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LATERAL DIFFUSION OF GRAMICIDIN S, M-13 COAT PROTEIN AND GLYCOPHORIN IN BILAYERS OF SATURATED PHOSPHOLIPIDS

MEAN FIELD AND MONTE CARLO STUDIES

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We have developed a general model that relates the lateral diffusion coefficient of one isolated large intrinsic molecule (mol. wt. $\gtrsim$1000) in a phosphatidylcholine bilayer to the static lipid hydrocarbon chain order. We have studied how protein lateral diffusion can depend upon protein-lipid interactions but have not investigated possible non-specific contributions from gel-state lattice defects. The model has been used in Monte Carlo simulations or in mean-field approximations to study the lateral diffusion coefficients of Gramicidin S, the M-13 coat protein and glycophorin in dimyristoyl- and dipalmitoylphosphatidylcholine (DMPC and DPPC) bilayers as functions of temperature. Our calculated lateral diffusion coefficients for Gramicidin S and the M-13 coat protein are in good agreement with what has been observed and suggest that Gramicidin S is in a dimeric form in DMPC bilayers. In the case of glycophorin we find that the 'ice breaker' effect can be understood as a consequence of perturbation of the lipid polar region around the protein. In order to understand this effect is is necessary that the protein hydrophilic section perturb the polar regions of at least approx. 24 lipid molecules, in good agreement with the numbers of 29–30 measured using ^{31}P-NMR. Because of lipid-lipid interactions this effect extends itself out to four or five lipid layers away from the protein so that the hydrocarbon chains of between approx. 74 and approx. 108 lipid molecules are more disordered in the gel phase, so contributing less to the transition enthalpy, in agreement with the numbers of 80–100 deduced from differential scanning calorimetry (DSC). An understanding of the abrupt change in the diffusion coefficient at a temperature below the main bilayer transition temperature requires an additional mechanism. We propose that this change may be a consequence of a 'coupling-uncoupling' transition involving the protein hydrophilic section and the lipid polar regions, which may be triggered by the lipid bilayer pretransition. Our calculation of the average number of *gauche* bonds per lipid chain as a function of temperature and distance away from an isolated polypeptide or integral protein shows the extent of statically disordered lipid around such molecules. The range of this disorder depends upon temperature, particularly near the main transition.

Introduction

A study of the translational and rotational diffusion of integral proteins in biological mem-

Abbreviations. DMPC, dimyristoylphosphatidylcholine, DPPC, dipalmitoylphosphatidylcholine

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branes is of importance in understanding the properties of such membranes. Most of the work of the last decade has been concerned with rotational diffusion [1], but recent years have seen an increase in studies of translational, or lateral, diffusion in reconstituted membanes [2–8] using the FRAP technique [9]. The dependence of the protein lateral diffusion coefficient, D_L, upon temperature can give information not only about properties of the lipid bilayer in which the protein is embedded, but also about the effects of the protein upon the bilayer. In the case of sufficiently high protein concentration information can also be obtained about direct protein-protein interactions. It is important, therefore, to construct theoretical models with which protein lateral diffusion measurements can be analyzed.

Our intention here is to construct a plausible, fairly general, model of the lateral diffusion of intrinsic molecules at very low concentrations in both the gel and fluid phases of a lipid bilayer in order to obtain an expression for D_L in terms of the properties of the protein and the state of the lipids. The concentration is assumed to be sufficiently low such that the diffusing objects are independent of each other. Of necessity we shall obtain a model with a number of parameters related to protein-lipid interactions. We shall eliminate as many of these as possible by using results of other experiments. We shall then study the lateral diffusion of Gramicidin S and the M-13 coat protein in reconstituted systems as a function of temperature. By contrasting these with the case of glycophorin, we shall study under what conditions a so-called 'ice breaker' effect might occur.

The calculation by Saffman and Delbruck [10,11], where an expression for D_L is related to the dimensions of an isolated cylindrical object moving in a thin sheet of viscosity μ, is not suited to the study undertaken here for two reasons. Bilayer viscosity changes when the temperature T falls below the main phase transition temperature T_c so that it is not possible to predict values of D_L for different temperatures unless one has a theory of the temperature dependence of μ. Possibly more important, however, is the assumption contained in Ref. 10 that μ is a constant everywhere in the thin sheet. There is evidence that the hydrophobic surface of an integral protein tends to induce static disorder in lipid hydrocarbon chains adjacent to it in the sense that they appear to be as disordered as chains in a pure fluid phase at the same temperature [12]. Although we would not suggest that bilayer viscosity depends only, if at all, upon hydrocarbon chain static order, it would seem rash to assume that it was independent of whether the chains were 'melted' or not. The possibility thus arises that for temperatures less than T_c the lipid viscosity μ is different close to a protein compared to its value far from the protein so that the expression for D_L in Ref. 11 does not necessarily hold.

In the next section we shall outline the model of the lipid bilayer which we shall use and describe how we shall include proteins within its framework. We shall then describe how this model can be adapted to Monte Carlo techniques and finally develop a description of protein lateral diffusion. We shall eliminate or evaluate a number of the parameters appearing in the model and present results for D_L calculated using both a Monte Carlo simulation and a mean-field approximation for the case of an isolated protein. We shall then compare our calculations to measurements performed on the M-13 phage coat protein [5], Gramicidin S [13], and glycophorin [8] in order to study in what way glycophorin could differ from the first two in its interaction with phospholipids. In the case of glycophorin we shall propose that competing effects may have to be considered in order to understand the temperature dependence of D_L in the lipid gel phase.

Theory

(a) General model

We shall make use of a model of interacting hydrocarbon chains which has been used before [14–16]. Each chain can exist in one of ten states: The all-*trans* ground state, g, possessing internal energy $E_g = 0$ and cross-sectional area $A_g = 20.4$ Å^2; eight 'intermediate' states 2,...,9, describing chain conformations which are approximately planar such as kinks or jogs near the ends of the chain. These states have internal energies E_n, areas A_n and degeneracies (the number of such states) D_n and can be explicitly enumerated. The philosophy of selecting them is that planar conformations may be relatively easy to excite when $T < T_c$; one

high-energy 'melted' state, e, with energy E_e, area $A_e \simeq 34$ Å^2 and degeneracy D_e very much larger than the degeneracies of the other states. This state dominates the system when $T > T_c$ in a pure bilayer. The effect of the water interactions which bring the bilayer into existence is included as an effective lateral pressure, Π_0, acting on the hydrocarbon chains. The Hamiltonian operator describing the interaction and internal energies of a pure lipid system is

$$\mathcal{H}_L = -\frac{J^M}{2} \sum_{<ij>} \sum_{nm} I_n I_m \mathcal{L}_{in} \mathcal{L}_{jm} + \sum_i \sum_n (E_n + \Pi_0 A_n) \mathcal{L}_{in} \quad (1)$$

Here J^M is the interaction strength between two nearest neighbour saturated all-*trans* chains each containing M carbon nuclei per chain, $I_n I_m$ describes the quadrupole-quadrupole interaction between two nearest neighbour chains, labelled i and j, in states n and m, respectively, and $\mathcal{L}_{in}$ is a projection operator for chain i in state n. The sum $<ij>$ denotes nearest neighbour interactions only.

