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Protein lateral movement in lipid bilayers. Stimulation studies of its dependence upon protein concentration

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A number of mechanisms have been proposed to account for the decrease in protein lateral diffusion coefficients in a lipid bilayer membrane, as the concentration of proteins is increased. One such mechanism is the steric hindrance (via, say, a hard-core repulsion) to the lateral movement of a protein due to the proximity of other proteins. Here a model is presented to study this effect alone. It is argued that the model will overestimate the effect being studied. The results of computer simulations show that such a mechanism will decrease the lateral diffusion coefficient by less than a factor of 20 below the zero-concentration limit, even when up to 81.7% of the bilayer surface is composed of integral proteins. This result supports the opinion (Kell, D.B. (1984) Trends Biochem. Sci. 9, 379) that such a mechanism cannot account for a decrease in the lateral diffusion coefficient by two or three orders of magnitude.

Introduction

Recently [1–3] there has been some discussion concerning possible mechanisms which can contribute to low rates of protein lateral movement. Amongst the mechanisms that might do so is the difficulty of moving laterally when other proteins block pathways simply ecause of their presence [2]. Although the points in favour of Ref. 2, and against Ref. 3, this mechanism are well-argued, no direct measurement of this effect alone could be quoted. As pointed out [1,2], one is not yet able to say what other mechanisms might be operating in a biological membrane. On the other hand, it is difficult to reliably reconstitute model membranes containing a very high concentration of integral proteins. Even in the latter case, one might not be certain that the only effect upon lateral motion is the blocking of a protein's path by other proteins.

In a case such as this, where the effect of a well-defined relatively simple mechansim is to be studied, a computer simulation of the effect can provide some insight. This paper will outline a model and argue that it represents, while not necessarily the 'worst case', at least a case in which such an effect might be overestimated. The results will then suggest how protein lateral movement depends only upon the presence of other proteins which are blocking movement pathways.

In the next section the model and the procedure for calculating distances moved will be described. Here the results of the simulation and its possible relation to lateral diffusion coefficients measured in real membranes will be outlined. In this section the defects of the model will be discussed, as well as how it does not, possibly, adequately model the lateral diffusion of proteins. It will be argued, however, that if the mechanism studied here were

the only one operating, then the results of this model should underestimate the lateral diffusion coefficient measured in real membranes.

The model and its limitations

We shall model a lipid bilayer, which is composed of lipids possessing some average property, containing integral proteins of one kind. There are no specific interactions between the lipids and the proteins, and the only interaction between the proteins are hard-core repulsions preventing one protein from penetrating another. This system will be modelled as a 'protein gas', viz. as a set of proteins free to move in a plane and subjected to random forces acting upon them. Thus, a single isolated protein will be taken as an object undergoing Brownian motion in two dimensions. The model presented here will be used to study how such motion changes as the protein concentration changes.

The plane of the hydrophobic region of the lipid bilayer is modelled as a triangular lattice with lattice constant, b (Fig. 1). The cross-section of the hydrophobic section of an integral protein is represented by a hexagon which covers n_H lattice sites. If M is the number of lattice sites adjacent to a single hexagon [4], then $n_H = 1 + M(M-6)/12$ so that n_H takes on the values 1, 7, 19, 37, ..., the first case representing a 'hexagon' composed of a single site. The centres and vertices of hexagons occupy lattice sites and hexagons may not share the same lattice sites, i.e., they may not 'overlap'. In order to model the effect of random forces acting on proteins the simulation proceeds as follows. Each hexagon is selected in turn in a random sequence, once and only once, 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 this is done. If this is not possible, then the hexagon remains in its position and another, randomly chosen, hexagon is selected and the procedure repeated. A sequence in which each hexagon is selected once and the moving procedure is carried out defines one Monte Carlo step. If a hexagon moves by one lattice constant then it has moved one step. It is clear that the number of steps moved by a given hexagon is less than or equal to the number of Monte Carlo steps performed. The computer program used was efficiently written in Fortran 77 and run on a Digital Equipment Corp. VAX 11/780 using the VMS operating system. The compiler optimizes Fortran 77 exceptionally well. The times taken to run the programs depend very sensitively upon the number of hexagons. These times typically ranged from about 2.5 CPU hours for low hexagon concentrations to about 45 CPU hours for the highest concentrations studied. Indeed, the maximum concentration studied was determined by the number of CPU hours which could be obtained in 7 days of continuous computing, this being the maximum time between system backups during which the computer was taken down.

