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Protein lateral movement in lipid bilayers. Monte Carlo simulation studies of its dependence upon attractive protein–protein interactions

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We have studied the random movement of hexagons on a triangular lattice where the only mechanisms hindering diffusion are (i) the hard-core repulsion between hexagons which block pathways and (ii) attractive interactions between pairs of hexagons which can give rise to hexagon aggregation. This is an extension of earlier work (Pink, D.A. (1985) Biochim. Biophys. Acta 818, 200–204) which studied how diffusion of hexagons on a lattice was affected only by (i). This system is a simple model of the movement of integral proteins, in the plane of a lipid bilayer, under the influence of random forces. In contrast to the case where (ii) was absent, we find that there are two kinds of behaviour determined by a critical attractive interaction energy, $K_v^*(c)$ less than 0 which depends upon the hexagon concentration, c, (i.e. upon lipid : protein ratio). For attractive interaction energies, K_v, lying between 0 and $K_v^*(c)$, the diffusion coefficient D is essentially constant, whereas if K_v is less than $K_v^*(c)$ then D decreases by orders of magnitude as K_v decreases. In the region where D changes rapidly with K_v, long-lived dynamical clusters, which is the form that the aggregation takes, appear. We conclude that, in contrast to hard-core repulsions between hexagons which can decrease D by up to about one order of magnitude depending upon the hexagon concentration, the attractive, cluster-inducing, interaction can decrease D by many orders of magnitude. We have related our results to measurements of the lateral diffusion of bacteriorhodopsin in DMPC bilayers at 30°C and predict that dynamical protein clusters or aggregates with lifetimes ranging from 1 to 10 ms should become apparent when the lipid : protein ratio falls below 100. As the ratio becomes smaller, the lifetimes of the clusters should increase.

Introduction

Recently there was some discussion on the extent to which proteins might block the movement of other proteins simply due to their presence providing a hard-core barrier [1–3]. A decrease in the diffusion coefficient would be expected as the concentration of proteins increased (i.e. as the lipid : protein ratio decreased) due simply to the blocking of diffusion pathways of a given protein, by all the other proteins. A model calculation was performed [3] in order to see how large this effect might be. The model represented the plane of the bilayer membrane as a two-dimensional lattice in the plane of which hexagons, representing the cross-sections of bilayer-spanning integral proteins, were free to move, with the requirement that no two hexagons would overlap. Because of the

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simplicity of the model, it was argued that the effect being considered might be overestimated compared to the behaviour of real proteins in a membrane. It was found that with about 50% of the plane surface covered by hexagons, the average value of a parameter, D, identified as a lateral diffusion coefficient, possessed a value about five times smaller than the value of D appropriate to a single unhindered hexagon. Even with 81.7% of the surface covered by hexagons, the average value of D was smaller than that for a single hexagon by a factor of about 20. Notwithstanding the great simplicity of the model, this result was taken to indicate that the blocking of diffusion pathways by proteins, which interact amongst themselves only by a hard-core repulsive interaction, could not account for the small values of diffusion coefficients measured in biological membranes. It was subsequently pointed out (Cherry, R., personal communication) that many integral proteins display aggregation, at sufficiently high protein concentration, in what appears to be a liquid-crystal fluid phase of the lipid bilayer. The question was raised concerning what effect might a mechanism, which gives rise to such aggregation, have upon the lateral diffusion coefficient of proteins (R. Cherry, personal communication). Here, we would like to extend the model described above in order to study what effect aggregation might have upon the parameter D. We shall do so by introducing an attractive interaction between the hexagons. Although it is unnecessary for us to assume a particular source of the interaction, one possible mechanism is that arising from the presence of electric charges on the protein. We shall therefore compare the interactions used here to the strength of a coulomb interaction between charges near the surface of proteins but we repeat that this may not be the mechanism giving rise to the attractive interaction which we use.

We have warned [3] about the limitations of this kind of model for studying the lateral motion of proteins. In the next section we shall briefly reiterate some of them and add further comments for and against the model and the simulation method.

We stress that this model should not be over-interpreted. The previous simulation [3] was intended to study solely the effect of the blocking of an object's diffusion pathways by the presence of other hard (moving) objects. Here we shall modify that model in one way only in order to study the changes that occur if the objects can stick to each other and aggregate, even though for a short time.

