Eur Biophys J (1992) 21, 305–311

European Biophysics Journal

A small protein in model membranes: a time-resolved fluorescence and ESR study on the interaction of M13 coat protein with lipid bilayers

Johan C. Sanders [1], M. Francesca Ottaviani [2], Arie van Hoek [1], Antonie J. W. G. Visser [3], and Marcus A. Hemminga [1]*

[1] Department of Molecular Physics, Agricultural University, Wageningen, The Netherlands
[2] Department of Chemistry, University of Florence, Firenze, Italy
[3] Department of Biochemistry, Agricultural University, Wageningen, The Netherlands

Received December 23, 1991/Accepted in revised form September 10, 1992

Abstract. Model membranes with unsaturated lipid chains containing various amounts of M13 coat protein in the α-helical form were studied using time-resolved fluorescence and ESR spectroscopy. The lipid-to-protein (L/P) ratios used were >12 to avoid protein-protein contacts and irreversible aggregation leading to β-polymeric coat protein. In the ESR spectra of the 12-SASL probe in dioleoyl phosphatidylcholine (DOPC) bilayers no second protein induced component is observed upon incorporation of M13 coat protein. However, strong effects are detected on the ESR lineshapes upon changing the protein concentration. The ESR lineshapes are simulated by assuming a fixed ratio between the parallel ($D_{\parallel}$) and perpendicular ($D_{\perp}$) diffusion coefficients of 4, and an order parameter equal to zero. It is found that increasing the protein concentration from L/P ∞ to L/P 15 results in a decrease of the rotational diffusion coefficient $D_{\perp}$ from 3.4×10^7 to 1.9×10^7 s^{-1}. In the time-resolved fluorescence experiments with DPH-propionic acid as a probe, it is observed that increasing the M13 coat protein concentration causes an increase of the two fluorescent lifetimes, indicating an increase in bilayer order. Analysis of the time-resolved fluorescence anisotropy decay allows one to quantitatively determine the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$, and the rotational diffusion coefficient $D_{\perp}$ of the fluorescent probe. The order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ increase from 0.34 to 0.55 and from 0.59 to 0.77, respectively, upon adding M13 coat protein to DOPC bilayers with an L/P ratio of 35. The rotational diffusion coefficient $D_{\perp}$ of the DPH-propionic acid probe decreases on incorporating M13 coat protein, in accordance with the ESR results. It is concluded that M13 coat protein in the α-monomeric state is not able to produce a long living lipid boundary shell and consequently an immobilization of the lipids. An overall effect on the lipids is induced, resulting in a reduction in the dynamics and an increase in average lipid order. The hydrophobic region of M13 coat protein is proposed to perfectly match the lipid bilayer, resulting in a relatively small distortion of the bilayer structure of the lipid system.

Key words: M13 coat protein – Lipid protein interaction – ESR spectroscopy – Time-resolved fluorescence spectroscopy – Order parameter – Diffusion coefficient

Introduction

The infection of *Escherichia coli* with the bacteriophage M13 starts with the entry of the phage via a sex pilus and the subsequent release of the viral DNA into the cytoplasm and storage of the major coat protein as an integral membrane protein in the cytoplasmic membrane of the host (Pratt et al. 1969). Newly synthesized coat protein is inserted into the membrane as a preprotein and is converted to mature protein with a molecular weight of 5240 Da, by cleavage with a leader peptidase. This protein and probably also the stored parental protein are used for the membrane-bound assembly of new bacteriophage particles (Model et al. 1982). The secondary structure of the coat protein in the bacteriophage, as determined by neutron scattering data and Raman experiments, is about 100% α-helical (Day 1969). It has been shown that in model membranes, M13 coat protein can adopt two different forms: an α-oligomeric and a β-polymeric form. The presence of either one of these forms critically depends on the headgroup type and the degree of saturation of the acyl chains of the lipids used (Nozaki et al. 1978; Fodor et al. 1981; Spruijt et al. 1989; Spruijt and Hemminga 1991).

The β-polymeric form is an aggregated β-sheet protein complex (Datema et al. 1987; Wolfs et al. 1989; Spruijt and Hemminga 1991; Van Gorkom et al. 1990), whereas the protein in the α-oligomeric form has a high amount of α-helix structure and forms only small aggregates, in dynamic equilibrium with protein monomers (Spruijt et al.

* *Correspondence to:* M. A. Hemminga, Department of Molecular Physics, Agricultural University, P.O. Box 8128, NL-6700 ET Wageningen, The Netherlands

1989; Spruijt and Hemminga 1991). It has been argued by Spruijt and Hemminga (1991) that this form is the biologically active state of the protein. This form has been studied recently using ^{2}H-NMR and ^{31}P-NMR spectroscopy (Sanders et al. 1991). In the present paper, we study the effects of M13 coat protein in the α-oligomeric form on bilayers consisting of DOPC with time-resolved fluorescence and ESR spectroscopy. These techniques are suitable for investigating changes in order and dynamics of the lipid chains upon incorporation of the M13 coat protein. The results obtained from both techniques can be compared directly, since they are both sensitive to motions on the nanosecond time scale. The lipid-to-protein (L/P) molar ratios taken are >12, because for an ideal single trans-membrane α-helix it is expected that 12 lipids will surround the protein (Wolfs et al. 1989; Spruijt and Hemminga 1991). In this L/P region, protein-protein contacts that may result in protein aggregation and the accompanying change in conformation, are avoided (Spruijt et al. 1989; Spruijt and Hemminga 1991).