When a single integral protein is introduced into the bilayer it can have two effects: It can interact via its hydrophobic surface with those hydrocarbon chains which are adjacent to it, the strength of these interactions depending upon which state the chain is in; it can interact via its polar region with the water and lipid polar groups. This interaction may be a long range interaction. It may also disrupt hydrocarbon chain packing by creating lattice dislocations in the gel phase. However, the hydrophobic surface of some integral proteins appears to induce static disorder in lipid chains adjacent to it [12] and this might militate against the formation of lattice defects in an otherwise gel phase. Here we shall ignore the possible formation of such defects. We shall simplify these interactions as follows: We shall assume that the protein hydrophobic surface is sufficiently 'rough' so as to distinguish only between the 'melted' state, e, and the other nine more extended states, and that the interaction between the protein surface and a chain in state e is sufficiently weak to be approximately zero. The effect of the possible perturbation of the water region by the polar sections of the protein will be taken as affecting the lateral pressure acting on the hydrocarbon chains. The Hamiltonian for the interaction between the lipid molecules and an isolated protein is thus,

$$\mathcal{H}_{LP} = K \sum_{<i>} \sum_{n \neq e} \mathcal{L}_{in} + (\Pi_1 - \Pi_0) \sum_j{}' \sum_n A_n \mathcal{L}_{jn} \quad (2)$$

Here K is the interaction energy between the protein and the lipid chains in states g, 2,...,9, with $<i>$ denoting a sum over those lipids which are adjacent to the protein. Π_1 is the effective pressure acting on the chains within some distance from the protein, the sum Σ' denoting that it is over some set of lipids near to the protein. It is evident that Π_1 may depend upon the extent and conformation of the protein polar structure. The total Hamiltonian is $\mathcal{H} = \mathcal{H}_L + \mathcal{H}_{LP}$. All the parameters here are known from previous calculations and fits to experiment, with the exception of the protein-lipid interaction variables, K, Π_1, and the range of the lateral pressure change due to the protein polar structure. We shall see later that K can be determined from ^{2}H-NMR results, but that Π_1 and its range can only be deduced from a study of the behavior of D_L.

(b) Simplification for Monte Carlo studies

In order to perform a Monte Carlo simulation it is convenient to have a model with as few internal states as possible. As described above, each lipid chain has ten internal states. A simplication can be obtained, however, as described in Ref. 17: We are interested in processes taking place over the characteristic time scale of the melting process. Now the time scale for transitions among the lowest lying states is of the order of $10^{-10} - 10^{-11}$ s, while that between the low-lying states and the 'melted' state, e, is of the order of 10^{-6} s. If we are concerned with processes taking place on the latter time-scale, then we can perform a thermal average over the states g, 2,...,9 to obtain an effective temperature-dependent ground state, G. This averaging would be trivial if either the states g, 2,...,9 did not interact, or if the interaction

energies were all the same. An inspection of Eqn. 1 shows that neither is true. Accordingly, we shall represent the interactions between these states by mean fields and perform a thermal average over the states g, 2,...,9 in order to obtain an effective temperature-dependent ground state, G. This is an improvement over the averaging performed in Ref. 17. We thus obtain a total Hamiltonian which looks like a sum of Eqns. 1 and 2, as before, but with the following differences: The sum over n labelling the chain states runs only over $n = G$ and e, and the parameters associated with the effective ground state G are now thermal averages over the low-lying states.

The labels of chains, i and j now refer to lattice sites at which the hydrocarbon chains are localized and Monte Carlo simulations will be performed as introduced by Metropolis and described in Ref. 18. A single Monte Carlo run involves visiting each site once only, randomly, and calculating, whether the chain state will change. Because this can change approx. every 10^{-6} s, this enables us to associate a time of approx. 10^{-6} s with each Monte Carlo run. In the next sub-section we shall describe how lateral diffusion may be simulated.

(c) Models of protein lateral diffusion

In Appendix A we show that the protein lateral diffusion coefficient, D_L, can be written as

$$D_L = C_0 < \mathfrak{D}^2 > \tag{3}$$

where $\mathfrak{D}$ is determined by the particular mechanism that we select for diffusion, and $< \ldots >$ signifies a thermal average over the states of the bilayer. It seems reasonable to assume that the protein will diffuse only if there is a sufficiently large amount of sufficiently melted lipid around it. This means that $< \mathfrak{D}^2 >$ will be proportional to P_m, the probability that there is on the average, a sufficiently melted region of lipid around the protein, and we rewrite Eqn. 3 as

$$D_L = D_0 P_m \tag{4}$$

In a later sub-section we shall study various forms of P_m.

When we study protein lateral diffusion using the Monte Carlo simulation, we shall use Eqn. A1 of Appendix A. Since all sites will be occupied either by lipid chains or by proteins, we shall model lateral diffusion by allowing a protein to move a distance equal to one lattice constant in some time, Δt. The sites left vacant by the movement of the protein will be filled by the chains whose sites have been occupied by the protein as it moved. The details of the mechanism of diffusion, and the displacement of lipid chains will be described in the next section.

(d) Evaluation of K

We shall first evaluate the constant K of Eqn. 2. ^{2}H-NMR studies show that the C-^{2}H bond order parameters at different positions along the 2-chain of dimyristoylphosphatidylcholine (DMPC) increase somewhat or not at all, with increasing polypeptide or protein concentration at $T = 30°C$, as long as this concentration is not too high [12,19]. The polypeptide, Gramicidin A′, used does not apparently possess a polar group which could introduce long range effects in the polar region. The similarity between the ^{2}H-NMR results for Gramicidin A′ and the integral protein cytochrome oxidase, at low concentrations, seem to suggest a similar conclusion for the protein. For molecules such as this it seems reasonable to choose $\Pi_1 = \Pi_0$ when the protein concentration is low. Further evidence for this conclusion is contained in Ref. 20 where a quantitative analysis of the ^{2}H-NMR data [12,19] suggests that isolated molecules of Gramicidin A′ or cytochrome oxidase do not perturb the polar region significantly.

We have calculated the C-^{2}H bond order parameters along a 2-chain adjacent to single proteins of various sizes in DMPC bilayers, and found that a value of $K \simeq -0.2 \cdot 10^{-13}$ erg gives results in good general agreement with Refs. 12 and 19. All thermodynamic calculations were performed using a mean-field approximation which has been described elsewhere [14,15]. This value of K will be used for all integral proteins. This means that we are assuming that, to a first approximation, the hydrophobic surfaces of proteins in contact with lipid molecules of the bilayer are equally 'rough' because it is this 'roughness' that K describes.

When the protein possesses polar sections that are apparently much larger than the hydrophobic sections that penetrate the bilayer we must con-

sider the possibility that it may perturb the surrounding polar region. This could result in a modification of the interactions in the polar region of the lipids which would lead to a lateral pressure Π_1, different from Π_0 in the neighborhood of the protein.

(e) Criteria for lateral diffusion to occur

We shall not concern ourselves with the details of the mechanisms whereby a protein diffuses through the bilayer but, as stated before, will relate D_L to the probability, P_m, that (a) a sufficient number of lipid molecules around the protein are (b) sufficiently melted.

With regard to (b) we observe from earlier calculations that the states g (the all-*trans* state), 2 (states with jogs near the end of the chain) and 5 (states with single kinks) dominate below the main phase transition temperature, T_c [14]. Accordingly we shall take a 'sufficiently melted' chain to be one in any of the ten states except these three. Thus, for each chain, the probability of being sufficiently melted is:

$$P_m = 1 - p_g - p_2 - p_5 \tag{5}$$

With regard to (a) we shall divide all the chains into layers around the single protein which will be diffusing in the bilayer. A 'sufficient number' of lipid chains will be N_1, N_2, ... from the first, second, ... layers. We thus obtain, for use in Eqn. 4, that $P_m = p_m(N_1, N_2, \ldots, N_k, \ldots)$. When a mean field approximation is used this probability factors in the following way,

$$P_m = p_m(1)^{2N_1} p_m(2)^{2N_2} \ldots p_m(k)^{2N_k} \ldots \tag{6}$$

where $p_m(k)$ is the probability that a chain in the kth layer will be sufficiently melted. The exponents will be $2N_1$, $2N_2$, ... if the protein penetrates both halves of the bilayer. If it penetrates only one half then these exponents must be divided by two. In practice we shall find that only the first layer need be melted to give results in accord with observation, so that we would have $N_2 = N_3 = \ldots 0$.

