brief communication

Lateral diffusion of membrane proteins in protein-rich membranes

A simple hard particle model for concentration dependence of the two-dimensional diffusion coefficient

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ABSTRACT A model for the effect of protein concentration on the rate of lateral diffusion of integral membrane proteins is presented, in which the proteins are represented by equivalent hard circular particles on a surface. As the density of particles increases, the probability of finding a vacancy immediately adjacent to a tracer particle into which it may diffuse decreases, resulting in a concomitant reduction of the tracer diffusion coefficient. Using scaled particle theory to calculate the concentration-dependent probabilities, a simple approximate result is obtained in closed form, that is compared with the results of previously published Monte Carlo lattice simulations and experimental observations.

INTRODUCTION

Experimental measurements of diffusion of membrane proteins within the plane of a synthetic membrane indicate that lateral diffusion coefficients decrease substantially as the total area of membrane occupied by protein increases (Peters and Cherry, 1982; Tank et al., 1982). It is estimated that a typical biological membrane contains intrinsic proteins occupying on the order of one-quarter of the surface area (Grasberger et al., 1986). Thus the effect of area occupancy on the surface diffusion of a membrane protein may have significant ramifications for the kinetics of a variety of biochemical processes thought to involve surface diffusion of one or more membrane protein species (Axelrod, 1983).

The tracer diffusion of several soluble proteins in aqueous solutions containing various amounts of different "background" protein species has recently been measured (Muramatsu and Minton, 1988). It was found that the dependence of tracer diffusion coefficients upon the concentrations and relative sizes of background species could in most cases be semiquantitatively described by an extremely simple model, wherein each of the protein species was represented by an equivalent hard sphere having a radius close to that calculated from simple steric considerations of molecular mass and partial specific volume. In this model it is assumed that a tracer molecule may diffuse only if an adjacent element of volume, into which it may diffuse, can be found which is free of any part of a background molecule. The rate of tracer diffusion decreases with increasing concentration of background species in accordance with the decreasing probability of finding such an element of free volume, calculated using scaled particle theroy.

The effect of area occupancy upon tracer diffusion in two dimensions has previously been treated by Monte Carlo simulation using a model wherein tracer and background particles hop between adjacent sites on a planar lattice (Pink, 1985; Saxton, 1986; and references therein). The purpose of the present communication is to present the two-dimensional analogue of the simple scaled particle model previously developed for diffusion in three dimensions, and to compare and contrast results obtained using this model with those previously obtained via Monte Carlo simulation and with the limited experimental data currently available.

DESCRIPTION OF MODEL

As the model described below is quite similar to that presented in Muramatsu and Minton (1988), it will be described here in condensed form; Muramatsu and Minton (1988) should be consulted for details. It is assumed that diffusion of an intrinsic protein in the plane of a membrane may be represented by a two-dimensional random walk in which the diffusing species undergoes a Brownian displacement of average distance Δr on an average of once every Δt seconds. A Brownian displacement involves movement of the diffusing (tracer) particle into an element of adjacent surface area termed the target area (shaded area in Fig. 1 *A*). When an additional background species is present in the membrane, there exists a probability that part of the target area will be occupied by parts of one or more background particles. The probability of undergoing a Brownian displacement during a given time interval is assumed to be proportional to the probability that the target area is entirely vacant of any part of a background particle. These considerations

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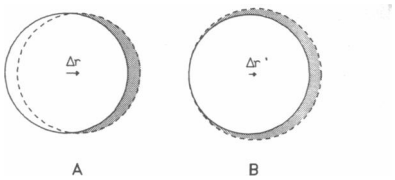

FIGURE 1 (*A*) Schematic representation of a two-dimensional Brownian displacement. Shaded target area, which must be free of background particles, is generated by translating the circular diffusing particle by distance Δr. (*B*) Approximation to target area shown in *A* generated by translating diffusing particle by $\Delta r'$ and simultaneously increasing its radius by the same amount. Figure reproduced from Muramatsu and Minton (1988).

lead to the relation (Muramatsu and Minton, 1988)

$$ln\, D/D_o = -\Delta G_b^*/RT, \tag{1}$$

where D is the tracer diffusion coefficient at finite concentration, D_o is the tracer diffusion coefficient in the dilute limit, and ΔG_b^* is the negentropic work required to vacate the target area of background particles. ΔG_b^* is approximated by $\Delta G_b'$, the negentropic work required to vacate an area of similar shape (shaded area in Fig. 1 *B*), generated by simultaneously translating the diffusing particle by $\Delta r'$ and increasing its radius by the same amount.