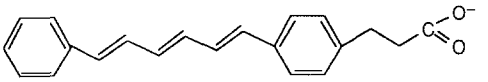
Materials and methods

Chemicals: DOPC was obtained from Sigma (St. Louis, USA). The fluorescent probe 3-(p-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid (DPH-propionic acid, Fig. 1A) was obtained from Molecular Probes, Inc. and spin labelled fatty acid (12-SASL, Fig. 1B) from SIGMA. All chemicals were used without further purification.

Sample preparation: Bacteriophage M13 was grown and purified as described elsewhere (Spruijt et al. 1989). After removing the chloroform with nitrogen gas, the desired amounts of lipids were lyophilized for at least 12 hours. If desired, DPH-propionic acid and 12-SASL were added to the chloroform solution at a molar ratio of 1:500 and 1:100, respectively. The lyophilized samples were solubilized in buffer (50 mM cholate, 10 mM Tris, 0.2 mM EDTA, pH 8.0). To the buffer solution the desired amount of protein in the same buffer was added. The coat protein was purified as described previously (Spruijt et al. 1989). Cholate was removed by extensive dialysis at 25 °C against a 100 fold excess of buffer (10 mM Tris, 0.2 mM EDTA, pH 8.0) for a total of 48 hours with buffer changes every 12 hours. The samples used for optical experiments were diluted to an $OD_{280\,nm}$ of 0.1. The corresponding reference samples without protein were prepared and diluted in the same way as the protein containing samples. The ESR samples were lyophilized, re-solubilized and concentrated using an Amicon Stirring cell. The L/P ratio of the samples was determined with a phosphate and protein determination. The conformation of M13 coat protein was checked with circular dichroism spectroscopy. An average of at least five scans was recorded at room temperature on a Jobin-Yvon Auto-Dichograph Mark V in the wavelength range 200–240 nm, using a sample cell with a 1-mm path length. The aggregation state was checked with HPLC as described by Spruijt et al. (1989).

ESR studies: ESR spectra were recorded on a Bruker ER 200 spectrometer with nitrogen gas flow temperature regulation (±1 °C). ESR settings were: 5 mW microwave power, 0.1 mT modulation amplitude, 200 ms time constant, 200 s scan time, 10 mT scan width and 339 mT centre field. Up to 12 spectra were accumulated to improve the signal to noise ratio. The ESR simulations were performed with a computer program developed by Schneider and Freed (1989). The parameters used for the simulation were as follows: components for the hyperfine coupling tensor (in mT) 0.62, 0.56, 3.25; components for the g-factor tensor 2.0088, 2.0061, 2.0027. The g-factor tensor components were similar to those used by other workers for the same type of radical (Meirovitch et al. 1984; Moser et al. 1989).

(A) DPH-propionic acid



(B) 12-SASL

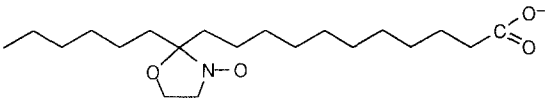


Fig. 1. Chemical formulas of DPH-propionic acid (**A**) and 12-SASL (**B**)

Time-resolved fluorescence studies: The experimental set-up for the polarized fluorescence decay measurements has been described previously (Visser et al. 1985). The excitation wavelength was 340 nm and the emission wavelength (437 nm) was selected by a filter (Balzer 437). The fluorescence decay was detected by time-correlated single photon counting (Phillips et al. 1985). The analysis was performed using a global analysis program, which uses a non-linear fitting least square procedure (Beechem and Graton 1989). The quality of the fit was determined by the weighted residuals and the chi-square.

After correction for the background fluorescence, the fluorescence decay ($I_f(t)$) of DPH-propionic acid in DOPC bilayers with various amounts of M13 coat protein was analyzed as a sum of exponentials:

$$I_f(t) = \sum_{i=1}^{2} \alpha_i e^{-t/\tau_i}, \quad (1)$$

in which τ_i is the fluorescence lifetime and α_i a pre-exponential factor. Typically, two exponential components were sufficient to describe the fluorescence decay.