Theory

The model which we shall use has been described before [3]. It consists of a triangular lattice of lattice constant b containing N_s sites together with hexagons distributed on the lattice so that (a) each hexagon centre, together with its six vertices, occupy lattice sites, and (b) different hexagons may not occupy the same lattice site. A hexagon occupies $n_H = 1 + M(M-6)/12$ lattice sites, where M is the number of sites adjacent to an isolated hexagon. In the model studied here we introduce an attractive interaction between any two vertices of two different hexagons when the vertices occupy sites which are nearest neighbours on the lattice. This is shown in Fig. 1 where the non-zero interactions are indicated by lines drawn between vertices. The strength of each such interaction is denoted by E_v. This is a crude model of a lipid bilayer membrane which contains bilayer-spanning integral proteins (hexagons) moving in the 'sea' of lipid molecules (unoccupied sites). The proteins are represented as undergoing Brownian motion due to random forces acting on them [3].

In order to model these random forces, the simulation proceeds as follows: A hexagon is

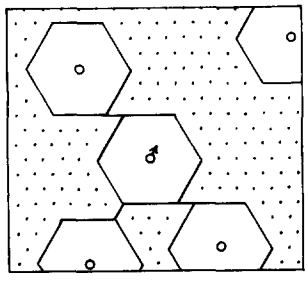

Fig. 1. Some hexagons with $M = 24$ on a triangular lattice. Solid bonds indicate interactions with energy $E_v = k_B T K_v$. The arrow at the centre of the hexagon shows in which direction the hard-core repulsive interaction permits it to move.

selected randomly and an attempt is made to move it by one lattice constant in a randomly chosen direction. If the move can be carried out without overlapping another hexagon then the following procedure is followed (Refs. 4, 5; for a simple description of the method used here, see, e.g., Ref. 6). Let the total energy of the distribution of hexagons be E_1 before the move is carried out, and let that of the new distribution, after the move is carried out, be E_2. Then if $E_2 \leqq E_1$ the move is carried out. If $E_2 > E_1$ then define $\Delta E = E_2 - E_1$ and select a random number R lying between 0 and 1. If $R < \exp(-\beta \Delta E)$, where $\beta = 1/k_B T$ with T being the absolute temperature the move is carried out. Otherwise, the move is not carried out and another hexagon is randomly selected. The procedure continues until each hexagon is selected once and only once. This constitutes one Monte Carlo step. This procedure ensures that the system approaches thermal equilibrium at a temperature T and remains in thermal equilibrium thereafter.

It is clear that the relevant parameter is not E_v, but $K_v = E_v/k_B T$. Thus, we shall report results simply for certain values of K_v. It is clear also that we are not considering any phase transitions brought about by changes in the states of the lipid molecules. This means that we are concerned with protein distribution and movement in a lipid bilayer membrane sufficiently far above the main lipid transition temperature so that all the lipids are in a disordered state.

The simulations were performed for hexagons with $n_H = 37$ ($M = 24$) on a triangular lattice of $(60)^2$ sites. Periodic boundary conditions were used and the procedure described above was followed. We found that, for the concentrations studied here, it was sufficient to use between $2 \cdot 10^5$ and $5 \cdot 10^5$ Monte Carlo steps. Although this is less than those used before [3] our results here are averages from between 4 and 18 independent simulations for each concentration and each value of K_v. As before, we recorded the square of the distance moved, in units of the lattice constant b, by the n-th hexagon at the end of k Monte Carlo steps, $r_n(k)^2$, as well as the number of steps moved, $s_n(k)$. As before, the periodic boundary conditions were ignored in calculating $r_n(k)^2$. Averages of these two quantities denoted by $r(k)^2$ and $s(k)$, were calculated,

$$r(k)^2 = \frac{1}{N_H} \sum_{n=1}^{N_H} r_n(k)^2$$

$$s(k) = \frac{1}{N_H} \sum_{n=1}^{N_H} s_n(k) \tag{1}$$

where N_H is the number of hexagons. In addition, a 'relative diffusion coefficient', $D(k)$, was calculated

