LECTURE

FLUCTUATIONS OF LIPIDS AND PROTEINS IN MEMBRANES

Fritz Jähnig

Max-Planck-Institut für Biologie, Correnssstrasse 38, 7400 Tübingen, Fed. Rep. of Germany

ABSTRACT

Proteins have a twofold effect on lipids in fluid membranes: they induce a tilt of the preferred axis of lipid orientation and they reduce the fluctuations of the lipid molecules about the preferred axis. This effect is maximal at the protein surface, where the tilt angle is 20^{o}-30^{o} and the order parameter 0.6 and, with increasing distance from the protein surface, decreases with a coherence length of about 15Å. This has been shown by fluorescence anisotropy measurements on oriented samples (1).

The perturbation of lipid order due to protein in turn affects protein behavior. First, this effect weakens the incorporation of proteins into membranes. The contribution to the free energy of binding is small, but the contribution to the enthalpy is large (2). Second, the lipid perturbation effect promotes aggregation of proteins in membranes. This has been demonstrated for the polypeptide melittin (3).

Below the lipid phase transition, proteins decrease the order of the surrounding lipids, but the boundary lipids do not fluctuate as strongly as above the phase transition. As a consequence, the internal protein fluctuations are reduced when passing through the lipid phase transition. This leads to a reduction of their activity as expressed by a

Key words: membrane lipids, membrane proteins, lipid fluctuations, protein fluctuations, lipid-protein interaction.

break in an Arrhenius plot. This correlation between lipid fluctuations, protein fluctuations and protein activity has been demonstrated by time-resolved fluorescence anisotropy measurements on lactose permease of E.coli (4).

INTRODUCTION

In characterizing the structure of membranes, a useful approach is the distinction between the mean structure and thermal fluctuations around that mean structure. The mean structure of membrane lipids is the bilayer with the lipid molecules oriented parallel to the bilayer normal. Around this mean orientation, the lipids undergo orientational fluctuations.

Positional fluctuations in the membrane plane lead to lateral diffusion. The bilayer structure, i.e. the mean structure, is responsible for the function of a membrane as a permeability barrier - the fluctuations are required for the fluidity of a membrane. The mean structure of membrane proteins, as far as their membrane-incorporated part is concerned, in most cases is given by a number of bilayer--spanning α-helices. In analogy to lipids, fluctuations may occur as positional and orientational fluctuations, the latter being composed of rigid-body and conformational fluctuations. Positional fluctuations, i.e. lateral diffusion of membrane proteins, and orientational fluctuations of whole proteins are necessary for all kinds of interactions between proteins. In addition, protein activity seems to require conformational fluctuations, as recognized in recent work on soluble proteins.

As is well known, fluorescence anisotropy (FA) measurements provide information on orientational fluctuations. To investigate the fluctuations of lipids, fluorescence probes are used which incorporate spontaneously between the lipid molecules and reflect their orientational fluctuations. Fluctuations of proteins may be studied via intrinsic or extrinsic fluorescence probes.

If a dispersion of membranes is investigated, the orientational fluctuations of the fluorophores which occur within the fluorescence lifetime are detected irrespective of their mean orientation. From the measured anisotropy r(t) one derives the relaxation time $\emptyset$ of the fluctuations and the residual anisotropy r_∞ at long times which yields the orientational order parameter

$$S = \langle P_2 \rangle = (3\langle \cos^2\theta \rangle - 1)/2$$

as a measure for the strength of the fluctuations. θ denotes the angle between the instantaneous orientation of a fluorophore and its mean axis of orientation, and the angular brackets represent the temporal average over the fluorescence lifetime (1, 2). More information is obtained from experiments on oriented membranes (3-6). First, the preferred or mean orientation of the fluorophores can be determined. Second, in addition to $\langle P_2 \rangle$ or $\langle \cos^2\theta \rangle$ one obtains the higher order parameter

$$\langle P_4 \rangle = (35\ \langle \cos^4\theta \rangle - 3\ \langle \cos^2\theta \rangle + 3)/8$$

or $\langle\cos^4\rangle$. This permits to construct an approximate form of the distribution of the fluorophore axes around the mean orientation.

For dispersed as well as oriented membranes, the temporal decay of the anisotropy is a complex process. Theoretically, for the fluctuations of a fluorophore in an anisotropic medium such as a membrane one expects a large number of relaxation times (7-9). Experimentally, one cannot resolve more than 2 or 3 relaxation times. The best approach, therefore, is to fit the theoretical expression for r(t) to the experimental data treating the rotational diffusion coefficients ($D_\perp$ and $D_\parallel$ in the case of a symmetric ellipsoid) as open parameters to be determined from the fit.

In this review of our work on FA three subjects will be discussed: (i) Lipid fluctuations - how are the fluctuations of different probes related to lipid fluctuations and what kind of membrane fluidity is provided by FA? (ii) Lipid-protein interaction - do proteins make lipids more fluid or more rigid? (iii) Protein fluctuations - how do membrane-incorporated α-helices fluctuate and how are their fluctuations coupled to lipid fluctuations?

