DIFFUSION OF MOLECULES ON BIOLOGICAL MEMBRANES OF NONPLANAR FORM

II. Diffusion Anisotropy

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ABSTRACT Molecules diffusing on nonplanar membranes, which have different amounts of corrugation in different directions, may experience dissimilar diffusion coefficients in each direction. Smith et al. (1979, *Proc. Natl. Acad. Sci. USA,* 76:5641–5644) measured diffusion anisotropy on fibroblast cell membranes in which the ratio of the diffusion coefficients, in different directions, was 0.27. In the present work we calculate the effect of anisotropic corrugation on the rate of diffusion of molecules on membranes. We find that part of the anisotropy reported by Smith et al. (1979, *Proc. Natl. Acad. Sci. USA,* 76:5641–5644) can be explained by the membrane nonplanarity, and we present the way of calculating this geometric factor.

INTRODUCTION

Molecules can have the ability to move laterally on biological membranes (1). A quantitative approach to measuring the lateral mobility of molecules on membranes is fluorescence photobleaching recovery (FPR) (2–6). The probed molecules in these experiments are either naturally or artificially marked with a fluorescent dye. Using laser light, small areas on the membrane are bleached. The bleached molecules move out of the areas in which they are initially localized and unbleached molecules move into the bleached areas. There exist two techniques of FPR. The first is spot FPR (4, 5), in which a circular area is bleached and the fluorescence recovery in it is measured vs. time. The more recent technique is that of pattern FPR (6), in which a number of areas are bleached on the membrane with a periodical pattern created by passing the bleaching beam through a Ronchi ruling. From the measured fluorescence intensity in the bleached areas at different times, a diffusion constant is calculated. In the usual interpretation of the results, membranes are considered as planar surfaces. The difficulty is, however, that in many cases biological membranes are not planar, but rather have blebs and microvilli.

In previous work (reference 8) we have calculated the effect of nonplanarity (in the form of microvilli) on the measured diffusion coefficient by spot photobleaching recovery. For spot FPR and for a model surface of $A \cos kx \cos ky$ ($k = 10\pi\ \mu\text{m}^{-1}$) we obtained the following results: Considering the surface (with microvilli length of 1 μm) to be planar results in a diffusion coefficient smaller than the real one by about a factor of 2. Because of the tortuous shape of the spot boundary, changing the length of the microvilli from small (0.5 μm) to longer (1, 2 μm) ones does not change the rate in which the bleached molecules leave the spot. These considerations are valid for short times, i.e., when the diffusion out of the bleached spot is dominated by molecules that were initially located in the neighborhood of the spot boundary. On the other hand, these calculations may represent experimental situations in which the diffusion coefficient is calculated from the half decay time, as is usually done in spot FPR. Experimental results of Dragsten et al. (9) and of Wolf et al. (10) are compatible with these calculations.

In this work we consider the effect of membrane nonplanarity on the measured diffusion coefficients using pattern FPR. One of the advantages of this method is that it can be used to measure diffusion coefficients along different directions on the cell surface. Smith et al. (7) found, by this method, that the diffusion of succinyl-concanavalin A receptors on the surfaces of adherent mouse fibroblast cells, having parallel stress fibers, is anisotropic. They have shown that the diffusion coefficient in the direction parallel to the direction of the cytoplasmic stress fibers, D_{fast}, appears to be approximately four times larger than that in the direction perpendicular to these fibers, D_{slow}. There are many possible reasons why the diffusion is slow in the direction perpendicular to the stress fibers. One such mechanism is the possible existence of cytoplasmic structures parallel to the stress fibers, which will slow down large scale motion in the transverse direction of membrane proteins that extend into the cytoplasm and interact with these structures. This is a physical effect. On the other hand, the geometry of the membrane shape affects the diffusion differently in each direction. If the membrane is folded in the direction perpendicular to the stress fibers, then the lateral diffusion of membrane proteins projected on a plane will appear to be slower in the direction perpendicular to the stress fibers than in the parallel direction. This will create an apparent diffusion anisotropy. In the following sections we calculate the possible effect of nonplanarity on the measured diffusion coefficient and apply the results to a membrane that is folded in one direction and therefore has a diffusion anistropy if the surface is considered to be a plane (this is assumed in FPR procedures). Then we examine what part of the diffusion anisotropy can be explained by geometric anisotropy in addition to other physical effects.