After correction for background fluorescence, the time-resolved fluorescence anisotropy decay of DPH-propionic acid in DOPC bilayers with various amounts of M13 coat protein was analyzed as a sum of exponentials, which were expressed in terms of four variables, $\langle P_2 \rangle$, $\langle P_4 \rangle$, $D_\perp$ and $r(0)$ (Ameloot et al. 1984). The parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ are the orientational order parameters expressed in Legendre polynomials of the sec-

ond and fourth rank. In view of the approximate cylindrical shape of the DPH-propionic acid probe, its rotational diffusion in the membrane is assumed to be axially symmetric with a parallel ($D_{\parallel}$) and perpendicular ($D_{\perp}$) diffusion coefficient. Because $D_{\perp}$ is much smaller than $D_{\parallel}$, for the analysis of the fluorescence experiments only $D_{\perp}$ needs to be taken into account (Ameloot et al. 1984). The initial anisotropy, $r(0)$, depends on the angle between the absorption and emission moments. This parameter was fixed to a value of 0.39 as has been obtained by other authors (Ameloot et al. 1984; Pottel et al. 1986).

Results

Protein studies: M13 coat protein is able to adopt two conformations, an α-helix or a β-sheet, depending on its lipid environment and history of preparation (Spruijt et al. 1989). Since the purpose of this study was to investigate the protein in an α-helix structure, care was taken to avoid protein-protein contacts and protein aggregation that may result in the formation of protein in a β-sheet conformation, by using L/P ratios >12. Therefore, the conformation and aggregation state of the protein in the lipid bilayers was carefully checked. The circular dichroism spectra of M13 coat protein show that the α-helix content of the protein is approximately 90%, whereas HPLC demonstrated the absence of large aggregates in the reconstituted samples. After the experiments the conformational and aggregation state of M13 coat protein was checked again. No changes could be detected, showing that the α-helical state of the protein is conserved at all L/P ratios used.

ESR: ESR spectra of 12-SASL in DOPC bilayers at 10 °C, which have a liquid crystalline transition temperature of −16 °C (Dijck et al. 1976), with various amounts of M13 coat protein are given in Fig. 2. At low L/P ratios no distinct bound component can be observed, as has been reported previously for the β-polymeric form of the M13 coat protein in similar bilayers (Datema et al. 1987; Wolfs et al. 1989), but strong effects on the lineshape are observed (Fig. 2). Similar, but somewhat less pronounced effects are observed for 14-SASL (results not shown). To describe the spectral changes found upon incorporation of M13 coat protein in terms of changes in order parameters and rotational diffusion coefficients, ESR spectra with and without protein were simulated using the program of Schneider and Freed (solid lines in Fig. 2).

The motional behaviour of the probe is found to be well described by an anisotropic Brownian diffusion. The choice of other motional models (free or jump diffusion) gave a less good fit. Axial diffusion is assumed with a fixed ratio between the parallel ($D_{\parallel}$) and perpendicular ($D_{\perp}$) diffusion coefficients ($D_{\parallel}/D_{\perp} = 4$), as found in simulations performed by Schneider and Freed (1989). All simulations were carried out with an order parameter equals to zero. The introduction of a non-zero order parameter was also tried, but did not result in an improved fitting of the experimental ESR spectra. The simulations resulted in a consistent set of data, in which the spectra at