Lipid Fluctuations

FA curves of four different probes in bilayers of dimyristoylphosphatidylcholine (DMPC) at 35°C are shown in Fig. 1. The probes are diphenylhexatriene (DPH), diphenyloctatetraene (DPO), triphenylamino-DPH (TMA-DPH), and trans-parinaric acid (tPnA). From the residual anisotropies r_∞ at long times the probe order parameters follow as

$$S^{\text{TMA-DPH}} \approx 0.6 > S^{\text{tPnA}} \approx 0.5 > S^{\text{DPH}} \approx S^{\text{DPO}} \approx 0.4.$$

If, in lowest approximation, the r(t) curves are fitted by single exponential decays, the probe relaxation-times are obtained as

$$\phi^{\text{DPO}} \approx 2\text{nsec} > \phi^{\text{DPH}} \approx \phi^{\text{TMA-DPH}} \approx 1\text{nsec} > \phi^{\text{tPnA}} \approx 0.5\text{nsec}.$$

The reason for the different order parameters of the probes and their different relaxation times must lie either in the different size and geometry of the probes or in different positions of the probes in the membrane with different lipid environments. The lipid order parameter S^L, as detected by deuterium magnetic resonance (DMR), is about 0.4 in the so-called plateau region which extends approximately from the carbonyl group to the tenth carbon atom of the chains and then decreases to a value of about 0.2 at the chain ends (10). Hence, probes at different positions along the membrane normal sense a different lipid environment, and, therefore, may differ in their own order. That such an effect exists is borne out by a comparison of TMA-DPH and tPnA. Both probes are expected to insert into a bilayer with their charged ends fixed in the polar head region of the lipids and with their hydrophobic part which carries the fluorophore penetrating between the lipid chains. In the case of TMA-DPH, due to the short connection between the charged moiety and the fluorophore the latter extends essentially over the plateau region of the lipid chains. tPnA, on the other hand, contains a spacer of 7 CH_2 groups between the charged moiety and the fluorophore, which

positions the fluorophore more towards the chain ends. Here the lipid order is lower and, therefore, the order parameter of tPnA is smaller than that of TMA-DPH.

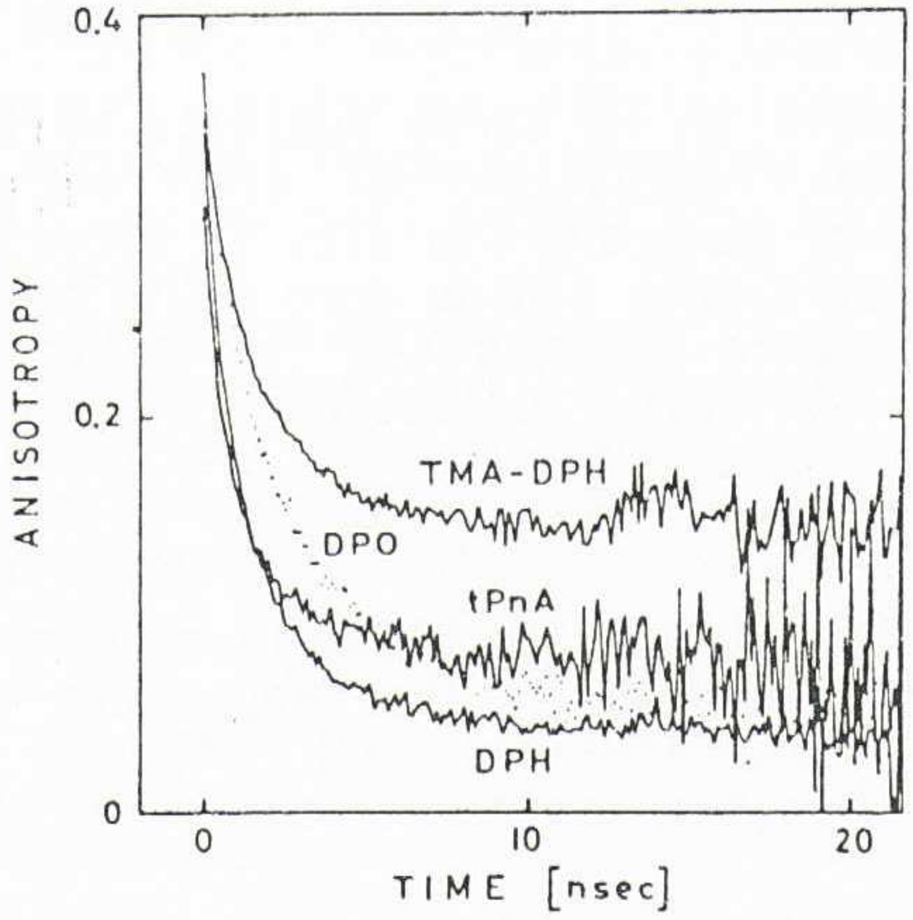

FIGURE 1

Fluorescence anisotropies r(t) of DPH, TMA-DPH, DPO, and tPnA in bilayers of DMPC at 35°C.

To assess the influence of probe size on the order parameter one may compare DPH and DPO. Although their positions cannot be as unambiguously fixed as for the charged probes TMA-DPH and tPnA, they should not be much different. Hence, their main difference seems to lie in the size. Nevertheless, their order parameters are equal, so that size seems to have a minor effect on probe order. Thus, the differences between probe order parameters arise mainly from different positions of the probes along the membrane normal and thus reflect the variation of lipid order along the membrane normal.

