Lateral diffusion in a mixture of mobile and immobile particles
A Monte Carlo study

Michael J. Saxton
*Plant Growth Laboratory, University of California, Davis, California 95616; and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720 USA

ABSTRACT The lateral diffusion coefficient for mixtures of mobile and immobile particles is obtained from Monte Carlo calculations of random walks by mobile tracers in the presence of immobile obstacles on a triangular lattice. The diffusion coefficient of the mobile species is obtained as a function of the area fractions of mobile and immobile species. The results are applied to diffusion of band 3 in the erythrocyte membrane, and indicate that obstruction of diffusion of mobile band 3 by band 3 and glycophorin attached to the membrane skeleton is not sufficient to explain the observed diffusion coefficient.

INTRODUCTION

Models of the concentration dependence of the lateral diffusion coefficient in membranes have treated two limiting cases, percolation and tracer diffusion. In percolation, mobile tracer particles are present at negligibly low concentration, and diffuse in the presence of a specified concentration of immobile obstacles (Eisinger et al., 1986; Saxton, 1987). There is a percolation threshold (Stauffer, 1985; Feder, 1988); at concentrations of obstacles above the threshold, long-range diffusion of tracers is blocked, and the long-range diffusion coefficient is zero. In tracer diffusion, all particles in the membrane are mobile and move with the same diffusion coefficient. There is no percolation threshold; diffusion is allowed at all concentrations, but the diffusion coefficient decreases as the concentration of diffusing particles increases (Pink, 1985; Saxton, 1987; Abney et al., 1989).

In many cell membranes, however, an intermediate case is likely, in which some species are immobilized, some species are freely diffusing, and the concentration of freely diffusing species is not necessarily negligible. This paper presents Monte Carlo calculations of lateral diffusion for mixtures of mobile and immobile particles. These calculations yield the diffusion coefficient of the mobile particles as a function of the concentrations of mobile and immobile particles.

METHODS

Monte Carlo calculations are carried out as described earlier (Saxton, 1987). Points or hexagons are placed randomly on a triangular lattice at the required concentration, and are randomly designated as tracers, which are mobile, or obstacles, which are immobile. In any particular run, the obstacles and the tracers are the same size. The tracers execute a random walk on the lattice, moving to unblocked nearest-neighbor sites. A jump by a tracer may be blocked by an obstacle or by another tracer. Point particles may not occupy the same lattice site, and hexagonal particles are not allowed to overlap, even at edges or vertices. The mean-square displacement of the tracers is obtained as a function of the number of time steps, and the diffusion coefficient is obtained as described earlier. Concentrations are expressed as area fractions, defined as the fraction of lattice points occupied by particles.

A 256 × 256 triangular lattice is used, with periodic boundary conditions. The length of a run is characterized by the number of Monte Carlo steps per particle (MCS/p), the average number of moves attempted per tracer. For point particles, the runs ranged between 1,000 and 2,000 MCS/p, and runs were repeated for 25–500 different initial configurations. For hexagons of radius 1, the runs were 1,500–10,000 MCS/p, and 25–500 repetitions; for hexagons of radius 5, the runs were 1,700–14,000 MCS/p, and 175–3,500 repetitions. Earlier runs (Saxton, 1987) were shorter, 200–400 MCS/p.

To examine the statistical error, repeated runs were made for a mixture of equal numbers of mobile and immobile particles with a total area fraction of 0.2. The sequence of random numbers was varied; the number of time steps was varied by a factor of 5 (for $R = 1$) or 2 (for $R = 5$); and the number of repetitions was varied by a factor of 2. For $R = 1$, $D^* = 0.4757 \pm 0.0048$ (mean ± SD, six runs), and for $R = 5$, $D^* = 0.4550 \pm 0.0077$ (five runs). The difference in D^* for $R = 1$ and $R = 5$ is small (<5%); in applications to cell membranes, the statistical error in D^* and the R dependence of D^* are small compared with the uncertainties in the observed D^* and the area fraction of obstacles. (The value $R = 5$ was chosen to provide an upper limit for the radius of band 3; see below.)