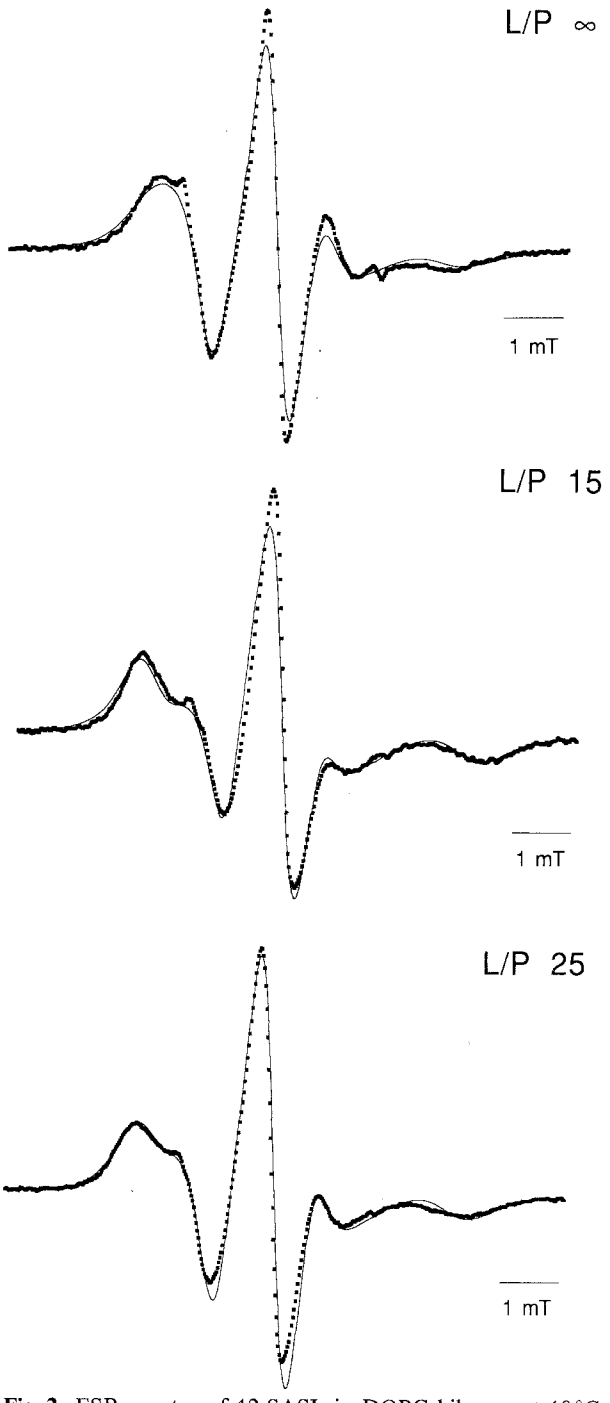


Fig. 2. ESR spectra of 12-SASL in DOPC bilayers at 10 °C with various L/P ratios (mole/mole) of M13 coat protein. The *dotted lines* are the experimental data and the *straight lines* represent the simulations. For the simulations the components for the hyperfine coupling tensor and g tensor were used as described in Material and methods. The values for $D_{\perp}$ are $3.4 \times 10^{-7}\ s^{-1}$ for L/P ∞, $2.4 \times 10^{-7}\ s^{-1}$ for L/P 25 and $1.9 \times 10^{-7}\ s^{-1}$ for L/P 15. The order parameter was taken to be zero in all spectra

the various L/P ratios were considered as single component spectra characterized by different values of $D_{\perp}$. The resulting values of $D_{\perp}$ at various L/P ratios are displayed in Fig. 3.

An important factor in the application of the present simulation model was the observation that the same ESR spectra were found for lower L/P ratios at higher temper-

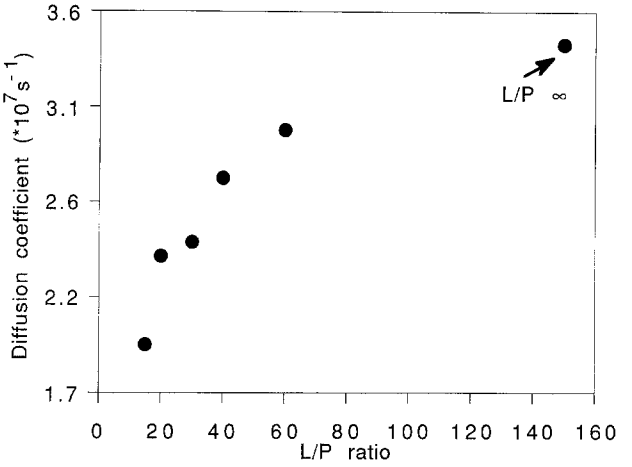


Fig. 3. The diffusion coefficient, $D_\perp$, obtained from the analysis of the ESR spectra of spin labels in DOPC bilayers at 10 °C with various amounts of M13 coat protein

Table 1. Fluorescence lifetimes (τ_i), the relative contribution (α_i) and the values for $\langle P_2 \rangle$, $\langle P_4 \rangle$, and $D_\perp$ of DPH-propionic acid in DOPC membranes with various amounts of M13 coat protein at 5 and 20 °C. The error in the values is estimated to be less than 5% for all parameters (10% for $D_\perp$)

L/P	T (°C)	α_1	τ_1 (ns)	α_2	τ_2 (ns)	$\langle P_2 \rangle$	$\langle P_4 \rangle$	$D_\perp$ ($\times 10^9\,s^{-1}$)
∞	5	0.47	2.94	0.53	6.00	0.34	0.59	0.10
55	5	0.43	3.10	0.57	6.21	0.39	0.62	0.09
35	5	0.45	3.28	0.55	6.42	0.55	0.77	0.08
∞	20	0.38	1.97	0.62	5.40	0.32	0.57	0.18
55	20	0.37	2.29	0.63	5.78	0.36	0.59	0.14
35	20	0.40	2.73	0.60	6.08	0.50	0.68	0.14

atures as compared to spectra observed at higher L/P ratios. This indicates that increasing the temperature compensates the effect of the protein on the ESR lineshape. For example, a temperature increase of 15 °C is compensated by increasing the protein concentration by a factor of two.

Time-resolved fluorescence: The fluorescence decay behaviour of DPH-propionic acid in bilayers of DOPC with various amounts of M13 coat protein is shown in Table 1. Two fluorescence lifetime components are sufficient to analyse the fluorescence decays, in agreement with previous reports (Stubbs et al. 1984). The origin of this multi-exponential behaviour is still unknown, because the photophysics of PDH-propionic acid have not yet been elucidated. However, this will not affect our analysis of the anisotropy decay, especially since the two lifetimes behave similarly under the various experimental conditions. For example, both lifetimes decrease with increasing L/P ratios, and increasing the temperature from 5 to 20 °C causes both lifetimes to decrease.

The parameters $\langle P_2 \rangle$, $\langle P_4 \rangle$ and $D_\perp$ obtained from the analysis of the anisotropy decay of DPH-propionic acid at various L/P ratios and temperatures are shown in Table 1 as well. As can be seen, a decrease of temperature causes the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ to increase. This also happens when the L/P ratio is lowered by an increasing protein concentration. An increase of the temperature from 5 to 20 °C leads to an increase of $D_\perp$ by nearly a factor of two; $D_\perp$ decreases only slightly upon increasing the protein concentration in the bilayers.