For the quantitative comparison between probe and lipid order we choose the probe TMA-DPH. It extends over the plateau region of the lipids and the order parameter is 0.6, considerably higher than the corresponding lipid order parameter $S^L \approx 0.4$. To explain this difference, one may consider the lipid fluctuations as a superposition of rigid-body motion and conformational transitions,

$$S^L = S^L(\text{rigid}) \cdot S^L(\text{conf}).$$

By definition, S^L(rigid) does not vary along the lipid chains and the decrease of S^L towards the chain ends is a consequence of increased conformational disorder. A rigid probe extended over the whole length of lipid chains would detect S^L(rigid). This also holds, if the probe is confined to the plateau region, whereas a probe located deeper in the bilayer senses an increasing contribution of conformational disorder. Thus, TMA-DPH is expected to reflect only the rigid-body motion of the lipids,

$$S^{TMA-DPH} \approx S^L(rigid)$$

and, therefore, its order is higher than the total lipid order as detected by DMR. Inversely, this assignment permits a determination of the two components of lipid order. Inserting S^L(rigid) = 0.6 and S^L = 0.4 in the above relation for S^L yields S^L(conf) = 0.6. This result means that lipid order in the plateau region originates about equally from rigid-body order and from conformational order.

According to DMR relaxation measurements (11), the relaxation time of lipid order varies in a similar way along the lipid chains as the lipid order parameter. Thus, if probe relaxation reflects lipid relaxation as did probe order reflect lipid order, the sequence of probe relaxation times corresponding to their magnitude would coincide with the sequence of order parameters. However, this is not the case, and another interpretation is required. The most obvious alternative is to explain the differences in relaxation time as differences in probe size: In a viscous medium, larger probes fluctuate more slowly than smaller ones. DPO is the largest probe, followed by TMA-DPH and DPH, and finally tPnA with the smallest fluorophore. Therefore, the relaxation times should decrease in this order, as actually found. Compared to lipid relaxation times $\phi^L \approx 0.1$ nsec,the shortest probe relaxation time $\phi^{tPnA} \approx 0.5$ nsec is still larger by a factor of 5. Lipid relaxation may therefore be considered as a relaxation of separate CH_2 groups. The size of a CH_2 group is about 7 times smaller than that of the tPnA fluorophore, sufficient to explain the shorter lipid relaxation time. But this picture of lipid relaxation is certainly an oversimplification, because the lipid CH_2 groups are linked and do not fluctuate independently.

Actually, one is not interested primarily in lipid or probe relaxation times, but in the viscosity underlying these relaxation processes. For the evaluation of relaxation times in terms of a viscosity the membrane cannot be considered as an isotropic viscous medium - the lipid orientational order must be taken into account. In lowest approximation (7, 9), only one relaxation time is considered which is expressed as

$$\phi = \langle\sigma\rangle / (6D_\perp) \qquad (1a)$$

with $D_\perp$ denoting the diffusion coefficient of the probe for rotation about an axis perpendicular to the long molecular axis and $\langle\sigma\rangle$ specifying the influence of lipid order. An approximate analytic expression is $\langle\sigma\rangle = (1+2S)\,(1-S)$ (12). Thus, the experimental value for ϕ can, if the experimental value for S is also used, be evaluated for $D_\perp$. Finally, from $D_\perp$ the viscosity $n_\perp$ for rotational motion about an axis perpendicular to the preferred axis of lipid orientation is derived.

Considering the probe as a symmetric ellipsoid of large axial ratio a/b the relevant relation is (13)

$$D_{\perp} = \frac{3kT}{16\pi a^3 \eta_{\perp}} \left(2\ln \frac{2a}{b} - 1\right). \qquad (1b)$$

For the case of DPH, the results for $D_{\perp}$ and $n_{\perp}$ are shown in Fig. 2. Two aspects are worth mentioning. At 35°C, i.e. in the fluid lipid phase, the viscosity is