When we perform a Monte Carlo simulation, since we have only two states, the effective ground state, G, and the excited state, e, we must choose the 'sufficiently melted' state to be e. In such a simulation we shall use the criterion that the protein will move to an adjacent site only if all of its nearest neighbours are melted. The direction that it moves in will be determined randomly.

It should be stressed that the criterion for lateral diffusion to occur is not related to the number of lipids which have some property, e.g. ^{31}P chemical shift anisotropy, changed due to their proximity to the protein. The latter are determined by the range of protein lipid interactions as well as correlation lengths in the bilayer. In the next section we shall deduce lower bounds for the number of phospholipids significantly influenced by a protein and make comparisons with experiment.

Results and Discussion

Proteins or polypeptides with small hydrophobic sections

We shall be concerned here initially with intrinsic molecules (proteins or polypeptides) having hydrophobic sections of molecular weight around 2000 if it spans the bilayer, or around 1000 if it penetrates only half of the bilayer. We shall calculate D_L for a single such molecule in a phospholipid bilayer. We shall compare our results to measurements made on very low concentrations of glycophorin [8] in DMPC. In the absence of data we shall assume that the diffusing unit of glycophorin is a monomer at very low concentrations, but the work of Van Zoelen et al. [21] and Yeagle and Romans [22] should be noted. Our general conclusions will not be invalidated if the diffusing unit of glycophorin is a dimer.

The case of glycophorin is particularly interesting because D_L appears to be essentially unchanged by the liquid-crystal to gel transition and remains high down to approx. 9°C below T_c in DMPC. At this temperature D_L decreases abruptly by about two orders of magnitude. We shall study under what conditions the first phenomenon, the so-called 'ice-breaker effect', can arise, and then try to propose a mechanism which might account for the abrupt change in D_L. We shall then study the case of Gramicidin S in DMPC [13] and attempt to discover whether the diffusing unit is a monomer or a multimer.

It must be noted that some studies of glyco-

phorin lateral diffusion in multibilayers of DMPC and egg phosphatidylcholine [23] using the FRAP technique have not detected the effects reported by Vaz et al. [8]. In the measurements of Wu et al. [23] the concentration of glycophorin is not reported, while Vaz et al. [8] used a lipid/glycophorin ratio of 4500:1. It is clear that in order to deduce a self-diffusion coefficient, the concentration should be sufficiently low to ensure that the diffusing units do not interact with each other.

It should also be stressed that we are studying the conditions under which the effects reported by Vaz et al. [8] can be due to short range protein-lipid interactions which depend upon the structure of glycophorin. Later on we shall remark briefly upon effects which are due to dislocations and similar defects introduced into a gel phase by the presence of an intrinsic molecule. Such effects would not, of course, be specific to glycophorin, but it has been pointed out that they cannot be excluded by our calculations (Sackmann, E., personal communication).

About nine lipid hydrocarbon chains can fit around the hydrophobic section of such an isolated intrinsic monomeric molecule described above, in each half of the bilayer (Appendix B). About fifteen chains can fit around this molecule as a second layer again in each half of the bilayer.

In the Monte Carlo simulation we allowed the hydrophobic section of the protein to occupy one site, and the six sites around it to be occupied by the chains of the first layer. Although the true number of such adjacent chains is about nine, instead of six as used here, in each half of the bilayer, we consider that the results obtained are qualitatively correct. We took the lipids to be dipalmitoylphosphatidylcholine (DPPC), chose $\Pi_0 = 30$ dyn/cm as before [15] and adjusted J[16] so that the first-order phase transition occurred at 41°C. We used triangular lattices of 900 or 400 sites with periodic boundary conditions. We allowed the lipids to come to thermal equilibrium over 500 Monte Carlo runs, and then allowed the protein to diffuse for 1500 runs and calculated the diffusion coefficient from it. The results are shown in Fig. 1. In all the Monte Carlo calculations the diffusion coefficient was arbitrarily set equal to $D_L = 2 \cdot 10^{-8}$ cm^2/s at 50°C.

Fig. 1A shows the choice of $K = -0.2 \cdot 10^{-13}$

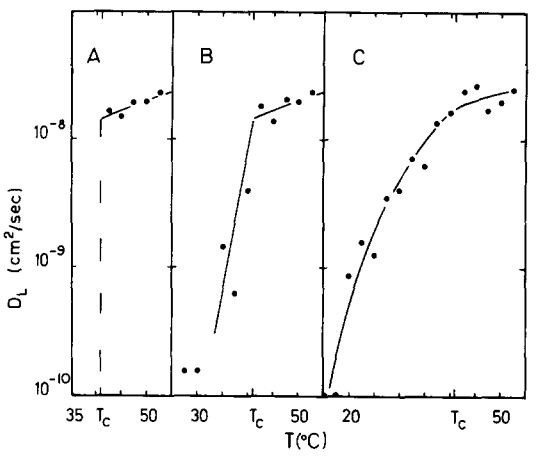

Fig 1 Calculated lateral diffusion coefficient of one small intrinsic molecule in a DPPC bilayer using Monte Carlo simulation (A) $K = -0.2\ 10^{-13}$ erg, $\Pi_1 = 30$ dyn/cm (B) $K = -0.2\ 10^{-13}$ erg, $\Pi_1 = 0$ (C) $K = 1.0\ 10^{-13}$ erg, $\Pi_1 = 30$ dyn/cm

and $\Pi_1 = 30$. This corresponds to a molecule which interacts with lipid chains like a protein hydrophobic surface but which does not possess a polar section large enough to perturb the lipids. We see that D_L falls off very slightly as T is decreased to T_c and then goes to zero. The value of zero simply means that D_L is less than 10^{-10} cm^2/s, the smallest value which we could calculate. This model should represent the behaviour of Gramicidin S but clearly does not give rise to the diffusion observed for low concentrations of glycophorin.

In order to mimic the effect of glycophorin we explored whether by changing Π_1 we could reproduce the 'ice breaker' effect. Fig. 1B shows the result of keeping $K = -0.2 \cdot 10^{-13}$ but choosing $\Pi_1 = 0$. This model represents a glycophorin molecule as having a hydrophobic section typical of any integral protein [12], but as having hydrophilic sections which directly affect the polar groups of those phospholipid molecules adjacent to it. The result of this latter interaction is assumed to 'reduce the packing' of the lipid hydrocarbon chains adjacent to the protein by reducing the effective lateral pressure from 30 dyn/cm to 0 dyn/cm. This choice of $\Pi_1 = 0$ is, of course, merely an approximation to what may be the effect of complicated dynamical interactions. Above T_c the results are essentially identical to the previous case.

For $T < T_c$, D_L is now large, but there is a definite change of slope at $T = T_c$. The reason for this is that in this model the protein directly affects only nearest neighbour sites. The interaction between the protein hydrophobic surface and the lipid chains in a bilayer is only a nearest neighbour interaction which we believe we have correctly described. The lack of success of this model in reproducing the observed 'ice breaker' effect of glycophorin must, therefore, lie in the restriction of Π_1 to influence only nearest neighbours: The polar group of glycophorin may be influencing the polar region of at least second nearest neighbour lipids.