Letting r_t represent the tracer radius, we obtain (Maramatsu and Minton, 1988)

$$\Delta G_b' = \Delta G_c(r_t + \Delta r') - \Delta G_c(r_t), \tag{2}$$

where $\Delta G_c(r)$ is the negentropic work required to create a circular cavity of radius r free of any part of a background particle in a two-dimensional fluid comprising hard disks of radius r_b occupying a fraction ϕ of total area. Scaled particle theory (Lebowitz et al., 1965) provides a simple closed form relation for approximate calculation of this quantity:

$$\Delta G_c(r)/RT = B_0 + B_1 r + B_2 r^2, \tag{3}$$

where

$$B_0 = -\ln(1-\phi)$$

$$B_1 = \frac{2}{r_b}\frac{\phi}{1-\phi}$$

$$B_2 = \frac{1}{r_b^2}\left[\frac{\phi}{1-\phi} + \left(\frac{\phi}{1-\phi}\right)^2\right].$$

Eqs. 2 and 3 are combined to yield

$$\Delta G_b'/RT = B_1\Delta r' + B_2(2r_t\Delta r' + \Delta r'^2). \tag{4}$$

Let Δr^* be that value of $\Delta r'$ such that $\Delta G_b' = \Delta G_b^*$, and assume for simplicity that it is independent of the concentration of background molecules, i.e., solely a property of the tracer species. Then Eqs. 1, 3, and 4 may be combined to yield

$$\ln D/D_o = -\left[\frac{x}{f}\left(2 + \frac{2+x}{f}\right)\right] Q - \left[\frac{x}{f}\cdot\frac{2+x}{f}\right]Q^2, \tag{5}$$

where

$$Q \equiv \frac{\phi}{1-\phi}$$

$$x \equiv \Delta r^*/r_t$$

and

$$f \equiv r_b/r_t.$$

The dependence of diffusion coefficient upon ϕ, the fraction of area occupied by background protein, is thus presented as a simple function of the ratio of the sizes of background and tracer species, and an adjustable scaling parameter, x, which is smaller than unity and assumed constant for a given tracer species.

RESULTS AND DISCUSSION

Calculations of D/D_o as a function of ϕ are plotted in Fig. 2 for various combinations of f and x. Two trends are immediately obvious. The dependence of D upon ϕ becomes stronger as the ratio of Brownian step size to tracer size becomes larger, and as the ratio of background species size to tracer size becomes smaller.

Consider the case of tracer diffusion of a single species of protein in a reconstituted membrane containing only that species (self-diffusion). Let us assume that at low resolution, the cross-section of the diffusing protein in the plane of the membrane may adequately be represented by

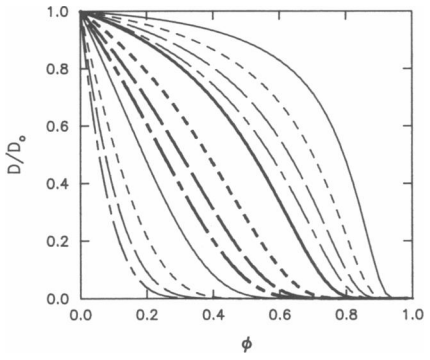

FIGURE 2 Dependence of D/D_o upon fractional area occupancy by hard circular particles, calculated via Eq. 5. Three sets of curves are shown; in each set the solid curve was calculated with $x = 0.1$, short-dashed with $x = 0.2$, *long-dashed* with $x = 0.3$, and dot-dashed with $x = 0.4$. The rightmost set of curves was calculated with $f = 3$, the middle set with $f = 1$, and the leftmost set with $f = \frac{1}{3}$.

a circle. For this case $f = 1$. One would expect that with increasing tracer size, the ratio of the Brownian step size to tracer size (that is, the parameter x) would decrease, and hence the dependence of D/D_0 upon ϕ as well. This result may be contrasted with that obtained by Saxton (1986) from Monte Carlo simulations of random walks of hexagons of various sizes on a triangular lattice. He reported that the concentration dependence of self-diffusion did not vary significantly with the size of the hexagon. Assuming that the size of individual hops on the lattice remains constant as the size of the hexagons increases, his result disagrees qualitatively with that of the present treatment.