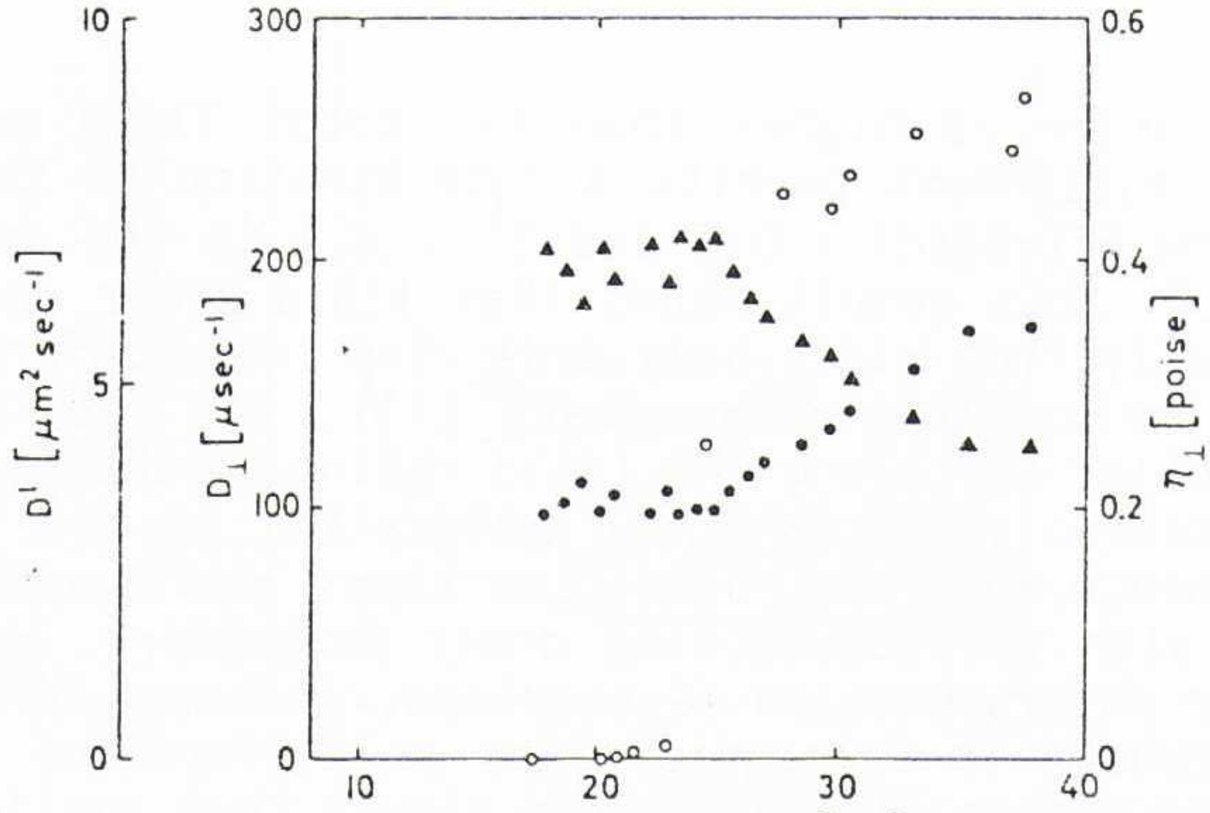

FIGURE 2

Temperature dependence of the rotational diffusion coefficient $D_{\perp}$ (●) of DPH in bilayers of DMPC, and of the corresponding viscosity $n_{\perp}$ (▲). For comparison, the temperature dependence of the lateral diffusion coefficient D^1 (o) of DMPC molecules is shown (data taken from ref. 32).

$n_{\perp}$ = 0.25 poise. This value is an order of magnitude lower than the viscosity for lateral diffusion derived from the lateral diffusion coefficient D^1. Secondly, the temperature dependencies of $D_{\perp}$ and D^1 (Fig. 2) are rather different: below the lipid phase transition D^1 is smaller by three orders of magnitude, whereas $D_{\perp}$ does not vary considerably across the phase transition. These two aspects indicate that a lipid membrane cannot be described adequately by only one viscosity - the different kinds of translational and rotational motion are governed by different viscosities. Fluidity of a membrane is usually identified with the fluidity, or inverse of the viscosity n^1, for lateral diffusion. The relevance of the fluidity for rotation, or inverse of the viscosity $n_{\perp}$, is less obvious. It might be correlated with the rate of conformational changes of membrane proteins which are required for their proper functioning, e.g. in transport.

Lipid-Protein Interaction

The question of whether proteins make a fluid bilayer more fluid or more rigid has been given different answers by different experimental techniques. In electron spin resonance (ESR) and FA experiments, a variety of proteins were found to increase the lipid order parameter, i.e. to make the lipids more rigid (14, 15). In DMR experiments, on the other hand, the lipid order parameter remained constant or even decreased slightly upon addition of protein (16, 17). To resolve this seeming contradiction the following model for lipid-protein interaction has been proposed (2, 15). At the surface of proteins the orientational fluctuations of lipids are restricted due to the rigidity of the protein structure. Furthermore, due to the uneven or tilted surface of proteins the mean axis of lipid orientation becomes tilted with respect to the membrane normal. Denoting the boundary values of the order parameter and the tilt angle by S_o and θ_o, respectively, and the unperturbed values by S_u and $\theta_u = 0$, the boundary conditions for immobilization and tilt become $S_o > S_u$ and $\theta_o > 0$, as illustrated in Fig. 3. With increasing distance from the protein molecules the perturbations of lipid order decrease exponentially with the coherence lengths ξ and λ,

$$S(r) = S_n + (S_o - S_n)[e^{-(r-R_o)/\xi} + e^{(r-2R+R_o)/\xi}] \qquad (2a)$$

$$\cos\,\theta(r) = 1 - (1-\cos\,\theta_o)[e^{-(r-R_o)/\lambda} + e^{(r-2R+R_o)/\lambda}]. \qquad (2b)$$

Here, R_o represents the protein radius and 2R the distance between the center of the protein molecule at r=0 and the centers of the surrounding protein molecules. Experimentally accessible are spatial averages of $S(r)$ and $\cos^2\theta(r)$. The average of $S(r)$, denoted S^f, represents the average of the fast lipid fluctuations which occur in 10^{-9} sec. The average of $(3\cos^2\theta(r)-1)/2$, denoted S^s, represents the order parameter of the spatial fluctuations of the mean axes. If one takes into account that lipid molecules undergo exchange between protein molecules via lateral diffusion, S^s can also be considered as the order parameter of temporal fluctuations, and since a typical time for lipid exchange is 10^{-5} sec, S^s represents the order parameter of slow lipid fluctuations. The total order parameter is given by a product,

$$S^{tot} = S^f \cdot S^s. \qquad (3a)$$

Proteins increase S^f and decrease S^s so that S^{tot} may remain constant due to a compensation of immobilization and tilt.