We have not built this change into our model, but instead we chose $K = 1.0 \cdot 10^{-13}$ and $\Pi_1 = 30$ to mimic the strong polar layer effects which might be causing local disorder of the lipid hydrocarbon chains. Fig. 1C shows the results. We see that now we do have the 'ice breaker' effect [8] with D_L being only slightly changed at T_c. We have studied a number of Monte Carlo simulations in which the requirements for lateral diffusion were varied but we have found essentially the same result: The 'ice breaker' effect can be understood as a consequence of the hydrophilic section of the protein affecting the lipid polar region so that the local pressure acting on the chains is reduced and they remain 'fluid' (represented by our static disorder) for temperatures below T_c. This result is essentially in agreement with the observations of Brûlet and McConnell [24] where the 'ice breaker' effect was predicted.

In Fig. 2 we show some typical distributions of lipid chain states for $T < T_c$ in one half of a bilayer. Fig. 2A shows the case $K = -0.2 \cdot 10^{-13}$ erg and $\Pi_1 = 30$ dyn/cm, and we see that there is some static disorder adjacent to the protein, but not sufficient to allow diffusion characteristic of $T > T_c$. Fig. 2B shows $K = -0.2 \cdot 10^{-13}$ erg and $\Pi_1 = 0$. Although there is now more static disorder near the protein it is insufficient to prevent a significant decrease in D_L from the values for $T > T_c$. In Fig. 2C with $K = 1 \cdot 10^{-13}$ erg and $\Pi_1 = 30$ dyn/cm, however, the effect of 'fluidizing' only the chains adjacent to the protein is propagated, via the lipid-lipid interaction, over distances up to four or more layers away from the hydrophobic segment. This is one mechanism

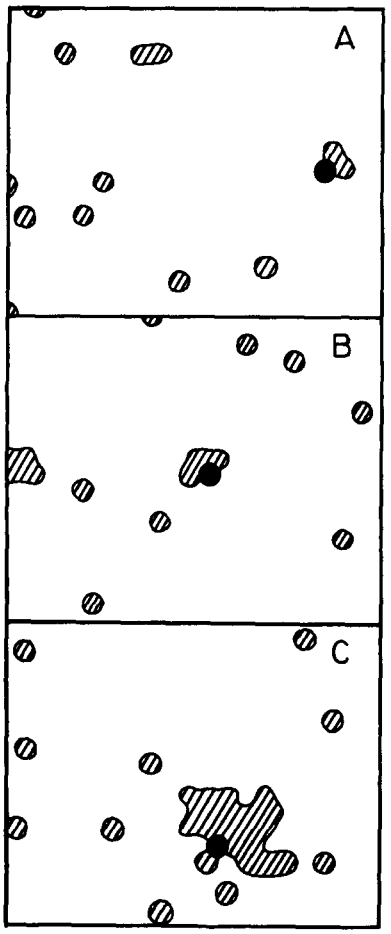

Fig. 2 Typical instantaneous distributions of sufficiently melted lipids (state e, cross-hatched areas) and gel-state lipids (state G, blank areas) on a portion of a triangular lattice from the Monte Carlo simulation of a small intrinsic molecule (filled circle) in a DPPC bilayer ($T_c = 41$°C) The temperature is 35°C (A) $K = -0.2 \cdot 10^{-13}$ erg, $\Pi_1 = 30$ dyn/cm (B) $K = -0.2 \cdot 10^{-13}$ erg, $\Pi_1 = 0$ (C) $K = 1.0\ 10^{-13}$ erg, $\Pi_1 = 30$ dyn/cm

whereby a value of D_L of the order of 10^{-8} cm^2/s can be obtained for $T < T_c$. It can be seen that it is not necessary that 'rigid' lipid molecules exchange sufficiently rapidly with those 'fluidized' by the protein. It is also unnecessary that domains of 'fluid' lipid should be connected in order to have a long 'fluid' path available to the protein. The protein creates its own region of fluidity as it moves and the diffusion limiting mechanism is the rate at which the protein can disorder the lipid chains as it approaches the boundary of the 'fluid' region which it has created. Our model is unable

to calculate this rate-limiting effect in a reliable way. However, bearing in mind that a value of $D_L = 10^{-8}$ cm^2/s implies that $(<r^2>)^{1/2} \simeq = 7$ Å during a time of approx. 10^{-7} s, we see that if a lipid chain can change from a 'rigid' to a 'sufficiently melted' conformation in approx. 10^{-7} s then a value of $D_L \simeq 10^{-8}$ cm^2/s for some $T < T_c$ is quite reasonable.

It can be seen, however, that the simulation does not show an abrupt change in D_L at some temperature below T_c, but that D_L decreases over a wide temperature range. In order to study the question of the origin of the abrupt decrease in D_L at $T \approx T_c - 9$°C for glycophorin in DMPC we considered an isolated protein surrounded by layers of DMPC chains and performed a mean field calculation. This is a much simpler calculation than performing Monte Carlo simulations. The mean fields, of course, depended upon how far the chain was from the protein. We studied six cases defined by the following conditions: (a) that the criterion for protein lateral diffusion to occur is that all nine chains in the first lipid layer around the protein in each half of the bilayer must be melted, and (b) that the perturbing effect of the protein's polar section extends out to either the second or the third lipid layer with the associated reduced pressure, Π_1, taken to be 0, 10 or 20 dyn/cm. In view of the molecular weight of glycophorin (approx. 31000) it seems unlikely that its hydrophilic section could cause a significant perturbation further away than the third lipid layer. These two sets of conditions are quite independent. Set (a) is simply what appear to be reasonable assumptions about under what conditions lateral diffusion can occur. Set (b) concerning the range of the perturbation of the hydrophilic section of glycophorin follows from our finding, in the Monte Carlo simulations, that in order to understand the high value of D_L for $T < T_c$ as a consequence of specific interactions between glycophorin and phospholipids (as distinct from nonspecific effects such as the formation of dislocations and similar defects as mentioned above), we must consider polar group perturbations which extend beyond those phospholipids adjacent to the glycophorin hydrophobic section. We thus chose (see Eqn. 6) (a) $N_1 = 9$ with all other $N_k = 0$, and (b) $\Pi_1 = 0$, $\Pi_1 = 10$ or $\Pi_1 = 20$ for either the first two layers of lipid, or the first three such layers, away from the isolated protein. The value of D_0 (Eqn. 4) was chosen so that $D_L = 2.0 \cdot 10^{-8}$ cm^2/s at $T = 40$°C, and the temperature dependence of D_L is shown in Fig. 3. Fig. 3A is for the protein polar section perturbing two layers of lipids, while Fig. 3B shows the effect if this perturbation extends to three layers.

The first result to note is that this mean field model gives results essentially like that of the Monte Carlo simulation of Fig. 1C. This is an important check which allows us to proceed with using the mean field approximation. The second result is that it is not possible to decide between whether the protein affects the lipid polar region for two layers or for three. The third result is that in no case do we see an abrupt change in D_L below T_c. Even in the most likely cases (curves c) D_L decreases from approx. $6 \cdot 10^{-9}$ to approx. 10^{-11} cm^2/s over a range of approx. 8°C which is far larger than the range of 1.5°C reported [8].