Discussion

During the infection process of *Escherichia coli* by the M13 bacteriophage, high amounts of old and newly synthesized M13 coat protein are stored in the cytoplasmic membrane of the host. Therefore, it is interesting to know how M13 coat protein affects the membrane properties. In this paper, model membranes with unsaturated lipid chains with various amounts of the M13 coat protein in the presumed biologically active α-monomeric form are studied with ESR and time-resolved fluorescence spectroscopy to probe the effect of the protein on the lipid bilayers. Care was taken to avoid protein-protein contacts (formation of α-oligomers) and the concomitant formation of β-polymers. It is known that β-polymers strongly affect the lipid membrane by giving rise to non-bilayer lipid structures around the aggregates (Sanders et al. 1992). Therefore, L/P ratios >12 were used and the reconstitution approach was taken as described by Spruijt et al. (1989) that conserves the α-helical monomeric state (Spruijt and Hemminga 1991). In the present study, the absence of protein aggregation is clearly demonstrated with HPLC and M13 coat protein is predominantly in an α-helical conformation, as shown by circular dichroism spectroscopy.

Diffusion coefficient: The changes in the ESR spectra of the 12-SASL spin probe upon introduction of M13 coat protein can be analyzed by a computer simulation in terms of a decrease in the rotational diffusion coefficient $D_\perp$. $D_\perp$ is assumed to be related to $D_\parallel$ by the equation $D_\parallel = 4D_\perp$ (see Results). The order parameter is fixed at zero. This is a good approximation since the order parameter found for the 12-SASL probe in our simulations is less than 0.1 (results not shown), in agreement with other studies (Wassall et al. 1990). Because of this low value, it is not possible to determine changes in order parameter induced by the protein with a sufficient accuracy. In addition, a variable order parameter would increase the number of parameters in the simulations, thereby increasing unnecessarily the complexity of the calculations. The rotational diffusion coefficient $D_\perp$ shows a non-linear dependence on the protein concentration (Fig. 3): a strong decrease is observed, especially at low L/P ratios. This effect may be expected, since on going to very low L/P ratios totally immobilized lipid would eventually result by squeezing the lipids between the aggregated α-oligomeric protein molecules (Peelen et al. 1992).

The fluorescent probe DPH-propionic acid also senses a decrease of the diffusion coefficient $D_\perp$ upon incorporating M13 coat protein. However, the effect of M13 coat protein on $D_\perp$ in this case is less when compared to the changes observed by ESR. Also the rate of the motion of the DPH-propionic acid probe is 2.5 times faster than the

rate of the 12-SASL probe. Although this small difference is difficult to explain in a quantitative way, it will be related to differences in size and molecular properties of the probes (see Fig. 1). These molecular differences of the probes also affect the order parameters as will be discussed below.

Order parameters: The molecular structure of the 12-SASL probe gives rise to a high flexibility, which arises from a trans-gauche isomerization of the acyl chain to which the nitroxide moiety is attached. This gives rise to an order parameter that is about zero. However, the DPH-propionic acid probe is a rigid molecule, as a result of the presence of the conjugated bonds (Fig. 1). Therefore, it senses the "average" order over different depths in the membrane. In Table 1 it can be seen that increasing the protein concentration causes an increase in both order parameters, $\langle P_2 \rangle$ and $\langle P_4 \rangle$, of the DPH-propionic acid probe. This indicates that the molecular order of the DOPC bilayers as sensed by the probe has increased upon incorporation of the M13 coat protein. The increase in order correlates with the observed increase of both lifetimes on adding protein to the bilayers (Table 1). It has been shown previously that lifetimes of DPH analogues show a remarkable correlation with order (Straume and Litman 1987). The increase of the lifetimes is found to result from a reduced water penetrability into the lipid bilayer by a reduced lipid flexibility (Best et al. 1987).

The value of $\langle P_4 \rangle$ is larger than the value of $\langle P_2 \rangle$ and shows the same variation with protein concentration as $\langle P_2 \rangle$. The increase of $\langle P_4 \rangle$ with increasing protein concentrations can be related to a decrease of the width of the angular distribution of the lipid probe in the membrane. If $\langle P_2 \rangle$ is plotted versus $\langle P_4 \rangle$ at various temperatures and at various protein concentrations, an almost linear relation is observed (Fig. 4). This suggests that changing the temperature is comparable to adding protein to lipid bilayers. This is in agreement with the finding from the ESR experiments, that increasing the protein concentration results in a change in diffusion coefficient, which can be compensated by increasing the temperature. From Fig. 4 it can also be seen that, in agreement with the findings of Best et al. (1987), the relation between $\langle P_2 \rangle$ and $\langle P_4 \rangle$ can not be considered as being described by the cone model. The relation between $\langle P_2 \rangle$ and $\langle P_4 \rangle$ is best described by a P_4-distribution model, indicating that the interaction energy of the DPH-propionic acid probe is proportional to P_4 (cos β), where β is the angle between the probe molecule and the normal to the bilayers. This is in agreement with results obtained by Pottel et al. (1986) for DPH molecules in the phospholipids DMPC and DPPC.