In ESR and FA, the time for a measuring process is of the order of 10^{-8} sec, therefore only the fast fluctuations are detected: S^f is obtained which increases due to protein. In DMR, on the other hand, the time for a measuring process is of the order of 10^{-4} sec, therefore all fluctuations are detected: S^{tot} is obtained which may remain constant or decrease slightly.

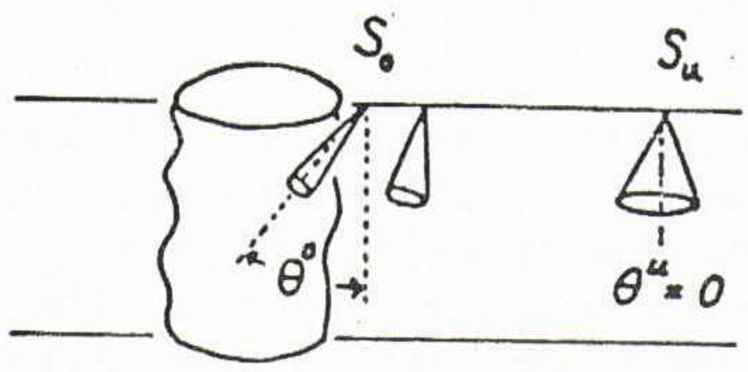

FIGURE 3

Schematic model for lipid-protein interaction. The cones symbolize the preferred axes of orientation and the extent of lipid orientational fluctuations. S_u and θ_u denote the order parameter and tilt angle of unperturbed lipids, S_o and θ_o those at the protein surface.

Although the above model for lipid-protein interaction offers a simple explanation for the different results obtained with different experimental techniques, one would like to measure the order parameters S^f and S^s directly by one and the same technique, e.g. FA. This is possible, if one works with oriented membranes instead of a dispersion of membranes. In this case, the orientation of the preferred axes is also observable. For a moment let us assume that fast fluctuations would not exist. If the fluorophores are oriented parallel to the membrane normal and the membrane normal is parallel to the polarization of the incoming light (Fig. 4), the anisotropy is maximal. It decreases if the membrane normal is rotated towards the direction of the incoming light. At an angle $\alpha = 45^o$ between the membrane normal and the initial polarization, the anisotropy becomes zero, because the orientation of the fluorophores is symmetric with respect to the parallel and perpendicularly polarized fluorescence light. However, if this symmetry is broken by the existence of a tilt of the preferred axes, the anisotropy at $\alpha = 45^o$ is finite. Thus, the angular dependence of the anisotropy is sensitive to the orientation of the preferred axes. This behavior is preserved, if fast fluctuations are present.

In the case of oriented membranes, information on the fluorophore orientation is contained in r_∞ as well as in r_o and, furthermore, is not restricted to the usual order parameter $S = \langle P_2 \rangle$, but includes the higher order parameter $\langle P_4 \rangle$. If fast and slow fluctuations exist, the total higher order parameter is also given by a product,

$$\langle P_4 \rangle^{tot} = \langle P_4 \rangle^f \cdot \langle P_4 \rangle^s, \qquad (3b)$$

in analogy to eq. (3a) for $\langle P_2 \rangle$. A detailed calculation shows that the angular dependence of r_∞ is governed by $\langle P_2 \rangle^f$, $\langle P_2 \rangle^s$, and $\langle P_4 \rangle^s$, whereas that of r_o is determined by $\langle P_2 \rangle^{tot}$ and $\langle P_4 \rangle^{tot}$. Hence, all four order parameters can be obtained from the angular dependencies of r_∞ and r_o.

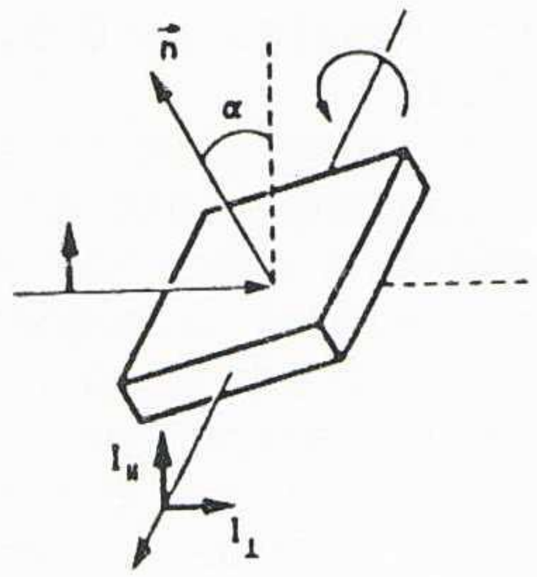

FIGURE 4

Geometry of FA measurements on oriented membranes.