We thus see that by some mechanism the hydrophilic section of glycophorin interacts with (this does not imply a bonding in any sense of the term) the lipid polar groups which lie within a certain distance of the protein, and so perturbs them, thereby reducing the effective lateral pressure on

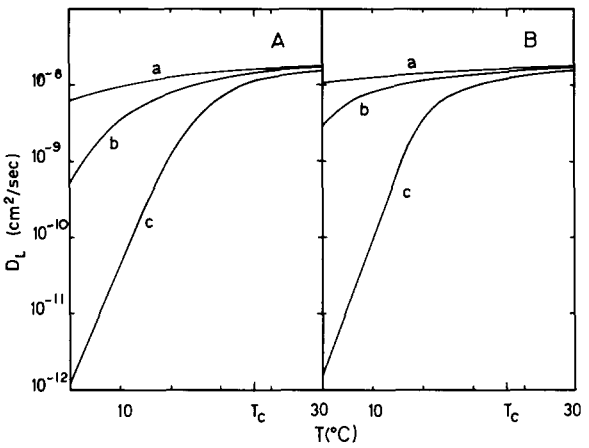

Fig 3. Calculated lateral diffusion coefficient of one glycophorin molecule in a DMPC bilayer using the model described in the text with a mean field approximation For all cases $K = -0.2 \cdot 10^{-13}$ erg, and $N_1 = 9$ Glycophorin polar section affects two lipid layers around the protein (A), and three lipid layers (B) In both cases: curve a, $\Pi_1 = 0$; curve b, $\Pi_1 = 10$ dyn/cm; curve c, $\Pi_1 = 20$ dyn/cm

their hydrocarbon chains. This is a mechanism whereby the 'ice breaker' effect can be understood.

If the abrupt decrease in D_L at $T \simeq T_c - 9°C$ in DMPC is due to specific glycophorin-DMPC interactions rather than a process taking place in the bilayer and independent of the protein, then we are led to make the following tentative proposal: When some of the lipid hydrocarbon chains, which are perturbed by the polar interactions referred to above, become sufficiently gel-like below T_c, this coupling is broken at a temperature which we denote by T_1, and the perturbation is reduced so that the effective lateral pressure on these chains, Π_1, becomes equal to Π_0 (the lateral pressure on those chains which are outside the range of the protein's hydrophilic section).

As the criterion for becoming 'sufficiently gel-like', we required that when the probability of a chain in the second or third layer to be sufficiently melted, $p_m(2)$ or $p_m(3)$, became small enough, then the coupling would be broken. In the case of the protein affecting two (three) layers we used $p_m(2)$ ($p_m(3)$) and we denoted this lower limit by $p_m(\text{lim})$. Not surprisingly, we found that $p_m(\text{lim})$ depended upon the value of Π_1. For the case that the protein perturbed two layers we found values of 0.89 ($\Pi_1 = 0$), 0.80 ($\Pi_1 = 10$) and 0.56 ($\Pi_1 = 20$), while for the case that the perturbation extended to three layers we obtained 0.89 ($\Pi_1 = 0$), 0.80 ($\Pi_1 = 10$) and 0.49 ($\Pi_1 = 20$). Later, we shall compare our results to measurements on DMPC and DPPC bilayers containing glycophorin using Raman and infrared spectroscopy and differential scanning calorimetry. There we shall see that the evidence is strong that the abrupt change in D_L is correlated with the lipid pretransition. This does not mean that our mechanism is wrong, but only that it is triggered in a way different to the one which we have proposed. We will, however, pursue this triggering mechanism in order to discover its predictions.

Fig. 4 shows these results for the three cases plotted in Fig. 3A. The results for the cases of Fig. 3B are similar and we are thus not able to decide between whether the protein affects the polar regions of two lipid layers or of three.

Fig. 5 shows our prediction for glycophorin in DPPC. Here we have chosen the case of two layers being affected though the choice of three layers

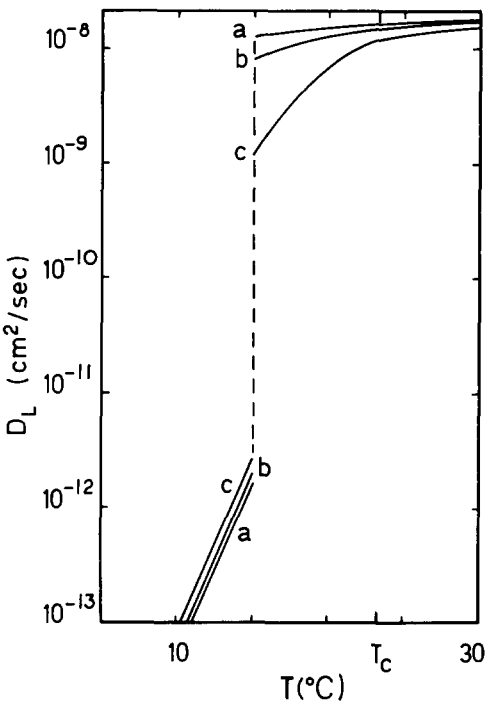

Fig 4 Calculated lateral diffusion coefficient of one glycophorin molecule in a DMPC bilayer using a mean field approximation with the protein polar section 'uncoupling' mechanism adjusted to operate at 15°C $K = -0.2\ 10^{-13}$ erg, and $N_1 = 9$, with the protein polar section affecting two lipid layers The values of Π_1 and $p_m(\text{lim})$ are curve a, 0 and 0 89, curve b, 10 dyn/cm and 0 80, curve c, 20 dyn/cm and 0 56

will give essentially the same results. Our calculation, which now has no free parameters, predicts that the dependence of D_L upon temperature for glycophorin in DPPC should look like that in DMPC but shifted upwards in temperature by an amount $T_c(\text{DPPC}) - T_c(\text{DMPC})$ (here 17.6°C). For this reason we have not performed calculations analogous to those of Fig. 4.

We should now be able to calculate the temperature-dependence of D_L for a variety of other proteins given their size (in order to estimate N_1) and by fitting the value of D_0 to one experimental point. Wu et al. [13] measured D_L for Gramicidin S in DMPC. The monomer has a molecular weight of 1141, and its dimensions are such that it penetrates one half of a DMPC bilayer [13]. Accordingly about nine chains can fit around one isolated such molecule. Fig. 6A shows the results of calculations by choosing D_0 so that $D_L = 2.2 \cdot 10^{-8}$/s at 40°C in approximate agreement with

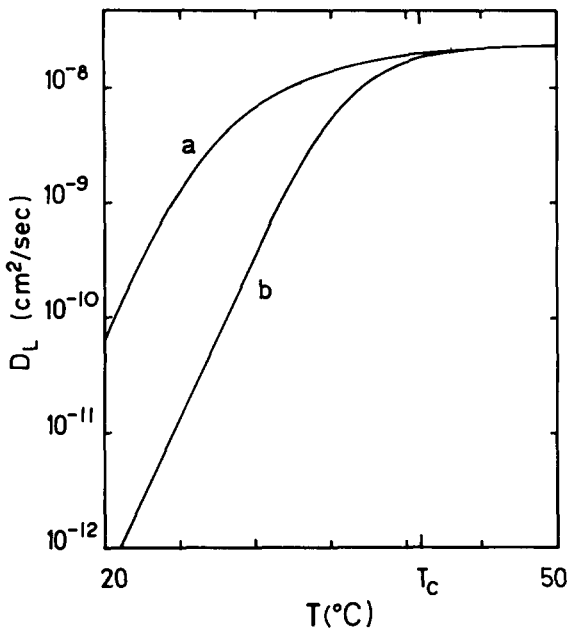

Fig 5 Calculated lateral diffusion coefficient of one glycophorin molecule in a DPPC bilayer using a mean field approximation with no 'uncoupling' mechanism operating $K = -0.2 \cdot 10^{-13}$ erg, and $N_1 = 9$, with the protein polar section affecting two lipid layers Curve a, $\Pi_1 = 10$ dyn/cm, curve b, $\Pi_1 = 20$ dyn/cm