$$D(k) = \overline{r(k)^2}/k \tag{2}$$

as well as the fractal dimensionality d_f of the paths taken by the hexagons in their movement,

$$\overline{r(k)^2} = A(k) \overline{s(k)}^{2/d_f} \tag{3}$$

The fractal dimensionality is a property of self-similar structures (here, the structure of the paths of the hexagons) [7,8]. These paths are statistically self-similar rather than geometrically so. Here $\overline{r(k)^2}$ and $\overline{s(k)}$ are values obtained by averaging the results of Eqn. 1 over several independent Monte Carlo simulations, at, of course, a fixed value of N_H.

The physical reason behind the definition of $D(k)$ is that each attempt to move through a distance equal to one lattice constant, b, is assumed to take place in some characteristic time Δt. The total distance moved is then related to $b^2 r(k)^2$ which takes place in a time $k\Delta t$. The ratio $b^2/\Delta t$ can be adjusted to give a value of the diffusion coefficient appropriate to a given protein concentration. Previously [3] the value of D typical of proteins in the fluid phase of a lipid bilayer when their concentration is very small (large lipid : protein ratio) was used to choose a value for $b^2/\Delta t$. Our definition of $D(k)$ thus differs in an inessential way from that given previously, $D = 2b^2 r(k)^2/k\Delta t$.

The computer program used was written in Fortran 77 and run on a Hewlett-Packard HP9000 series 550 computer using the HP-UX operating system. Although the simulations would have run faster had they been written in C, the availability of this system, with two CPUs each with its own on-board accelerator being equivalent, for our

purposes, to two VAX 11/780's, enabled us to attain sufficiently rapid throughput. The times for each simulation typically ranged from about 4 CPU hours to about 15 CPU hours per processor. We used our own random number generator with a cycle of approx. 2^{31} numbers instead of that provided with the system which had a cycle of length approx. 2^{15}.

In order to see what range of values of K_v might be realistic let us study the strength of an attractive interaction arising via electrostatic forces. The potential energy, of two charges $\lambda_1 e$ and $\lambda_2 e$ a distance r apart in a medium possessing a dielectric constant κ is

$$V = \frac{\lambda_1 \lambda_2 e^2}{4\pi\varepsilon_0 \kappa r} \tag{4}$$

where e represents the electronic charge and the other constants have their usual meanings. If the charges are about 6 Å apart then (in units of 10^{-13} ergs);

$$V = 38.4 \frac{\lambda_1 \lambda_2}{\kappa} \tag{5}$$

If $\lambda_2 = -\lambda_1 = -1$ and if an effective κ ranges from $\kappa \approx 80$ to $\kappa \approx 1$ then V ranges from $V \approx -38.4$ to $V \approx -0.48$. If the temperature is $T = 303.2$ K, about 7 K above the main lipid transition temperature of a bilayer of pure DMPC, then, for the range of V above, $V/k_B T$ ranges from ≈ -91.7 to ≈ -1.1.

It is unlikely that the expression of Eqn. 4 is anything else than a guide to the order of magnitude of possible attractive interactions between proteins via electrostatic effects. The numbers obtained from it will be used only to estimate the range of interactions that we might consider.

Finally, we want to reiterate some of our reservations concerning this kind of system as a model for studying protein lateral diffusion in lipid bilayer membranes. First, however, it is sometimes suggested that a Monte Carlo simulation simply generates a sequence of states of the system from which one can calculate static equilibrium properties, and that there is no reason to think that such a sequence represents a development of the system in time through a succession of time-ordered states. While the first statement is true, in the kind of simulation described here in which for a given configuration, the state of the system differs from the state of the previous configuration by a small amount, the second statement is not necessarily valid. A more elaborate justification for the use of Monte Carlo methods to study diffusion can be found in Ref. 9.

It might be suggested that the cross-sections of proteins are not hexagons and, indeed, some authors seem to feel that circles better represent the average shape of that part of a protein which penetrates the hydrophobic region of the bilayer. We are not aware of any results that suggest that this is so. Indeed it is interesting that one possible cross-section suggested for the human erythrocyte Band 3 dimer is a hexagon [10].