We have performed steady-state FA measurements with DPH in oriented membranes of DMPC containing melittin, a polypeptide of 26 amino acid residues (6). With increasing melittin concentration the anisotropy at $\alpha = 45^{o}$ was found to increase which indicates an increasing tilt of the preferred axes of lipid orientation. For the quantitative analysis we took the relaxation time from time-resolved measurements on dispersed membranes and fitted the angular dependence of the steady-state anisotropy

$$r_s(\alpha) = \frac{r_o(\alpha) - r_\infty(\alpha)}{1 + \tau/\phi} + r_\infty(\alpha) \qquad (4)$$

with the four order parameters as the open variables. Since $\langle P_4 \rangle^f$ appears only in r_o, it could not be determined with sufficient accuracy. The results for S^f, S^s, and the product S^{tot} are shown in Fig. 5. Upon addition of melittin S^f increases indicating partial immobilization of lipids, whereas S^s decreases indicating partial tilting. Both effects compensate in S^{tot} which, therefore, remains constant. These results represent a clear confirmation of the above model for lipid-protein interaction.

The analysis of the data on oriented membranes yields further information. Since for the slow fluctuations the two order parameters $\langle P_2 \rangle^f$ and $\langle P_4 \rangle^s$ are known, an approximate distribution function can be derived. Equivalently, by using the special distribution of eq.(2b) the characteristic parameters θ_o and λ may be determined. The result is $\theta_o = 30^{o}$ and $\lambda = 15\text{Å}$. This means that at the protein surface the lipids are tilted by 30^{o} and this perturbation falls off with a coherence length of 15Å, corresponding to about three rings of lipid chains. The coherence length ξ of the fast fluctuations, at the moment, can only be estimated and turns out to be of the same order of magnitude as λ (18). Using $\xi = 15\text{Å}$,

$\langle P_2 \rangle^f$ can be evaluated for S_o which results as 0.5. This value is higher than the unperturbed order parameter $S_u = 0.36$.

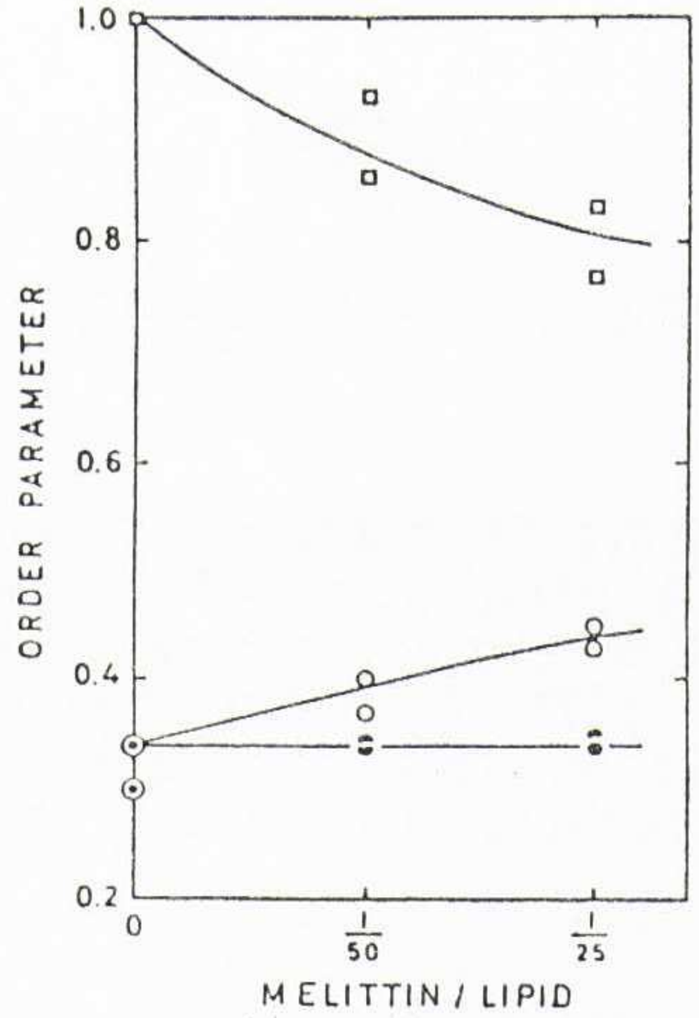

FIGURE 5

The fast, slow, and total order parameters S^f(o), S^s(□), and S^{tot}(●) of DPH as a function of the molar ratio melittin/DMPC at 35°C.

These results were derived for melittin, but the analysis of published data for other proteins shows that the finding $S_o = 0.5$ seems to be of more general validity (15). Hence, the immobilization of lipids by proteins due to their rigidity may be a universal feature. Concerning the protein-induced tilt of lipids the situation is unclear. Melittin spans the bilayer in the form of a bent α-helix with the helical segments being oriented at angles of about 30° relative to the membrane normal (19). Therefore, it is not astonishing that the lipids are tilted by 30°. Whether non-tilted α-helices also induce a tilt of the lipids, simply due to their uneven surface, remains to be investigated.

Protein Fluctuations

The bilayer-spanning segments of many proteins adopt an α-helical conformation (20). Therefore, we started out to study the possible fluctuations of simple α-helices in membranes. One example for such a helix is melittin which forms a bent α-helix of 20 residues and a random C terminal segment of 6 residues. The helix is hydrophilic along one side and hydrophobic along the opposite side. Therefore, several helices, actually four, aggregate in such a way that the hydrophobic sides are in contact with the lipids and the hydrophilic sides face each other to avoid this contact (21). Evidently, such a structure represents a pore through the membrane, as illustrated in Fig. 6. In this case, fluctuations of the helices might be correlated with the opening and closing of the pore. Further examples of simple α-helices are two synthetic polypeptides of the following amino acid sequences (22).