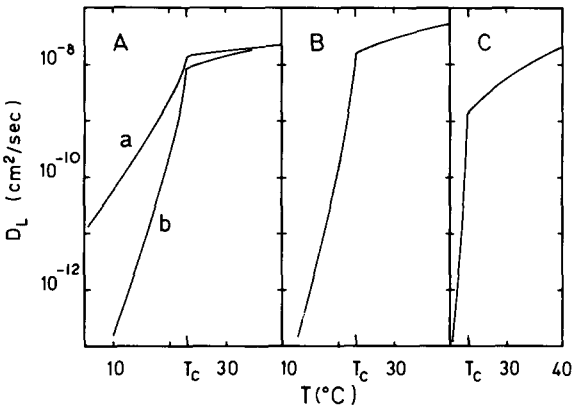

Fig 6 Calculated lateral diffusion coefficients of three intrinsic molecules in a DMPC bilayer with only one such molecule present $K = -0.2 \cdot 10^{-13}$, $\Pi_1 = 30$ dyn/cm (no protein perturbation of the lipid polar layer occurs) (A) Gramicidin S $N_1 = 9$ Curve a, D_L for monomers with $D_0 = 4.7 \cdot 10^{-8}$, curve b, D_L for dimers with $D_0 = 1.0 \cdot 10^{-7}$. This case gives values closer to those observed [13] (B) M-13 phage coat protein $N_1 = 11.6$, $D_0 = 3.7 \cdot 10^{-7}$ (C) Hypothetical bilayer-spanning protein of molecular weight 40000. $N_1 = 26$, $D_0 = 1.6 \cdot 10^{-6}$

Ref. 13. Curve a is based on the assumption that Gramicidin S is a monomer in the bilayer while curve b is the result obtained if it assumes a dimeric form in the bilayer. A comparison with Fig. 2A of Ref. 13 seems to suggest the latter possibility: At 20°C, for example, the measurements give $D_L \approx 10^{-10}$ cm²/s, while the calculation gives $2.3 \cdot 10^{-10}$ cm²/s.

Encouraged by the results above we considered the case of the M-13 phage coat protein (molecular weight 5260) in DMPC [5]. We calculated that approximately 11–12 hydrocarbon chains in each half bilayer could fit around one isolated such molecule. Choosing D_0 so that we obtain $D_L \simeq 5.2 \cdot 10^{-8}$ cm²/s at 40°C in rough agreement with [5] we see from Fig. 6B that at 25°C D_L has fallen to $2 \cdot 10^{-8}$ cm²/s which is somewhat lower than $3 \cdot 10^{-8}$ cm²/s as measured. We are not aware of any measurements performed for $T < T_c$. Our calculation predicts that D_L should fall to approx. 10^{-10} cm²/s at 20°C and approx. 10^{-12} cm²/s at 15°C. We shall, however, raise objections in the conclusion to taking our calculated values too seriously at very low temperatures.

Fig. 6C shows the calculation of D_L for a hypothetical protein of molecular weight 40000 with D_L fitted to $2 \cdot 10^{-8}$ cm²/s at $T = 40$°C. In this case approx. 26 lipid chains can fit around one isolated protein in each half of the bilayer, we assume that it spans the bilayer, and that it does not have a polar group to induce an 'ice breaker' effect.

Fig. 7 shows the average number of *gauche* bonds per lipid chain, $< n_g >$, as a function of layer away from the surface of glycophorin in DMPC (A) and DPPC (B), for four temperatures where we assumed that the protein hydrophilic section affected two lipid layers. In both cases (curves c) we see that the 'ice breaker' effect extends to about the fourth layer just below T_c, this being a consequence of the increase in correlation length near phase transitions. For temperatures above T_c the 'ice breaker' effect is restricted to the two directly affected layers. Note also that at sufficiently low temperatures below T_c (curves d) the chains adjacent to the protein are significantly disordered statically. In our model this results not from affects of the protein hydrophilic section, but from the 'roughness' of the protein hydrophobic

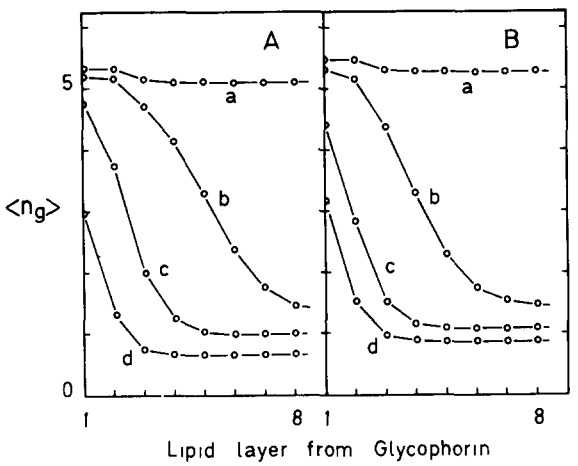

Fig 7 Average numner of *gauche* bonds per lipid chain as a function of temperature and distance, in lipid layers, away from one isolated glycophorin molecule with the protein polar section affecting two lipid layers (A) DMPC bilayer Curve a, $T=30°C$, curve b, $T=23°C$, curve c, $T=17°C$, curve d, $T=5°C$ (B) DPPC bilayer Curve a, $T=48°C$; curve b, $T=40°C$, curve c, $T=32°C$, curve d, $T=24°C$ Note the temperature dependence of the extent of perturbed lipid

surface, as we can see from a comparison with the effects due to proteins which do not perturb the polar region as glycophorin apparently does.

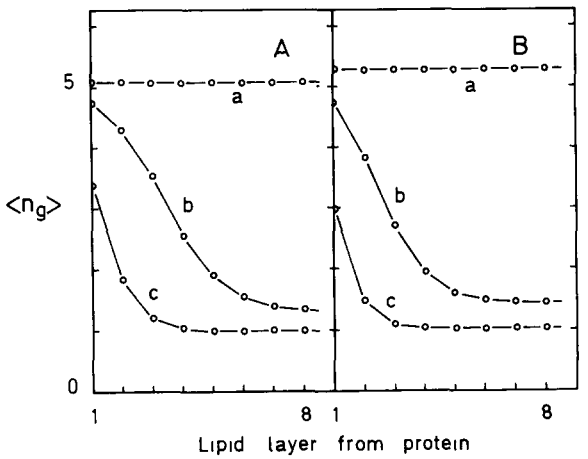

Fig 8 Average number of *gauche* bonds per lipid chain as a function of temperature and distance, in lipid layers, away from one isolated intrinsic molecule which does not have a hydrophilic section perturbing the lipid polar region as described here These results should hold for Gramicidin S and the M-13 phage coat protein (A) DMPC bilayers Curve a, $T=30$ C, curve b, $T=23°C$, curve c, $T=17°C$ (B) DPPC bilayers a, $T=48°C$; curve b, $T=40°C$; curve c, $T=30°C$

Fig. 8 shows a similar calculation of $< n_g >$ for the other cases considered: Gramicidin S, the M-13 phage coat protein and the hypothetical protein of molecular weight 40000. It is a prediction of this model that the static hydrocarbon chain order as a function of distance away from polypeptides or integral proteins such as these should be independent of the size of the molecule, and should depend only upon the 'roughness' of the molecule's hydrophobic surface as long as its molecular weight is greater than 1000. The results shown in this figure are essentially identical to those of calculations performed using analytical methods and Monte Carlo simulations to study proteins and cholesterol in lipid bilayers (Boothroyd, A. et al., unpublished data).