The weakness of representing the motion of a protein in the plane of the bilayer by an object moving on a lattice has been mentioned [3]. In that paper, it was considered that the effect of this would be to yield a value for D smaller than that which would be obtained from a more realistic model. It has been assumed that each Monte Carlo step represents one average time interval, Δt, and that Δt is independent of both protein concentration and protein–protein interaction.

The objection might be raised that there is no provision in the model for a cluster of hexagons to move as one unit because each hexagon is selected in turn and an attempt is made to move it. While this objection might not be important in the case that the attractive protein–protein interaction is zero, in the case considered here, where because of a strongly attractive interaction hexagons may remain aggregated for a large number of Monte Carlo steps, it may be unrealistic to have no motion of an aggregate as a unit. To move one of these aggregation units as a whole, however, would require not only translating it, but rotating it as well. Since such an aggregate would, in general, not possess the symmetry of the lattice, then it would have to be distorted in order to carry out the latter movement. Furthermore, an aggregate undergoing motion as a result of random forces would not move as far, in a unit time, as a single hexagon. In both of the motions described we would have to present rules for moving such aggregates. In view of the crudeness of the model and in view of the fact that we are interested in

changes involving orders of magnitude, we considered that to refine the model in this way was inappropriate. The value of D from our simulation will then be lower than that obtained from an experiment.

Results

The fraction of the area of the lattice covered by the hexagons, f_A, and the hexagon concentration, c, are defined as

$$f_A = n_H N_H / N_s$$

$$c = N_H / (N_s - n_H N_H + N_H) \quad (6)$$

where N_s is the total number of sites on the lattice, and n_H and N_H were defined earlier. The relations between f_A and c are

$$f_A = n_H c / (1 + (n_H - 1) c)$$

$$c = f_A / (n_H - (n_H - 1) f_A) \quad (7)$$

The number n_H represents, approximately, the cross-sectional area of the protein in units of the cross-sectional area represented by a unit cell of the lattice. We studied various concentrations of hexagons on the $(60)^2$ lattice ranging from $N_H = 10$ ($c = 0.0031$) to $N_H = 54$ ($c = 0.0326$). The values of N_H used range from $f_A = 0.103$, to $f_A = 0.555$ respectively, the last named being characteristic of some biological membranes. These concentrations were studied for different values of K_v ranging from $K_v = 0.0$, to $K_v = -4.0$. The distribution of hexagons were initialized for 10^4 Monte Carlo steps and averages were computed over $5 \cdot 10^5$ monte Carlo steps for $N_H = 10$ and $2 \cdot 10^5$ Monte Carlo steps for the other cases.

The fractal dimensionalities of the paths followed by the hexagons (Eqn. 3) were calculated from plots of $\log(\overline{r(k)^2})$ against $\log(\overline{s(k)})$ and yielded results in accord with a single value of $d_f = 2$. Values of A as a function of concentration for $K_v = 0$ were in accordance with the results reported previously [3].