In Fig. 5 the angular distribution function, calculated using information theory, is given for a sample with and without protein (Ameloot et al. 1984). From Fig. 5, it can be observed from the maximum at $\beta = 0°$, that the protein orders the fluorescent probes along the normal to the bilayer. Also a small maximum at $\beta = 90°$ is observed in Fig. 5. This maximum probably arises from an artefact as a result of not taking into account a higher expansion in order parameters of the distribution function and can be disregarded (Best et al. 1987).

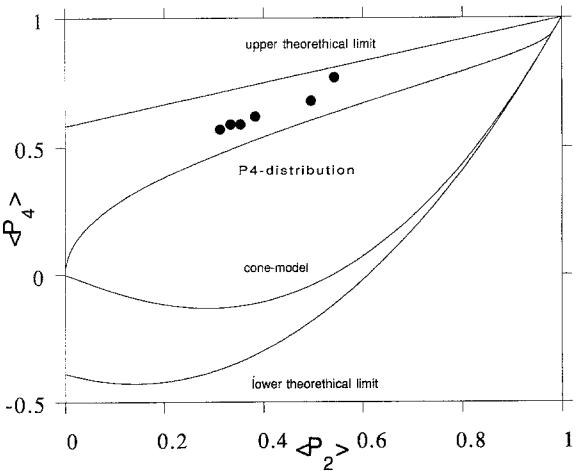


Fig. 4. $\langle P_2 \rangle - \langle P_4 \rangle$ plot, using the data of Table 1. The upper and lower theoretical boundaries for $\langle P_2 \rangle - \langle P_4 \rangle$ are shown. In addition, the dependencies are shown for the $\langle P_4 \rangle$-distribution and the cone model

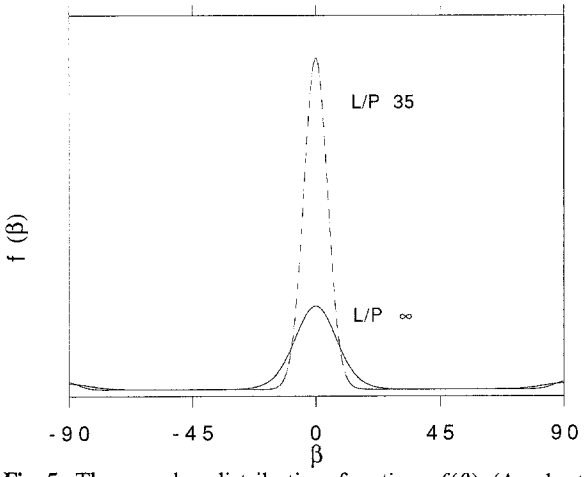


Fig. 5. The angular distribution function, $f(\beta)$ (Ameloot et al. 1984), constructed from the experimental values of $\langle P_2 \rangle$ and $\langle P_4 \rangle$ using information theory applied to DPH-propionic acid in DOPC bilayers with and without M13 coat protein

Phospholipid model membranes consisting of DOPC specifically deuterated in both chains at the 11-position (1,2-[11,11-$^2H_2$]-DOPC) containing various amounts of M13 coat protein in the α-helical from have been studied with ^{2}H-NMR spectroscopy (Sanders et al. 1992). These experiments indicate that on the ^{2}H-NMR time scale the order in the bilayer is decreased in the presence of the coat protein. This shows that ^{2}H-NMR spectroscopy is much more sensitive in detecting small differences in the order parameter than is ESR spectroscopy on the 12-SASL probe. Whereas, the order of the lipid bilayers, as seen by the fluorescent DPH-propionic acid probe, increases upon incorporation of the M13 coat protein, the ^{2}H-NMR experiments show the opposite. This difference may be due to the presence of bulky amino acid side chains of M13 coat protein in the hydrophobic membrane region. These groups will provide protrusions with holes in between, that will be sensed by the deuterium nuclei on the flexible acyl chains. This may result in an

increased disorder when more M13 coat protein is introduced in the phospholipid system. However, the relatively long, rigid fluorescent probe will only experience the "average" protein surface and sense an increased order. Previously, protrusions and holes have been proposed to play a role in the packing of M13 coat protein incorporated in membranes (Dunker and Jones 1978). Bulky side chains may be provided by the aromatic amino acids of M13 coat protein (tyrosine 21 and 24, tryptophan 25, and phenylalanine 42).