Finally, it should be noted that the distance from an isolated protein out to which a perturbation of the lipid hydrocarbon chains can be observed, is temperature-dependent, particularly near T_c, as can be seen in Figs. 7 and 8.

Comparisons with other measurements

Recent measurements using differential scanning calorimetry (DSC) [25], ^{31}P-NMR [22,25] and ^{13}C-NMR [26] have been interpreted in the following way: That from 80 to 100 phospholipid molecules are perturbed by each glycophorin molecule so that they do not take part in the gel to liquid crystal transition as measured by DSC; that 29 DMPC molecules are immobilized, or nine molecules of dioleoylphosphatidic acid are very strongly immobilized, in their phosphate region as detected by ^{31}P-NMR; and that about 30 lipid molecules are immobilized as measured using ^{13}C-NMR.

We have found that in order to understand the lateral diffusion of glycophorin, within the framework of our model, the polar regions of at least two layers of phospholipid molecules must be sufficiently perturbed around each such molecule. With approx. 9 lipids in the first layer and approx. 15 in the second (on both sides of the bilayer) our model requires that the polar regions of at least 24 phospholipid molecules must be perturbed by the glycophorin hydrophilic section. While we cannot say whether this perturbation would lead to immobilization on the ^{31}P-NMR time scale, our numbers are in accord with those measured.

According to our model, this number will not

necessarily be that which would be detected using DSC. To see this, refer to Fig. 7 curves (b). We see that just below the main phase transition temperature about four layers of lipid chains are significantly perturbed. Although we do not share the view that these are 'removed' from the cooperative gel-liquid crystal transition, it is clear that because they are more statically disordered than lipid chains far from the protein, they will contribute less to the transition enthalpy. From geometric considerations (Appendix B) there are about 74 lipid molecules on both sides of the bilayer in these four layers. Accordingly, DSC will detect this number as making a reduced contribution to the transition enthalpy. If we had assumed that the glycophorin hydrophilic section perturbed the polar region of three lipid layers then we would find that about 108 lipid molecules per glycophorin molecule would be detected, using DSC, as making a reduced contribution to the transition enthalpy. It is encouraging that these two numbers are in general agreement with the range of 80 to 100 deduced from DSC measurements [25].

Our calculations are in general agreement with very recent ^{1}H-NMR measurements by Ong et al. [27] who find that approx. 40 lipid molecules are directly affected by each glycophorin molecule in small unilamellar vesicles. Our results are also in general agreement with the measurements by Taraschi and Mendelsohn [28] on glycophorin/DPPC bilayers using raman spectroscopy as well as with those by Mendelsohn et al. [29] on glycophorin-DMPC unilamellar vesicles using Raman and infrared spectroscopy. Taraschi and Mendelsohn [28] find that at least 135 lipid molecules have their phase transition behaviour altered by each glycophorin molecule. This is somewhat higher than, but in general agreement with, the results of Van Zoelen et al. [25] and our calculation. Their measurements, however, were done for relatively low lipid/glycophorin ratios (1000:1 to 125:1), and their number of 135 lipid molecules is deduced from their observations at the highest concentrations used. At such concentrations questions of protein-protein interactions and lateral phase separation must be taken into account, and our calculation is for one diffusing unit.

However, Taraschi and Mendelsohn report no indication of a peak in the specific heat, as measured using DSC [28], near the temperature of approx. $T_c - 9°C$ corresponding to which the large change in D_L was reported to occur [8] over approx. 1.5°C. It is true that the latter measurement was performed on DMPC bilayers, but it seems unlikely that a result of this sort would not occur in DPPC as well. The way in which our 'uncoupling' mechanism is triggered must lead to a prediction of a peak in the specific heat at $T \approx T_c - 9°C$. Furthermore, as described, the triggering is a purely local effect since it depends only upon the order of the chains in the neighbourhood of the protein and so this peak must persist to higher protein concentrations unless the protein keeps all of the lipids fluid. In the absence of such a peak [28] the way in which our mechanism is triggered, but not necessarily the mechanism itself, must be called into doubt. It will be recalled that we assumed that when the probability of lipid chains, in a given layer around the protein, to be 'sufficiently melted' became too small, then the 'uncoupling' mechanism would be triggered. If, however, the triggering was related to long-range effects, such as changes in lateral packing, other than that due to the local ordering of the chains, at the pretransition then our mechanism would not predict a peak in the specific heat because at sufficiently high protein concentration the pretransition vanishes.

The recent measurements by Mendelsohn et al. [29], are, as far as we can see, essentially in accord with our calculations on DMPC bilayers. Fig. 7A shows that at 5°C the static hydrocarbon chain order is similar to that of a pure lipid except in the first layer around the protein (curve d). In the fluid phase (curve a) the protein has an insignificant effect upon this order.

One question which we have not studied is whether large multimers of glycophorin would diffuse rapidly below T_c. It would appear that the problem would be essentially the same as that of monomers.

Conclusions

We have presented a model for the lateral diffusion of isolated integral proteins or polypeptides (with a molecular weight greater than 1000) in phospholipid bilayers, which relates the diffusion coefficient, D_L, to the static lipid hydrocarbon

chain order. We have used this model in Monte Carlo simulations or in mean-field approximations to calculate the temperature dependence of D_L for Gramicidin S, the M-13 coat protein, and glycophorin in DMPC and DPPC bilayers. We have been concerned with the protein-lipid interactions which might give rise to the behaviour observed, and have not studied the contribution of gel-state defects, such as dislocations, to lateral diffusion.

We find that, at the concentrations studied, the change in D_L of Gramicidin S at T_c is in accord with those molecules being in dimeric forms in DMPC bilayers. We have predicted the temperature dependence of D_L for the M-13 coat protein for $T < T_c$ in DMPC bilayers. We have also calculated the average number of *gauche*-bonds per lipid hydrocarbon chain for various lipid layers around such proteins at different temperatures.

We find that the 'ice-breaker' effect of glycophorin in DMPC bilayers, viz. the small reduction in D_L as T decreases from T_c to $T_c - 9°C$, can be understood if the hydrophilic section of the protein perturbs the polar regions of neighbouring lipids, thereby causing a reduction in the effective lateral pressure acting in their hydrocarbon chain region. We find that at least approx. 24 lipid molecules must be directly affected by such a mechanism which is in accord with the numbers 29 or 30 detected by ^{31}P-NMR spectroscopy. Because of the interaction between hydrocarbon chains, the effect of this perturbation can extend outwards to four or five layers of lipid hydrocarbon chains away from the protein. From geometrical considerations, this means that between approx. 74 and approx. 108 phospholipid molecules will make a reduced contribution to the transition enthalpy, in general agreement with the numbers of 80 to 100 deduced from DSC.

Our mechanism, however, cannot account for the abrupt decrease in D_L for glycophorin in DMPC bilayers reported to occur as the system is cooled through $T \approx T_c - 9°C$, and an understanding of it required an additional mechanism. If this mechanism is related to the structure of glycophorin, and is not a consequence of changes in the bilayer independent of the protein, then we propose that the abrupt change in D_L is a consequence of a 'coupling-uncoupling' mechanism involving the protein hydrophilic section and the phospholipid polar regions. It appears most likely that this mechanism is triggered by changes in lipid packing at the bilayer pretransition.