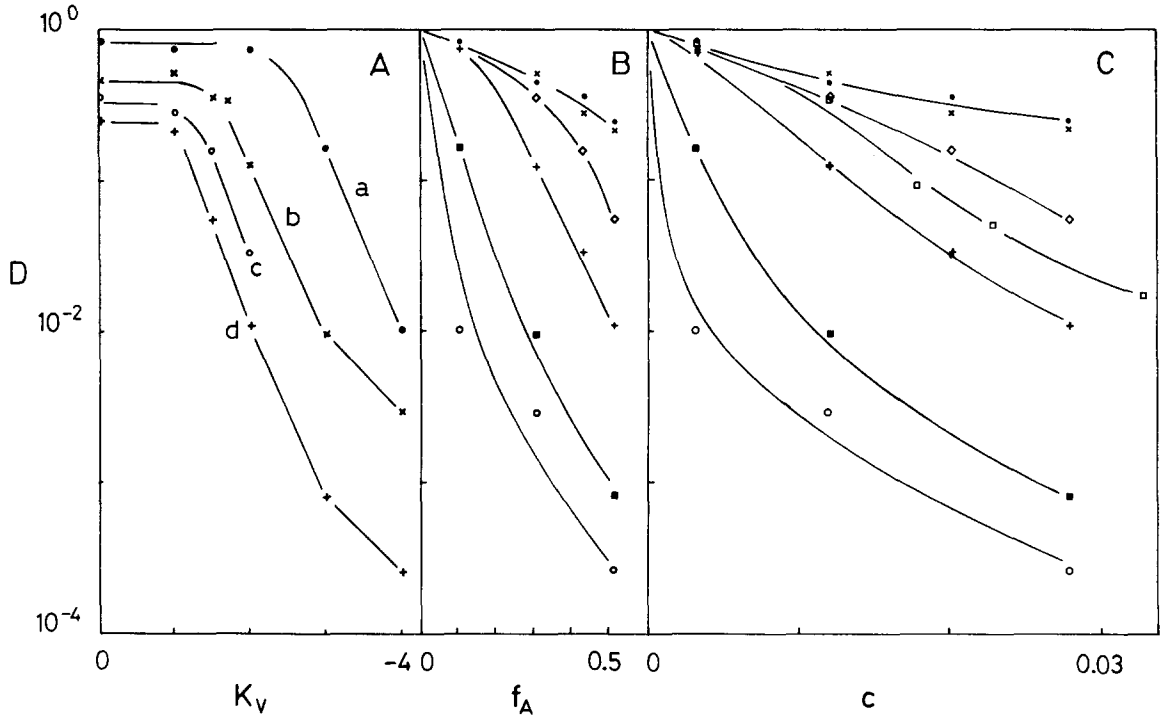

Fig. 2. (A) The dependence of D (Eqn. 7) upon K_v for different hexagon concentrations: (a) $f_A = 0.103$; (b) $f_A = 0.308$; (c) $f_A = 0.432$; (d) $f_A = 0.514$. (B) The dependence of D upon f_A for different values of K_v: 0 (●); −1.0 (×); −1.5 (◇); −2.0 (+); −3.0 (■); −4.0 (○). (C) The dependence of D upon hexagon concentration, c, for different values of K_v: 0 (●); −1.0 (×); −1.5 (◇); −1.7 (□); −2.0 (+); −3.0 (■); −4.0 (○). In all cases the solid lines are intended only as a guide to the eye.

We defined D to be

$$D = \lim_{k \to \infty} D(k) \tag{8}$$

where $D(k)$ is defined in Eqn. 2. Values for D were obtained by plotting $D(k)$ as a function of k and estimating the asymptote. In view of the comments regarding the limitations of the model, the values of D obtained are probably lower than those which would be obtained from a more realistic model. Fig. 2 shows the results for D as a function of K_v for different hexagon concentrations (Fig. 2A) and as a function of f_A (Fig. 2B) or as a function of c (Fig. 2C) for different values of K_v. These show that for a given hexagon concentration there are essentially two regions as a function of K_v (Fig. 2A): As K_v increases from $K_v = 0$, D remains essentially constant until some critical value at which it decreases rapidly as K_v increases further. The critical value of K_v, $K_v^*(c)$, depends upon the hexagon concentration c, and the shapes of the curves in Fig. 2B and Fig. 2C are a consequence of this. This is seen in, for example, Fig. 2C where, for $K_v = 0$, D is lower by only a factor of 4 at $c = 0.0278$ ($f_A = 0.514$) compared to the value at $c = 0$. However, as k_v increases, the shapes of the curves change until at $K_v = -4.0$, D decreases by two orders of magnitude as f_A increases from 0 to 0.015. Various critical values of $K_v^*(c)$ are: $K_v^*(0.0031) \approx -2.0$, $K_v^*(0.0119) \approx -1.5$ and $K_v^*(0.0278) \approx -1.0$.