RESULTS

Diffusion coefficients are obtained from calculations of random walks of point or hexagonal particles on a triangular lattice. The only interaction between particles is a hard-core repulsion. As before (Saxton, 1987), a lipid corresponds to a single lattice point, with radius $R_L = 0$ and area $A_L = 1$, and a protein corresponds to a hexagon of radius R and area $A(R) = 3R^2 + 3R + 1$. The radius

*Address for correspondence.

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is defined as the distance (in lattice constants) from the center to a vertex, and the area is defined as the number of lattice points within, or on the perimeter of, a hexagon. Concentrations are expressed as area fractions, and the diffusion coefficient D^* is normalized so that $D^* = 1$ when the concentration of obstacles is zero and the concentration of tracers approaches zero.

For example, the area of a hexagon of radius 1 is 7. If the fraction of lattice sites occupied by centers of hexagons is 0.05, the area fraction occupied is 0.35. The lattice constant for the triangular lattice is $\ell_{TRI} = 0.8$ nm, so the area occupied by one lipid is $(\sqrt{3}/2)\ell_{TRI}^2 = 0.554$ nm^2, and a hexagon of area 7 corresponds to a protein of area 3.88 nm^2.

If c_M is the area fraction of mobile particles and c_I is the area fraction of immobile particles, then the total concentration of particles is $c_T = c_M + c_I$. In Fig. 1, calculated diffusion coefficients D^* are given as a function of c_M for various values of c_T. Fig. 1 *a* shows the results for point particles, and Fig. 1 *b* shows the results for hexagons of radius 1 and 5.

Note that at any fixed c_M and c_T, there is a significant difference in D^* between hexagons and point particles, but little difference between hexagons of radius 1 and hexagons of radius 5. Similar results were obtained earlier for tracer diffusion; the R-dependence was small for R between 1 and 16 (Saxton, 1987).

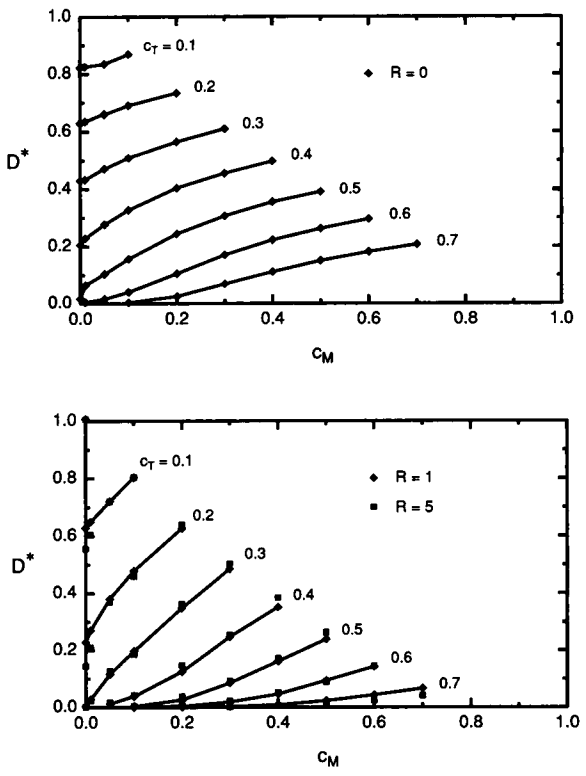

FIGURE 1 Calculated lateral diffusion coefficients D^* as a function of the area fraction c_M of mobile particles for the indicated total area fractions c_T of mobile and immobile particles. (*a*) Point particles. (*b*) Hexagons of radius 1 and 5. The lines join points for radius 1.

DISCUSSION

The results may be applied to lateral diffusion of the anion transport protein band 3 in the normal erythrocyte. A comparison of D in normal and spectrin-deficient spherocytic (S^-) mouse erythrocytes showed that D(normal) ~ $1/50D(S^-)$ (Sheetz et al., 1980).

Several explanations of the reduced diffusion coefficient have been proposed. First, diffusion may be obstructed by the membrane skeleton (Cherry et al., 1976; Koppel et al., 1981; Tsuji and Ohnishi, 1986; Tsuji et al., 1988; Saxton, 1989, 1990*a*). Here the membrane skeleton provides a series of transient barriers which obstruct diffusion by blocking the cytoplasmic domain of band 3. This domain is elongated; its length has been estimated as 25 nm (Low, 1986). Second, diffusion may be hindered by transient binding of mobile proteins to proteins immobilized by attachment to the membrane skeleton (Elson and Reidler, 1979; Koppel, 1981; Golan, 1989). Third, diffusion may be hindered by the high concentration of protein of the membrane, some bound to the membrane skeleton and some freely diffusing. The first two mechanisms are reviewed by Golan (1989) and by Saxton (1990*b*).