$(AlaVal)_5Trp(AlaVal)_4AlaGluAla$

$Trp(AlaVal)_4AlaGluAla.$

The longer one denoted P22 consists of 22 residues, enough to span the lipid bilayer in α-helix conformation. Except for Glu21 at the C terminus the residues are hydrophobic, and a strong tendency for aggregation in the membrane should not exist. Thus, P22 is expected to exist in membranes as α-helical, bilayer-spanning monomers (Fig. 5). The shorter polypeptide denoted P12 has only 12 residues and should exist in membranes as an α-helical, monolayer-spanning monomer (Fig. 5).

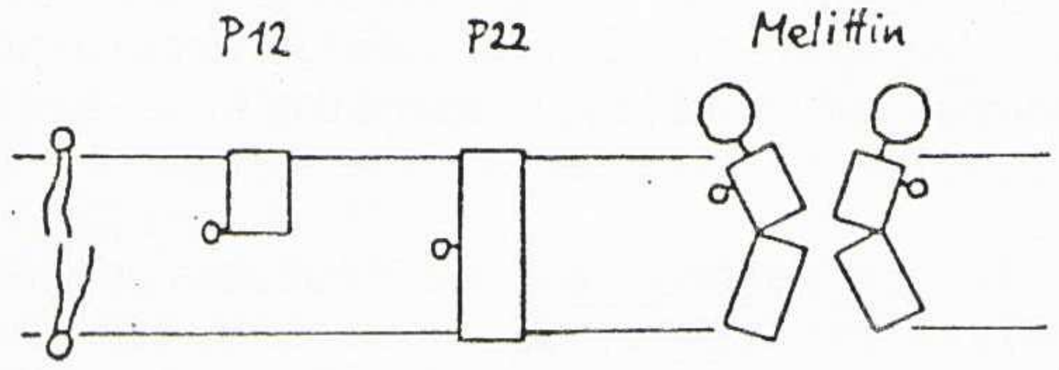

FIGURE 6

Schematic representation of three polypeptides in membranes: P12 consisting of 12 amino acid residues, P22 consisting of 22 residues, and melittin consisting of 26 residues and aggregated in the form of tetramers. The small spheres represent Trp residues.

Our aim was to detect the fluctuations of these different α-helices by FA measurements on the Trp residues. The synthetic polypeptides have a Trp residue approximately in the middle of the bilayer, and melittin has a Trp at position 19 which is located approximately at the height of the glycerol backbone of the lipids (23).

Time-resolved FA measurements on melittin in fluid membranes of DMPC yielded a relaxation time of 3 nsec and an order parameter $S \approx 0.75$. Up to now, the synthetic polypeptides have been studied only by steady-state FA measurements. If the steady-state anisotropy is evaluated for an order parameter assuming a relaxation time of 3 nsec, one obtains $S \approx 0.5$ for P12 and $S \approx 0.9$ for P22. This means that the monolayer-spanning polypeptide P12 fluctuates essentially like a lipid molecule, whereas the bilayer-spanning polypeptide P22 does not fluctuate considerably. The order parameter for melittin lies in between, i.e. the melittin helix undergoes some fluctuations. In analogy to lipids, one may envisage two kinds of fluctuations: rigid-body fluctuations and conformational fluctuations. Compared to the bilayer-spanning α-helix of P22 rigid-body fluctuations may be facilitated in the aggregated state of melittin. Conformational fluctuations may occur in the melittin helices due to the bend in the middle of the helix. At present, we cannot decide between these two possibilities for fluctuations of melittin. What these results seem to indicate, however, is that the observed fluctuations are fluctuations of the helices and not of the Trp side chains relative to the helices. In the latter case, the order parameters measured for the three polypeptides would not differ so much. Furthermore, fluctuations of the Trp side chains are expected to relax in 100 psec (24) whereas the melittin fluctuations occurred in the range of nsec (25). The observation on melittin that aggregated helices undergo orientational fluctuations may be relevant for more complex membrane proteins.

Whether or not slower fluctuations of helices in membranes occur is difficult to decide with FA on Trp residues because of the short life time of the Trp fluorescence. For this reason we employed an extrinsic fluorescence probe, namely pyrene which has a fluorescence life time component of about 100 nsec. Pyrene has not yet been attached to one of the above polypeptides, but to lactose permease of E.coli. The site of attachment is Cys148 which according to structural models of this protein is a member of a bilayer-spanning α-helical segment (26), and according to quenching studies is located at the height of the C5 carbon atoms of the lipid chains (27).