The behaviour of D can be understood by looking at configurations of the hexagons on the lattice. Fig. 3 shows configurations for $N_H = 10$ ($c = 0.0031$) for values of $K_v = 0$, -2.0, -3.0 and -4.0 at Monte Carlo step numbers 10^5, $3 \cdot 10^5$ and $5 \cdot 10^5$. For the first two values of K_v, no marked clustering is observed. For $K_v = -3.0$ we see the formation of dynamic clusters: aggregates that exist for 10^3–10^4 Monte Carlo steps and break up only to reform elsewhere on the lattice. This is even more marked for $K_v = -4.0$ where aggregates are the rule. In this case the clusters exists for 10^4–10^5 Monte Carlo steps. A comparison with Fig. 2 shows that it is near $K_v^*(0.0031) \approx -2.0$ that clusters begin to persist for a relatively large number of Monte Carlo steps, and that D shows a sudden decrease as c increases. Similar behaviour is seen in Fig. 4 for $N_H = 30$ and Fig. 5 for $N_H = 50$ which show the configurations at $4 \cdot 10^4$, $12 \cdot 10^4$ and $2 \cdot 10^5$ Monte Carlo steps. For $N_H = 30$ ($c = 0.0119$) small clusters can be seen forming when $K_v = -1.5$, while for $K_v = -2.0$ we can see the large clusters which exist for $\gtrsim 10^4$

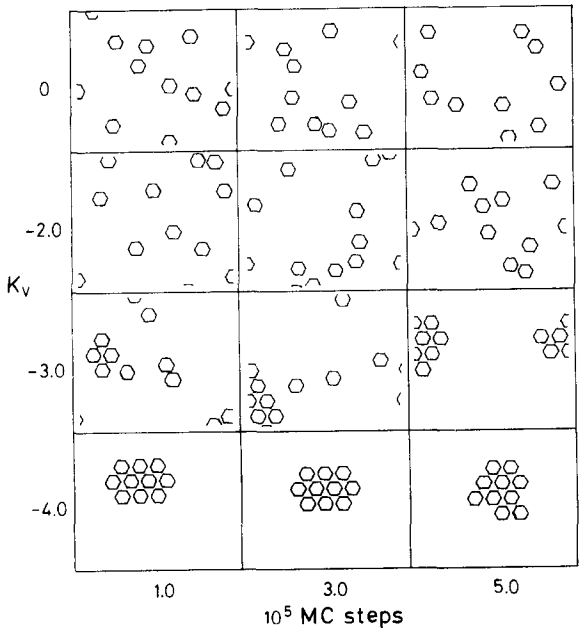

Fig. 3. Typical instantaneous hexagon configurations for $N_H = 10$ ($f_A = 0.103$, $c = 0.0031$) as a function of K_v (vertically) at given Monte Carlo steps (horizontally, in units of 10^5 steps).

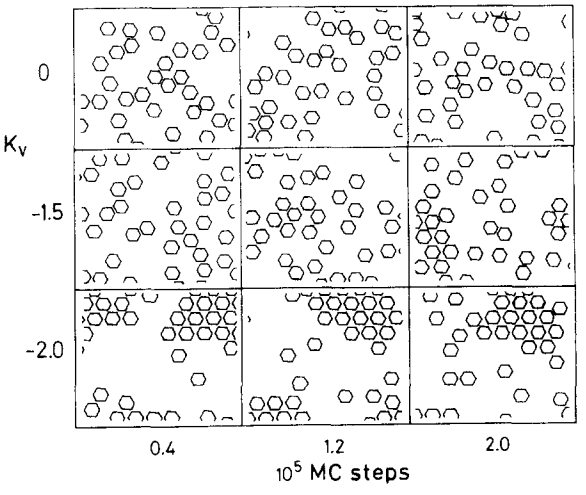

Fig. 4. Typical instantaneous hexagon configurations for $N_H = 30$ ($f_A = 0.308$, $c = 0.0119$) as a function of K_v (vertically) at given Monte Carlo steps (horizontally, in units of 10^5 steps).

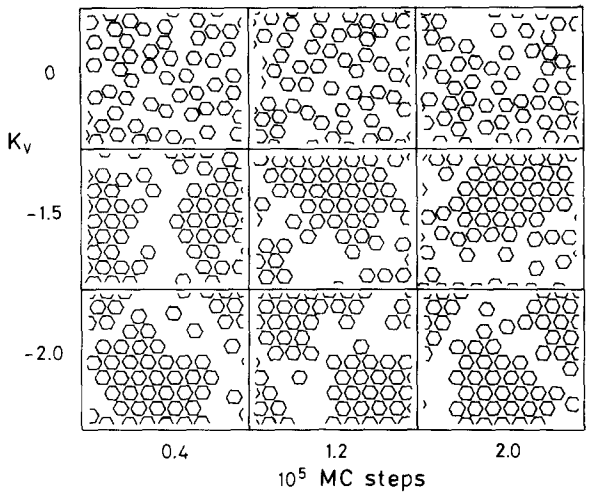

Fig. 5. Typical instantaneous hexagon configurations for $N_H = 50$ ($f_A = 0.514$, $c = 0.0278$) as a function of K_v (vertically) at given Monte Carlo steps (horizontally, in units of 10^5 steps).