General conclusions: As observed from the ESR experiments in this paper, M13 coat protein in the α-monomeric state at high L/P ratios (L/P > 12), is not able to induce a long living lipid boundary shell and consequently an immobilization of the lipids. This is in contrast with ESR studies performed by Peelen et al. (1992) on M13 coat protein in the α-oligomeric state at low L/P ratios (L/P < 12), where an immobilized component was observed of which the intensity depended on the M13 coat protein concentration (Peelen et al. 1992). For this system, it was estimated that about four lipids bind per protein monomer (Peelen et al. 1992). This low number of boundary lipids indicates that protein-protein contacts take place at these low L/P ratios (Wolfs et al. 1989) and that lipid sites are shared by two or more protein monomers. Protein-protein contacts result in the formation of α-oligomers. This effect gives rise to an immobilized ESR spectral component, as has also been observed in studies with the β-polymeric M13 coat protein (Datema et al. 1987; Wolfs et al. 1989).

A long range perturbation of α-oligomeric M13 coat protein at low L/P ratios has been observed by Peelen et al. (1992): A fluid component, which was characteristic of a lower temperature than at which the corresponding spectra of the lipid protein systems was recorded, had to be used in the spectral simulations. This indicates that also in the case where protein-protein contacts take place, M13 coat protein induces an overall lipid effect, similar to that found for the monomeric form.

M13 coat protein in the α-helical conformation has a rigid trans-membrane α-helix exposing a hydrophobic surface to interact with the lipid chains. In general, it has been assumed that immobilization of lipids by proteins originates from the rigidity of the protein backbone of the membrane bound domain of the protein (Jost et al. 1973; Marsh 1981; Devaux 1983). Based on our experiments, it is clear that not only the rigidity of the trans-membrane part of the protein is important, but also the size of the hydrophobic surface of the protein region: a single M13 coat protein molecule is not able to cause a strong immobilization, in contrast to α-oligomers consisting of aggregated protein molecules with an α-helix conformation.

When small trans-membrane α-helical M13 coat protein monomers are incorporated in lipid systems, an overall effect on the lipids in induced, resulting in a reduction of the dynamics and an increase in average order of the lipids experienced by the rigid fluorescent DPH-propionic acid probe. This overall effect of the protein on the lipid bilayer can be reversed by increasing the temperature. In this respect the effect of the M13 coat protein resembles that observed upon introducing rigid amphipathic molecules, such as cholesterol, in lipid bilayers (Marsh and Smith 1973; Schreier-Muccillo et al. 1973; Hemminga 1975). This conclusion is in agreement with the observation that the hydrophobic region of M13 coat protein perfectly matches the lipid bilayer, resulting in a relatively small distortion of the bilayer structure of the lipid system (Sanders et al. 1992).

Acknowledgements. This research was supported by the Netherlands Foundation of Biophysics with financial aid from the Netherlands Organization of Scientific Research (NWO) and by the commission of the European Communities, contract nr. ST2J-0088.

References

Ameloot M, Hendrickx H, Herreman W, Pottel H, van Cauwelaert F, van der Meer W (1984) Effect of orientational order on the decay of the fluorescence anisotropy in membrane suspensions. Experimental verification on unilamellar vesicles and lipid/α-lactalbumin complexes. Biophys J 46:525–539

Beechem JM, Graton E, Ameloot M, Knutson JR, Brand L (1989) The global analysis of fluorescent intensity and anisotropy decay data; second generation theory and programs. SPIE 909, p. 70–81

Best L, John E, Jähnig F (1987) Order and fluidity of lipid membranes as determined by fluorescence anisotropy decay, Eur Biophys J 15:87–102

Datema KP, Wolfs CJAM, Marsh D, Watts A, Hemminga MA (1987) Spin-label electron spin resonance study of bacteriophage M13 coat protein incorporation into mixed lipid bilayers. Biochemistry 26:7571–7574

Day LA (1969) Circular dichroism and ultraviolet absorption of a deoxyribonucleic acid binding protein of filamentous bacteriophage. J Mol Biol 39:265–297

Devaux F (1983) ESR and NMR studies of lipid-protein interactions in membranes. Biol Magn Reson 5:183–299

Dunker AK, Jones TC (1978) Proposed knobs-into-holes packing for several membrane proteins. Membr Biochem 2:1–16

Fodor SPA, Dunker AK, Ng YC, Carsten D, Williams RW (1981) Lipid-tail group dependent structure of the fd gene 8 protein. In: Seventh. biennial Conference on Bacteriophage Assembly, pp. 441–455 (Dubow MS, ed) Alan R Liss Inc, New York

Hemminga MA (1975) ESR study of the mobility of the cholestane spin label in oriented lecithin-cholesterol multibilayers. Chem Phys Lipids 14:141–150

Jost PC, Griffith OH, Capaldi RA, Vanderkooi, GA (1973) Lipid-protein and lipid-lipid interactions in cytochrome oxidase model membranes, Proc Natl Acad Sci USA 70:480–484

Marsh D (1981) In Membrane spectroscopy (E. Grell. Ed) pp. 51–142, Springer Verlag, New York

Marsh, D Smith ICP (1973) Interacting spin label study of the fluidizing and condensing effects of cholesterol on lecithin bilayers. Biochim Biophys Acta 298:133–144

Meirovitch E, Nayeem A, Freed JH (1984) Analysis of protein-lipid interactions based on model simulations of electron spin resonance spectra. J Phys Chem 88:3453–3465