Time-resolved FA studies on pyrene-labeled lactose permease in fluid membranes of DMPC revealed two relaxation times, $\phi_1 = 9$ nsec and $\phi_2 = 40$ nsec (28). The amplitudes b of the two relaxation processes are of comparable magnitude, but they differ in their temperature dependence across the lipid phase transition. Whereas b_1 remains constant, b_2 becomes smaller by a factor of 2 below the phase transition, i.e. these fluctuations are strongly restricted below the phase transition. This difference in the temperature dependence of the two relaxation amplitudes would be difficult to understand, if both relaxation processes were considered as fluctuations of the helix to which pyrene is attached. Therefore, one tends to attribute the fast relaxation to fluctuations of pyrene relative to the helix and the slow relaxation to fluctuations of the helix. Pyrene bound to Cys148 was found previously to be of limited accessibility from the lipid phase (27), so that the weak coupling between pyrene and lipid fluctuations would be conceivable. The fluctuations of the helix bearing the pyrene, on the other hand,

should be sensitive to the lipid fluctuations, because this helix is strongly hydrophobic on the side opposite to Cys148 and therefore should be in immediate contact with the lipids.

Such a coupling between protein and lipid fluctuations is of special interest in the case of lactose permease, because a strong coupling between protein activity and lipid fluctuations has been known for a long time - the transport activity of lactose permease decreases drastically below the lipid phase transition (29). Presumably, this coupling between protein activity and lipid fluctuations is mediated by protein fluctuations: protein activity requires a conformational change; a conformational change occurs on the basis of small and fast conformational fluctuations of the protein, and these are only possible if the surrounding lipids also fluctuate (30). This way of understanding the functioning of proteins in terms of conformational fluctuations has been introduced for soluble proteins, but should apply equally well for membrane proteins. Up to now, only very few studies, however, have been reported on conformational fluctuations of membrane proteins (31) and further investigations are urgently required.

REFERENCES

1 - HEYN M.P. FEBS Lett. 108:359,1979.
2 - JAHNIG F. Proc. Natl. Acad. Sci. USA 76:6361,1979.
3 - CHAPOY L.L. and DUPRE D.B. J. Chem. Phys. 69:519,1978.
4 - SZABO A. J. Chem. Phys. 72:4620,1980.
5 - VAN DER MEER B.W., KOOYMAN R.P.H. and LEVINE Y.K. Chem. Phys. 66:39,1982.
6 - VOGEL H. and JAHNIG F. Proc. Natl. Acad. Sci. USA. 82:0000,1985.
7 - KINOSHITA K., KAWATO S. and IKEGAMI A. Biophys. J. 20:289,1977.
8 - ZANNONI C. Mol. Phys. 42:1303,1981.
9 - VAN DER MEER B.W., POTTEL H., HERREMAN W., AMELOOT M., HENDRICKX H. and SCHRODER H. Biophys. J. 46:515,1984.
10 - SEELIG A. and SEELIG J. Biochemistry 13:4839,1974.
11 - BROWN M.F., SEELIG J. and HÄBERLEN U. J. Chem. Phys. 70:5045,1979.
12 - VAN BLITTERSWIJK W.J., VAN HOEVEN R.P. and VAN DER MEER B.W. Biochim. Biophys. Acta. 644:323,1981.
13 - MEMMING R. Z. Phys. Chem. 28:168,1961.
14 - KNOWLES P.F., WATTS A. and MARSH D. Biochemistry 18:4480,1979.
15 - JAHNIG F., VOGEL H. and BEST L.Biochemistry 21:6790,1982.
16 - SEELIG A. and SEELIG J. and HOPPE SEYLER'S Z. Physiol. Chem. 359:1747,1978.
17 - OLDFIELD E., GILMORE R., GLASER M., GUTOWSKY H.S., HSHUNG J.C., KANG S.Y., KING T.E., MEADOWS M. and RICE D. Proc. Natl. Acad. Sci. USA 75:4657,1978.
18 - JAHNIG F. Biophys. J. 36:329,1981.
19 - VOGEL H., JAHNIG F., HOFFMANN V. and STUMPEL J. Biochim. Biophys. Acta 733:201,1983.
20 - JAHNIG F. Proc. Natl. Acad. Sci. USA 80:3691,1983.
21 - VOGEL H. and JAHNIG F. - manuscript submitted
22 - HARTMAN P., VOGEL H. and JAHNIG F. - manuscript in preparation.
23 - VOGEL H. FEBS Lett 134:37,1981.
24 - ICHIYE T. and KARPLUS M. Biochemistry 22:2884,1983.
25 - GEORGHIOU S., THOMPSON M. and MUKHOPADHYAY A.K. Biochim. Biophys. Acta

642:429,1981.
26 - WRIGHT J.K., DORNMAIR K., MITAKU S., MOROY T., NEUHAUS J.M., SECKLER R., VOGEL H., WEIGEL U., JÄHNIG F. and OVERATH P. Ann. N.Y. Acad. Sci. 456:326, 1985.
27 - MITAKU S., WRIGHT J.K., BEST L. and JÄHNIG F. Biochim. Biophys. Acta 776: 247,1984.
28 - DORNMAIR K. and JÄHNIG F. - manuscript in preparation.
29 - WRIGHT J.K., RIEDE I. and OVERATH P. Biochemistry 20:6404,1981.
30 - BRAMHALL J. and JÄHNIG F. Biochim. Biophys. Acta 690:310,1982.
31 - KAWATO S., YOSHIDA S., ORII Y., IKEGAMI A. and KINOSHITA K. Biochim. Biophys. Acta 634:85,1981.
32 - WU E.S., JACOBSON K. and PAPAHADJOPOULOS D. Biochemistry 17:3936,1977.