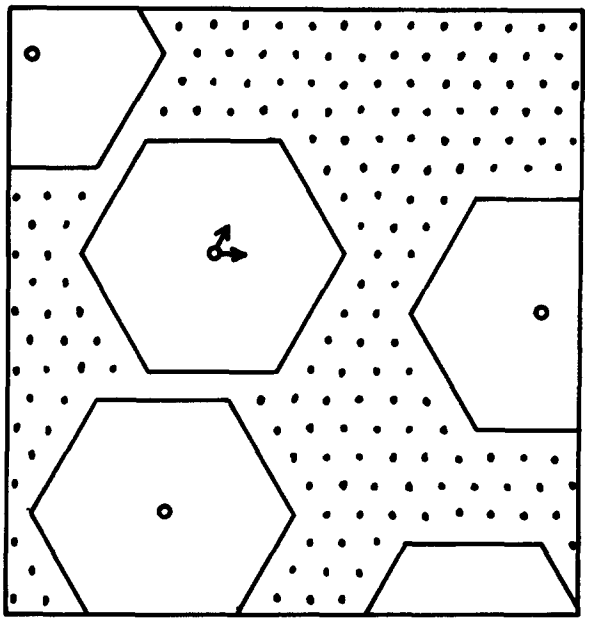

Fig. 1. The lattice constant, b, is the distance between two nearest neighbour sites (dots) of the triangular lattice. Hexagon centres are indicated by open circles. The number of lattice sites adjacent to a single isolated hexagon is $M = 30$. If $b = 6$ Å then each lattice site represents, approximately, one lipid hydrocarbon chain and the hexagons represent bilayer spanning proteins of molecular weight approx. 45 000. The hexagon with arrows has been selected to attempt to move in the course of a Monte Carlo step. It is able to move only in the two directions shown by arrows, the four other directions being blocked by two hexagons. If the direction randomly chosen in which to move is not one of the two arrowed directions, then that hexagon does not move during that Monte Carlo step.

Simulations were performed for hexagons with $n_H = 61$ on a lattice of $(100)^2$ sites. Periodic boundary conditions were used. Hexagons were distributed randomly, where practical, and 1000 Monte Carlo steps were performed in order to initialize the distribution. At the end of this procedure the positions of the hexagon centres defined their initial positions. A sufficiently large number of Monte Carlo steps were then performed, ranging between $6.6 \cdot 10^5$ and $1.8 \cdot 10^6$ Monte Carlo steps. The distance moved, in units of the lattice constant, by the nth hexagon at the end of the k Monte Carlo steps, $r_n(k)$, as well as the number of steps moved, $s_n(k)$, were recorded. In calculating r, the periodic boundary conditions were ignored in that as a hexagon crossed a boundary it moved onto another equivalent lattice. Averages of r and s were calculated:

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

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

where N_H is the number of hexagons.

In order to relate r and s to distances moved and times elapsed for proteins moving in a membrane the following assumption was made: Each Monte Carlo step represents an attempt to move an average distance b during an average time interval, Δt, under the influence of the random forces described above. The absolute value of the lattice constant, b, is chosen so that the ratio $b^2/\Delta t$ will yield a value of