The dependence of D/D_0 upon fractional site occupancy ϕ^* of hexagons on a triangular lattice, calculated by Pink (1985) and Saxton (1986) for hexagons of comparable size, is plotted in Fig. 3. The results of the two independently performed simulations agree reasonably well out to a fractional area occupancy of 0.6. Above that value, the value of log (D/D_0) calculated by Saxton decreases much more rapidly with increasing area occupancy than does that calculated by Pink.

Four curves, representing the dependence of D/D_0 calculated using Eq. 5 with $f = 1$ and five different values of x, are also plotted in Fig. 3. The value of ϕ used in calculating these curves has been taken to be equal to 0.907 times the value of fractional site occupancy used for the lattice simulations to partially take into account the fact that hexagons can completely fill a plane, whereas hexagonal close packing of circles only fills 0.907 of planar area. It is evident that the dependence of D/D_0 upon fractional area occupancy calculated according to Eq. 5 cannot be made to agree with the results of the simulations over the entire range of ϕ for any fixed value of x. Reasonable agreement can, however, be achieved over the range $0 < \phi^* < 0.3$.

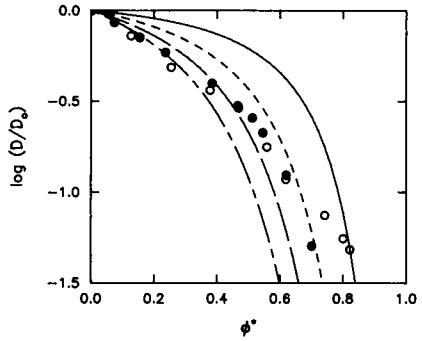

FIGURE 3 Dependence of log (D/D_0) for two-dimensional self-diffusion upon fractional site occupancy by hard hexagons (Pink, 1985; Saxton, 1986). The corresponding fractional occupancy of a surface by hard circular particles is $\phi = 0.907\,\phi^*$ (see text). (*Open circles*) results of Pink (1985). (*Filled circles*) results of Saxton (1986) for $R = 4$ (notation of Saxton [1986]). Smooth curves calculated using Eq. 5 with $f = 1$ and $x = 0.1$ (*rightmost curve*), 0.2, 0.3, and 0.4 (*leftmost curve*).

Both the lattice model (Pink, 1985; Saxton, 1986) and the present model predict concentration dependences of the self-diffusion coefficient that are qualitatively smaller than observed experimentally for two different membrane proteins (Peters and Cherry, 1982; Tank et al., 1982). Our results thus confirm previous assertions (Pink, 1985; Saxton, 1986) that factors other than excluded volume, such as protein-protein interactions, must be contributing substantially to the concentration dependence reported in Peters and Cherry (1982) and Tank et al. (1982).

At the present time we are unaware of any experimental data or any other model describing the effect of increasing concentration of one species of membrane protein upon the tracer diffusion coefficient of a second species of membrane protein, with which the present model may be compared.

The present model and its three-dimensional analogue differ from previous two-dimensional and three-dimensional free volume theories of diffusion (O'Leary, 1987, *a* and *b*) in two important respects. In the theories of O'Leary it is assumed that the element of free volume that must be created for a Brownian jump to take place is circular (or spherical), and must have a radius equal to that of the tracer species. Here and in Muramatsu and Minton (1988), this element of free volume is not assumed to be circular (or spherical), and its volume is only a fraction of that of the tracer. The relations used in the present work and in Muramatsu and Minton (1988) to calculate the negentropic work of creating the requisite element of free volume are significantly different from those used by O'Leary and are based on what we feel is a more reasonable physical model of diffusive motion.

There are several possible reasons why the predictions of the scaled particle model are at variance with the results of the lattice model simulations. These include (*a*) unphysical quantization of translation and/or orientation imposed by the lattice model and (*b*) oversimplification of the present model deriving from the assumption that the parameter Δr^* is independent of background concentration and/or neglect of correlation between the probabilities of successful Brownian displacements at time t and time $t + \Delta t$. Because currently available data are insufficient to indicate which of the two approaches yields a more realistic result, we hope that the present communication will stimulate development of a reliable experimental model for tracer diffusion of hard particles in a two-dimensional fluid.

Received for publication 22 July 1988 and in final form 24 October 1988.

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