To review the structure of the membrane skeleton very briefly, spectrin tetramers form the bonds of a network, and actin oligomers form the nodes; approximately six tetramers are bound to each node. Ankyrin links spectrin to band 3, and band 4.1 links spectrin to glycophorin. These connections to integral proteins attach the network to the cytoplasmic surface of the membrane (Goodman et al., 1988; Bennett, 1989; Lux and Becker, 1989). Both dimers and tetramers of band 3 may be present (Jay and Cantley, 1986).

To estimate the effect of high concentrations of band 3 on lateral diffusion, we need to estimate the area fraction of band 3. Weinstein et al. (1979) proposed that intramembrane particles (IMP's) are band 3 tetramers, and found the diameter of an IMP to be 6.6 nm. Using this value and the range of densities of IMP's given by Weinstein et al. (1979), we obtain area fractions between 0.09 and 0.11. Golan et al. (1984) estimated the area fraction to be 0.17; we assume a value of 0.2 as an upper limit. A protein of radius 3.3 nm corresponds to a hexagon of radius 4.03.

An estimate of the fraction of immobile band 3 can be obtained from the stoichiometry. An erythrocyte has 1×10^5 ankyrin molecules and 1×10^6 monomers of band 3. One ankyrin binds one band 3 monomer (Goodman et al.,

1988). So if band 3 is a tetramer, there would be 2.5×10^5 tetramers, of which 1×10^5, or 40%, are bound. This is the same fraction obtained in rotational diffusion measurements (Nigg and Cherry, 1980), though the situation is in fact more complicated (Clague et al., 1989). From Fig. 1 *b*, if 40% of an area fraction of 0.2 is immobile, then $D^* = 0.49$. But even if 95% of band 3 were immobile, the diffusion coefficient would only be reduced by a factor of about one-fifth, not the factor of one-fiftieth observed.

One modification to the model would be to include the effect of boundary lipid. Assume a circular shell of boundary lipid rigidly attached to band 3, and assume the radius of a lipid to be $r_L = 0.42$ nm (giving the area of 0.554 nm^2 used earlier). Then the area of an IMP increases by a factor of $(r_P + 2r_L)^2/r_P^2 = 1.57$, and an area fraction of $c_T = 0.20$ increases to 0.31. From Fig. 1 *b*, D^* is still much larger than the experimental value unless practically all of the protein is immobilized.

The effect of glycophorin is not enough to account for the observed diffusion coefficient. The area occupied by the hydrophobic chain of glycophorin is negligible. If the area per chain is 0.34 nm^2 (Edholm and Johansson, 1987) and the total number of copies of glycophorin A, B, and C is 730,000 (Goodman et al., 1988), then the area fraction occupied by the chains is only 0.0018.

The contribution from the headgroup, however, is significant. Rüppel et al. (1982) found that the headgroup of glycophorin A interacts with ~100 lipids (for high concentrations of glycophorin A, when it is in the three-dimensional configuration). If there are 2.2×10^8 lipid molecules in one leaflet of the membrane (Lux and Becker, 1989), and the headgroups of all the glycophorins are the same size, then the total area fraction occupied by glycophorins is 0.33. Consider the combined effect of the glycophorins and band 3. If the area fraction of glycophorins is 0.33, and 70% is mobile, as observed (Golan, 1989), and the area fraction of band 3 is 0.20, and 50% is mobile (Golan, 1989), then $c_T = 0.53$, $c_M = 0.33$, and from Fig. 1 *b*, $D^* = 0.09$, still a factor of 4.5 higher than observed.

So, according to the model, obstruction of diffusion by a combination of mobile and immobile band 3 and glycophorin does not account for the observed diffusion coefficient. Obstruction of diffusion by the membrane skeleton, or transient binding of mobile band 3 to immobile band 3, or both, may be involved (Golan, 1989; Saxton, 1990*b*).

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