Monte Carlo steps. Finally the random distributions for $N_H = 50$ ($c = 0.0278$) when $K_v = 0$ are replaced by configurations displaying clustering when $K_v = -1.5$. Again, these are dynamic, existing for 10^3–10^4 Monte Carlo steps. When $K_v = -2.0$ however, the clusters exist for a great number of Monte Carlo steps.

Finally, Fig. 6 shows data reported by Peters and Cherry [11] for the lateral diffusion coefficient, D_L, of eosin-labelled bacteriorhodopsin in DMPC measured using the FRAP technique and replotted by us. The data are taken from their Fig. 3 at 30°C from which we have estimated, by

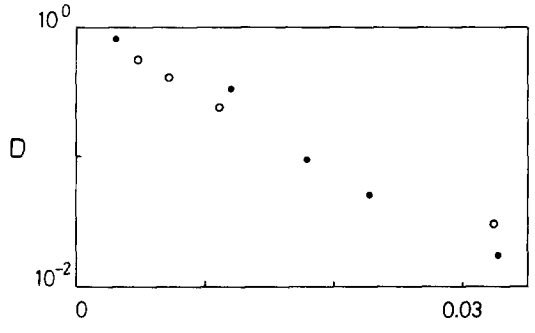

Fig. 6. The dependence of D upon c. Results of the simulation for $K_v = -1.7$ (●). Data of Peters and Cherry [11], (with extrapolation to $c = 0$) normalized to $D = 1.0$ at $c = 0$, for bacteriorhodopsin in DMPC bilayers at $T = 30$°C (○).

extrapolating their data, the $c \to 0$ limit of their D_L which we find to be approx. 4.8 $\mu m^2/s$. In order to compare their data with our calculations we have scaled their data so that the value of D_L at $c = 0$ is defined to be 1.0. Included in Fig. 6 is a plot of our simulations for $K_v = -1.7$ and it can be seen that the agreement is encouraging. We stress, however, that any detailed numerical agreement between the measured data and our calculations should not be taken too seriously because of the comments made earlier. The comparison is only intended to show that the model appears not to contain gross errors. However, our calculations predict that for a concentration of bacteriorhodopsin, c, greater than $c \approx 0.01$ (lipid to protein ratio less than ≈ 90–100) in DMPC at $T \approx 30$°C, some dynamic clustering should be observed which will increase and persist for longer times as c increases.

Conclusions

There have been a number of suggestions (Refs. 1, 2 and references cited therein) concerning mechanisms which might cause integral proteins in biological membranes to exhibit lateral diffusion coefficients two to three orders of magnitude lower than values expected from a consideration of the movement of a single such protein. In a previous paper [3] it was shown, by using the simple model described here, that it was unlikely that the blocking of a protein's diffusion pathways by the presence of other proteins could account for the decrease. It was found that hexagons moving on a lattice had their diffusion coefficient reduced by a factor of approx. 20 below the value of that at $c = 0$, when 81.7% of the lattice is covered by hexagons. It was argued that this result was a 'worst case scenario'.

Here we have addressed the question of whether an attractive interaction between the hexagons on the lattice can modify the diffusion coefficient of the hexagons sufficiently to suggest that the diffusion coefficient observed for integral proteins can be understood as a consequence of such a mechanism. We have not suggested a particular source for the interaction, but one which suggests itself is the possibility of an electrostatic interaction between opposite charges on two integral proteins.

We performed a rough calculation to see what range of attractive interaction might be reasonable if this were the mechanism. However, we see no reason why other mechanisms should not give rise to attractive interactions of the magnitudes which we have used here.