It would be quite futile for us to speculate upon details of the mechanism whereby the glycophorin hydrophilic section interacts with the lipid polar groups and becomes decoupled from them under certain conditions, as we have proposed. A detailed study of the polar region of the lipids around this protein is needed.

Even if our proposed coupling and decoupling turns out to be wrong, this would not affect the validity of our model of protein lateral diffusion (Eqns. 4 and 5), and the calculations shown here, except possibly those shown in Fig. 4. It should be stressed that our model of protein lateral diffusion is for one protein. At higher concentrations the effect of protein-protein interactions must be considered.

We have cautioned against accepting our calculated values of D_L at low temperatures, sufficiently far below T_c, too readily. This is because we have not taken into account factors which may enhance diffusion such as the formation of dislocations and other defects created in the gel phase by the presence of a sufficiently large intrinsic molecule.

Finally, it should be noted that by putting $\Pi_1 = 0$, 10 or 20 dyn/cm for the case of glycophorin we are only approximating what might be an interaction involving many dynamical variables. If our proposal concerning the existence of this effect is correct, then a more elaborate treatment of it will be necessary.

Appendix A

In order to relate an expression for protein lateral diffusion coefficient, D_L, to the properties of the lipid bilayer in which the protein is incorporated, we start by considering a random walk performed by the protein's centre of mass. In some characteristic time, Δt, the protein moves a distance d, via a mechanism identified simply as $\mathfrak{D}$, in a direction given by the randomly-directed unit vector $R(\hat{r})$. At the ith step the displacement vector is

$$\vec{r}_i = d\mathfrak{D}R(\hat{r}_i) \tag{A1}$$

where $\hat{r}_i$ denotes a unit vector. By summing over all the steps to obtain the total displacement $\vec{r}$, and averaging $r^2 = \vec{r} \cdot \vec{r}$ over a random path we find that

$$\overline{r^2} = \sum_{ij} d^2 \mathcal{D}^2 \overline{R(\hat{r}_i) \cdot R(\hat{r}_j)}$$

$$= d^2 \sum_i \mathcal{D}^2 \qquad \text{(A2)}$$

The last sum is over a random path through the bilayer which will sample the states of the lipid at that temperature. If $\mathcal{D}$ does not depend directly upon the state of the protein, then we can rewrite Eqn. A2 as

$$\overline{r^2} = d^2 N \langle \mathcal{D}^2 \rangle$$

where N is the number of steps taken. This yields

$$D_L = \frac{\overline{r^2}}{4N\,\Delta t} = \frac{d^2}{4\,\Delta t} \langle \mathcal{D}^2 \rangle \equiv C_0 \langle \mathcal{D}^2 \rangle \qquad \text{(A3)}$$

The factor C_0 must be determined empirically.

Appendix B

We estimated the approximate number of lipid chains which can fit around an isolated intrinsic molecule by assuming that the portion of the molecule which is inside the bilayer, possessing a mass M_I, has a volume proportional to this mass such that the same constant of proportionality relates the volume of a lipid molecule to its mass, M_L. Then, if the cross-section area of the lipid molecule is A_L and that of the intrinsic molecule is A_I, with h being half the thickness of the bilayer, we have

$$2hA_I/M_I = hA_L/M_L \qquad \text{(B1)}$$

When the intrinsic molecule spans only half of the bilayer, the factor of 2 is omitted. A_L is given by $2A_c$ where A_c is the area of a lipid chain. If the cross-sections of the chain and the intrinsic molecule are taken to be effectively circular, then we can define radii

$$R_c = (A_L/2\pi)^{1/2}, \quad R_I = (A_I/\pi)^{1/2} \qquad \text{(B2)}$$

The approximate number of hydrocarbon chains N_c which can fit around one isolated such molecule is then

$$N_c = \pi(R_c + R_I)/R_c \qquad \text{(B3)}$$

For example, in the case of glycophorin $M_I \simeq 2500$ [8], and $M_L \simeq 680$ with $A_c \simeq 30$ Å^2, on the average, for DMPC, so that $A_I \simeq 110$ Å^2 and $R_I \simeq 6$ Å. Thus, $N_c \simeq 9$ as we have used.

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References

1 Cherry, R J (1979) Biochim Biophys Acta 559, 289–327
2 Derzko, Z and Jacobson, K (1978) Biophys J 21, 204a
3 Vaz, W L C, Wu, E-S and Jacobson, K (1979) Biophys J 25, 178a
4 Vaz, W L C, Jacobson, K, Wu, E-S and Derzko, Z (1979) Proc Natl Acad Sci USA 76, 5645–5649
5 Smith, L M, Smith, B A and McConnell, H M (1979) Biochemistry 18, 2256–2259
6 Smith, L M, Parce, J W, Smith, B.A and McConnell, H M (1979) Proc Natl Acad Sci USA 76, 4177–4179
7 Schindler, M, Osborn, M.J and Koppel, D E (1980) Nature (London) 283, 346–350
8 Vaz, W L C, Kapitza, H G, Stumpel, J, Sackmann, E and Jovin, T.M (1981) Biochemistry 20, 1392–1396
9 Axelrod, D, Koppel, D E, Schlessinger, J, Elson, E and Webb, W W (1976) Biophys. J 16, 1055–1069
10 Saffman, P G (1976) J Fluid Mech 73, 593–602
11 Saffman, P G and Delbruck, M (1975) Proc Natl Acad Sci USA 72, 3111–3113
12 Rice, D M, Meadows, M D, Scheinman, A O, Goni, F M, Gomez, J.C, Moscarello, M A, Chapman, D and Oldfield, E (1979) Biochemistry 18, 5893–5903

13 Wu, E-S, Jacobson, K, Szoka, F. and Portis, A, Jr (1978) Biochemistry 17, 5543–5550
14 Pink, D A, Green, T and Chapman, D (1980) Biochemistry 19, 349–356
15 Cailie, A, Pink, D A, DeVerteuil, F and Zuckermann, M J (1980) Can J Phys 58, 581–611
16 DeVerteuil, F, Pink, D A, Vadas, E and Zuckermann, M J (1981) Biochim Biophys Acta 640, 207–222
17 Pink, D A, Georgallas, A and Zuckermann, M J (1980) Z Physik B. 40, 103–110
18 Binder, K (1979) Monte Carlo Methods in Statistical Physics (Binder, K, ed), Springer-Verlag, New York
19 Rice, D M and Oldfield, E (1979) Biochemistry 18, 3272–79
20 Pink, D A, Georgallas, A and Chapman, D (1981) Biochemistry 20, 7152–7157
21 Van Zoelen, E J J, Verkleij, A J, Zwaal, R F A and Van Deenen, L L M (1978) Eur J Biochem 86, 539–546
22 Yeagle, P L and Romans, A (1981) Biophys J 33, 243–252
23 Wu, E-S, Low, P S and Webb, W W (1981) Biophys J 33, 109a
24 Brûlet, P and McConnell, H M (1976) Biochem Biophys Res Commun 68, 363–368
25 Van Zoelen, E J J, Van Dijck, P W M, De Kruijff, B, Verkleij, A J and Van Deenen, L L M (1978) Biochim Biophys Acta 514, 9–24
26 Utsumi, H, Tunggal, B D and Stoffel, W (1980) Biochemistry 19, 2385–2390
27 Ong, R L, Marchesi, V T and Prestegard, J H (1981) Biochemistry 20, 4283–4292
28 Taraschi, T and Mendelsohn, R (1980) Proc Natl Acad Sci USA 77, 2362–2366
29 Mendelsohn, R, Dluhy, R, Taraschi, T, Cameron, D G and Mantsch, H H (1981) Biochemistry 20, 6699–6706