPC, assuming a spin label orientation perpendicular to the membrane normal. This value is much smaller than that calculated for the monomer, and corresponds to protein aggregates of

maximally 85 monomer units (approx. 50 units in DMPG), numbers which are of the same order as those found by Wolfs et al. [8] using chromatography. A corresponding value of $D_{R\|} \leq 0.7 \cdot 10^5$ s^{-1} can be deduced from the STESR integral for DMPC, corresponding to a minimal aggregate size of approx. 14 monomer units, which is fully consistent with the values obtained by gel permeation chromatography [8].

Looking now at the effect of removing the polar end tails of the protein, increased effective rotational correlation times are observed in the gel phase. This is in agreement with an enhanced extent of aggregation [18]. In the fluid phase, the effective rotational correlation times are of the same order as found for the native protein, although the outer splitting, observed in the conventional ESR spectra, is decreased by removing the ends. This could indicate that the segmental (wobbling) mobility of the protein within the membrane is increased, since the protein now lacks the two hydrophilic sites, whereas the overall rotational diffusion about the membrane normal is relatively unchanged.

In summary, the ESR results on the spin-labeled M13 coat protein indicate a clear sensitivity of the aggregation state of the protein to the lipid environment. The dependence on the lipid composition correlates rather well with measurements on the DMPG- and DMPC-reconstituted protein in cholate [8]. It is possible that part of the difference observed in segmental mobility may also reflect subtle changes in protein conformation, although the overall secondary structure does not change with lipid composition [27].

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