$$D = 2b^2\overline{r(k)^2}/k\Delta t \qquad (2)$$

in the range of 10^{-9} to 10^{-8} cm^2/s [5,6] for a single hexagon moving on the lattice. Here $\overline{r(k)^2}$ represents the average of $r(k)^2$ over a sufficient number of simulations. Thus, if we let b represent the average distance between lipid chains in a melted phase, which is about 6 Å [7], then $\Delta t \approx 10^{-6}$ s. In this case each hexagon represents a bilayer-spanning protein of molecular weight about 45 000. We shall refer to two other quantities which will describe the packing of hexagons and the average structure of the paths followed by the hexagons. They are f_A, the fraction of the area of the lattice covered by the hexagons

$$f_A = n_H N_H / N_s \qquad (3)$$

where N_s is the total number of lattice sites, and the fractal dimensionality (Ref. 8; also, for example, Ref. 9), d_f, of the paths defined by

$$r(k) = As(k)^{1/d_f} \qquad (4)$$

where k is sufficiently large that d_f is a constant independent of k, and A is an amplitude.

Some of the defects of this model are clear. To model the lateral motion of one kind of protein in a lipid bilayer one could perform a molecular dynamics simulation of a three-dimensional mixture of objects representing pairs of lipid hydrocarbon chains connected by a glyceride backbone, and the hydrophobic region of proteins. A more tractable calculation can be done if one performs a molecular dynamics simulation of a two-dimensional mixture of 'soft' shapes representing the average cross-sections of lipid molecules and protein hydrophobic sections. In order to obtain good statistics on the motion of the proteins at high concentrations, one would have to make use of about 10^4 objects representing lipids and about 10^2 objects representing proteins moving for a simulated time of at least 10^{-2} s. In such a simulation the proteins could move in ways which are not allowed by the model described here. For example, since a reversal of the path is selected with a probability of 1/6 here, and since such a reversal is unlikely in a more realistic model, larger values of r^2 should be obtained in a given time than those given by the model here. Thus, the model used here might yield values of D smaller than those obtained from a more realistic model. Such a result is of use, however, if one is studying the question of to what extent a high concentration of proteins inhibits protein lateral movement simply due to the blocking of diffusion pathways, because it can indicate lower limits.

Results and Conclusions

Table I shows some of the results of simulations carried out for various numbers of hexagons, N_H,

TABLE I

VALUES OF A^2 (IN UNITS OF b^2) FOR $d_f = 2$, AND D (FOR $b = 6$ Å AND $\Delta t = 10^{-6}$ s) AS A FUNCTION OF HEXAGON CONCENTRATIONS

N_H	f_A	A^2	k (Monte Carlo steps)	$r(k)$	D (10^{-9} cm^2/s)
1		1.000			7.200
60	0.366	0.458	$6.6 \cdot 10^5$	503.71	2.768
90	0.549	0.297	$8.0 \cdot 10^5$	384.40	1.330
130	0.793	0.17	$1.58 \cdot 10^6$	301.81	0.415
134	0.817	0.17	$1.80 \cdot 10^6$	300.91	0.362

on a lattice of $N_s = 10^4$ sites. Log-log plots of the data to obtain values of A^2 and d_f gave values for d_f ranging from 2.02 ($N_H = 60$) to 2.05 ($N_H = 134$). A simulation of a single random walker, for which d_f is known to be 2, yielded a value of $d_f = 2.02$ from 80 trials each of $5 \cdot 10^6$ steps. These results have been interpreted as indicating that $d_f = 2$ in all cases studied. With this interpretation, the value of A^2 is easily obtained using Eqn. (4). The second and third columns show the fractional area of the lattice covered by the hexagons and the amplitude A^2 in units of b^2. The values of A^2 are calculated from paths involving between $5.16 \cdot 10^5$ and $5.47 \cdot 10^5$ steps. A fuller discussion of these results will be presented elsewhere. The fourth and fifth columns show the number of Monte Carlo steps and the corresponding values of $r(k)$ in units of the lattice constant, b.