We performed Monte Carlo studies to obtain the lateral diffusion coefficient of hexagons, of concentration c, moving on a triangular lattice. We chose the size of a hexagon to correspond to a bacteriorhodopsin molecule, if each lattice site corresponds to a lipid hydrocarbon chain in one side of a bilayer membrane. We have, however, reiterated our reservations about uncritically taking over the results obtained here in order to use them to understand the details of real proteins moving in real membranes. We have pointed out further defects of this model which arise when long-lived clusters form due to the attractive interaction between heagons. We have argued, however, both here and elsewhere [3] that these deficiencies of the model should represent a 'worst case scenario': that if a simulation was performed on a realistic (three-dimensional) bilayer composed of realistic models of lipid molecules and integral proteins ,then the values of D should be greater than those observed here.

We find the following results for the hexagons of the size considered here:

(i) In the case of only a hard-core repulsion between hexagons and zero attractive interaction, K_v, D is decreased by a factor of approx. 4 as the area of the lattice covered by hexagons ,f_A, increases from 0 to 0.5. This behaviour is followed if K_v lies in the range of about $-1.0 \lesssim K_v \lesssim 0$. For K_v lying between approx. -1.3 and -2.0, D is decreased by one or two orders of magnitude over that range of f_A. For $K_v \lesssim -3.0$, D is reduced by a least two orders of magnitude as f_A increases from 0 to approx. 0.3. There are thus, essentially, three types of behaviour (Fig. 2) as K_v changes. In the last case, the model is least reliable for reasons given above concerning the absence of movement of clusters of hexagons as a single unit.

(ii) Clusters of hexagons are formed the properties of which depend upon K_v and c (or f_A). These clusters are dynamic in that they break up and others are formed as the simulation proceeds. The larger f_A is and the more negative K_v is, the larger and 'longer-lived' are the clusters. Previously, [3] a time of 10^{-6} s was chosen as a reasonable average time during which an attempt is made to move each hexagon a distance of approx. 6 Å. This was based upon the picture that the hexagons used here would correspond to trans-bilayer proteins of molecular mass $\geqslant 25\,000$ daltons. On the basis of this, our calculations predict that, for example, when c lies between approx. 0.02 and 0.03 and K_v lies between approx. -2.0 and -1.5, clusters can exist for approx. 10^4 Monte Carlo steps which corresponds to approx. 10 ms. At these higher concentrations, a freeze-fracture micrograph of the plane of the bilayer might be interpreted as phase separation of lipids and proteins. Such dynamical clusters are not manifested for $c \leqslant 0.005$ and K_v lying between approx. -2.0 and 0. (See Fig. 2) for these regions.) For a stronger interaction, $K_v \leqslant -3.0$, large long-lived ($\geqslant 0.1$ s) clusters appear to be formed.

(iii) The measurements of Peters and Cherry [11] on bacteriorhodopsin in DMPC at 30°C are well-reproduced by our simulation with $K_v \approx -1.7$. Notwithstanding our reservations concerning the reality of the model, our results suggest that they should observe dynamic clusters for lipid : protein ratios less than about 90–100 with lifetimes of $\geqslant 1$ to 10 ms, and that the cluster size and lifetime should increase as the lipid : protein ratio decreases further.

We had seen previously [3] as well as here (Fig. 2) that simply the blocking of a hexagon's diffusion pathways by the presence of other moving hexagons, with no other interactions present, cannot account for a decrease in D by two or three orders of magnitude, even at very high concentrations. However, we conclude from this study that the presence of attractive forces between integral proteins, of a strength well within the range attributable to electrostatic and, probably, other interactions, can account for the observed decrease in lateral diffusion coefficient as the concentration is increased. A model of proteins in lipid bilayer membranes which does not take such attractive interactions into account must therefore be regarded as less than complete.

Finally, these results might be tested. If it is possible to fabricate bilayer-spanning molecules,

both essentially similar except that one kind possesses charges so that any pair of the latter will experience an attractive interaction, while pairs of the other do not, then a mechanism for aggregation might be identified, and, if so, the results of a measurement of D should be predictable by this model, if it has any validity.

Acknowledgement

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