Model P, McGill C, Mazur B, Fulford WD (1982) The replication of bacteriophage f1: gene V protein regulates the synthesis of gene II protein. Cell 29: 329–335

Moser M, Marsh D, Meier P, Wassmer K-H, Kothe G (1989) Chain configuration and flexibility gradient in phospholipid membranes. Comparison between spin-label electron spin resonance and deuteron nuclear magnetic resonance, and identification of new conformations. Biophys J 55:111–123

Nozaki Y, Reynolds JA, Tanford C (1978) Conformational states of a hydrophobic protein. The coat protein of fd bacteriophage, Biochemistry 17:1239–1246

Pottel H, van der Meer BW, Herreman W, Depauw H (1986) On the significance of the fourth-rank orientational order parameter of fluorophores in membranes. Chem Phys J 102:37–44

Peelen SJCJ, Sanders JC, Hemminga, MA, Marsh D (1992) Stochiometry, selectivity, and exchange dynamics of lipid protein interaction with bacteriophage M13 coat protein studied by spin label electron spin resonance – Effects of protein secondary structure. Biochemistry 31:2670–2677

Phillips D, Drake RC, O'Connor DV, Christensen RL (1985) Time correlated single-photon counting (TCSPC) using laser excitation, Anal Instrum NY 14:267–292

Pratt D, Ozagoloff H, Beadoin J (1969) Conditional lethal mutants of the small filamentous coliphage M13. Virology 39:42–53

Sanders JC, Poile TW, Spruijt RB, van Nuland NAJ, Watts A, Hemminga MA (1991) A NMR investigation on the interactions of the α-oligomeric form of the M13 coat protein with lipids, which mimic the Escherichia coli inner membrane. Biochim Biophys Acta 1006:102–108

Sanders JC, Poile TW, Wolfs CJAM, Hemminga MA (1992) Formation of non-bilayers structures induced by M13 coat protein depends on the conformation of the protein. Biochim Biophys Acta 1110:218–224

Schneider DJ, Freed JH (1989) Calculation of slow motional magnetic resonance spectra: a user guide. In Biological Magnetic Resonance, volume 8: Spin labelling, Berliner, JL, Reuben J (Eds.) 7, pp 1–76, Plenum Press, New York

Schreier-Muccillo S, Mards D, Dugas H, Schneider H, Smith ICP (1973) Spin-probe study of the influence of cholesterol on motion and orientation of phospholipids in oriented multibilayers and vesicles, Chem Phys Lipids 10:11–27

Spruijt R, Wolfs CJAM, Hemminga MA (1989) Aggregation-related conformational change of the membrane-associated coat protein of bacteriophage M13. Biochemistry 28:9159–9165

Spruijt RB, Hemminga MA (1991) The in situ aggregational and conformational state of the major coat protein of bacteriophage M13 in phospholipid bilayers mimicking the inner membrane of host *Escherichia coli*. Biochemistry 30:11147–11154

Straume M, Litman BJ (1987) Equilibrium and dynamic structure of large, unilamellar, unsaturated acyl chain phosphatidylcholine vesicles. Higher order analysis of 1,6-diphenyl-1,3,5-hexatriene and 1-(4-(trimethylammonio)phenyl)-6-phenyl-1,3,5-hexatriene anisotropy decay. Biochemistry 26:5113–5120

Stubbs CD, Kinosita K, Munkonge F, Quinn PJ, Ikegami A (1984) The dynamics of lipid motion in sarcoplasmic reticulum membranes determined by steady-state and time-resolved fluorescence measurements on 1,6-diphenyl-1,3,5-hexatriene and related molecular. Biochim Biophys Acta 775:374–380

van Dijck PWM, De Kruijff B, van Deenen LLM, De Gier J, van Demel RA (1976) The preference of cholesterol for phosphatidylcholine in mixed phosphatidylcholine/phosphatidylethanolamine bilayers. Biochim Biophys Acta 455:576–587

Van Gorkom LCM, Horváth LI, Hemminga MA, Sternberg B, Watts A (1990) Identification of trapped and boundary lipid binding sites in M13 coat protein/lipid complexes by deuterium NMR spectroscopy. Biochemistry 29:3828–3834

Visser AJWG, Ykema T, van Hoek A, O'Kane DJ, Lee J (1985) determination of rotational correlation times from deconvoluted fluorescence anisotropy decay curves. Demonstration with 6,7-dimethyl-8-ribityllumazine and lumazine protein from *Photobacterium leiogathi* as fluorescent indicators. Biochemistry 24:1489–1496

Wassall SR, Yang RC, Wang L, Phelps TM, Ehringer W, Stillwell W (1990) Magnetic resonance studies of the structural role of vitamin E in phospholipid model membranes. Bull Magn Reson 12:60–64

Wolfs CJAM, Horváth LI, Marsh D, Watts A, Hemminga MA (1989) Spin-label ESR of bacteriophage M13 coat protein in mixed lipid bilayers. Characterization of molecular selectivity of charged phospholipids for the bacteriophage M13 coat protein in lipid bilayers. Biochemistry 28:9995–10001