The last column shows values of D for $b = 6$ Å and $\Delta t = 10^{-6}$ s. Their ratio has been chosen so that for the case of a single hexagon the value of D is approximately equal to values of the lateral diffusion coefficient reported for proteins at low concentrations [5]. Clearly, however, the ratio $b^2/\Delta t$ can be changed from this value, but this will not affect the ratios of different values of D. The effect of the blocking of pathways for the movement of hexagons, on the part of other hexagons, can be seen in the decrease in D as the concentration of hexagons increases. With 54.9% of the area of the lattice covered by hexagons, the value of D is about 5-times smaller than the value for a single hexagon. What is striking is that with 81.7% of the area covered, which is a concentration above that ($f_A \approx 0.805$) at which the hexagon-hexagon pair correlation function shows that a structural packing transition, similar to that of the random close packing of hard discs, has occurred (Laidlaw and Pink, unpublished data), the value of D is reduced

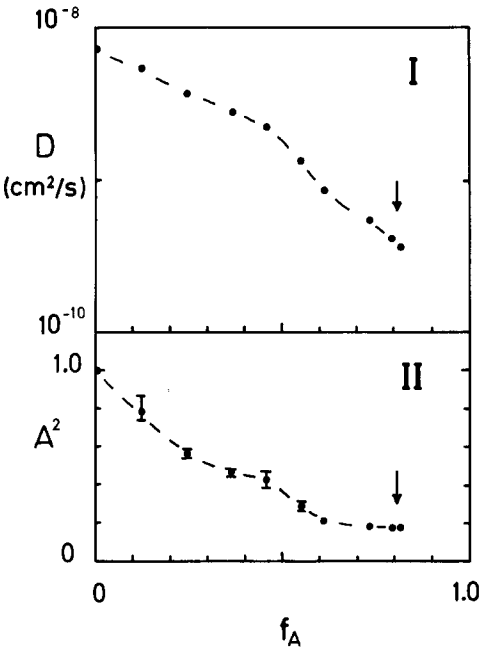

Fig. 2. (I) D (Eqn. 2) as a function of f_A, the fraction of sites occupied by hexagons (Eqn. 3). Note the apparent change in slope near the percolation limit around $f_A \approx 0.5$. The arrow indicates the concentration at which a random close packing-like transition occurs. (II) A^2 (Eqn. 4) as a function of f_A. Note that there appears to be no change at the packing transition at $f_A \approx 0.805$, but that there is an apparent change at $f_A \approx 0.5$. In both cases the dashed line is simply to aid the eye. Note that while D is determined by the dependence of r^2 upon k, the number of attempts made by each hexagon to move by one lattice constant, A^2, is determined by the dependence of r^2 upon $s(k)$, the number of moves actually made in k attempts.

by only a factor of about 20 from the value for $N_H = 1$.

More of the data are shown in Fig. 2 to bring out details of the dependence of D and A^2 upon f_A. In Fig. 2I, D is shown plotted on a logarithmic scale and one sees that there appears to be a change in slope at $f_A \approx 0.5$, which is the neighbourhood of the percolation limit for this system [10]. Fig. 2II shows the dependence of A^2 (Eqn. 4) upon f_A. Here the change near $f_A = 0.5$ seems clearer and there appears to be no change through the packing transition at $f_A \approx 0.805$, indicated by the arrows.

Above, it has been argued that this model may overestimate the effect of the blocking of protein movement pathways by other proteins in a real membrane. Clearly, if the values of A^2, obtained from a more realistic model, are larger than those given here, then $r(k)$ will be correspondingly larger except, of course, for the case $N_H = 1$. This means that the lateral diffusion coefficients which would be obtained from a more realistic model will be larger than the values obtained here for D. It should be noted that, with $\Delta t = 10^{-6}$ s, the number of Monte Carlo steps performed correspond to measurement times of between 0.66 and 1.8 s.

The results of the simulations reported here thus support the view [1,3] that such a mechanism will not contribute substantially to the reduction of the protein lateral diffusion coefficient in biological membranes. In particular, it is difficult to see how a value of about 10^{-11} cm^2/s can be achieved by this mechanism alone for $f_A \approx 0.5$, which is typical of biological membranes.

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