LARGE SCALE DIFFUSION ON A PLANAR PERIODIC ONE-DIMENSIONAL MANIFOLD

We consider a surface that can be described as a plane which is corrugated in one direction. The corrugation is in the direction perpendicular to the direction of the stress fibers in the mouse fibroblasts on which the diffusion measurements were done by Smith et al. (7). We choose the corrugated and the noncorrugated directions to be x and y, respectively. The diffusion problem on this surface can be split into a simple linear diffusion along the noncorrugated direction y, and a one-dimensional diffusion on a curved line lying in the plane xz. In the forthcoming paragraphs we discuss the diffusion problem along this curved direction.

The curved line in the xz plane can be described by the equation:

$$z = f(x) . \tag{2a}$$

It is assumed that $f(x)$ is a continuous function of x and is periodic with a period ℓ. Because of the topological equivalence of any reasonable curve to a straight line, the problem of diffusion on a curved line can be transformed to the solution of a regular one-dimensional diffusion equation:

$$C_t = D\, C_{ss} , \tag{2b}$$

where $C_t \equiv \partial C/\partial t$, $C_{ss} \equiv \partial^2 C/\partial s^2$, D is the diffusion coefficient along the curved line, and s is the natural parameter of the line, i.e., the length of the line measured from some arbitrary point (say $x = 0$). In this case,

$$s = \int_0^x (1 + f_x^2)^{1/2}\, dx , \tag{2c}$$

where $f_x \equiv \partial f/\partial x$. Substituting Eq. 2c into Eq. 2b yields the following diffusion equation:

$$C_t = D[C_{xx}/(1 + f_x^2) - C_x f_x f_{xx}/(1 + f_x^2)^2] , \tag{2d}$$

where $f_{xx} \equiv \partial^2 f/\partial x^2$, $C_x \equiv \partial C/\partial x$ and $C_{xx} \equiv \partial^2 C/\partial x^2$. This equation corresponds to Eq. I.14 of reference 8. We would like to compare the diffusion along the curved line $f(x)$ to a diffusion along a straight line by transforming Eq. 2d to a diffusion equation of a form $C_t = D_{eff} C_{xx}$ and compare D_{eff} with D. It can be shown (Aizenbud, B. M., and N. D. Gershon, manuscript to be submitted for publication) that for such a line and in large scale experiments (where the measurement scale is much larger than ℓ or the results are averaged over a scale comparable with the period, as it is in FPR measurements) the diffusion equation 2d can be replaced by:

$$C_t = D_{eff}\, C_{xx} , \tag{2e}$$

where

$$D_{eff} = (\ell/L)^2 D \tag{2f}$$

and L is the actual length of the line contained in one period:

$$L = \int_0^\ell (1 + f_x^2)^{1/2}\, dx . \tag{2g}$$[1]

Thus the ratio of the diffusion coefficients in the curved and planar directions, D_{slow}/D_{fast}, is given by

$$D_{slow}/D_{fast} = D_{eff}/D = (\ell/L)^2 , \tag{2h}$$

where only the geometrical effect is included in D_{slow}. This relation can be obtained simply if we restrict our consideration to steady state processes (Hardt [11] and Aizenbud, B. M., and N. D. Gershon, manuscript to be submitted for publication. This supplemental material can be obtained from the authors upon request). As is demonstrated in the next section, this relation is useful in determining how a nonplanarity of a surface along one axis changes the observed diffusion.

GEOMETRICAL FACTOR OF ANISOTROPY

In this section we take the result (Eq. 2h), apply it to diffusion along a periodic line, and calculate the diffusion anisotropy on a model surface that results from corrugation of the membrane in only one direction. To estimate the effective diffusion coefficient, we have to calculate L, the

[1]Cohen et al. (12) considered one-dimensional diffusion with a periodic diffusion coefficient. They showed that at large scales (compared with the period of the diffusion coefficient) the diffusion process can be described in terms of an effective diffusion coefficient D_{eff}, such that $1/D_{eff} = \langle 1/D \rangle$, where the angular brackets denote an average. The problem considered here cannot be transformed to theirs.

length of the line contained in one period. This result can then be applied to calculate the ratio of the diffusion coefficients in two perpendicular directions in a surface that is wavy in one direction (Fig. 1), as observed on a projection of the surface on a plane. FPR measurements are performed over relatively large areas of the membrane, and therefore these results can be used to estimate the effect of nonplanarity in one direction on the observed diffusion anisotropy in such surfaces.

We consider a membrane that is curved and periodic only along the x direction (Fig. 1) and can be described by

$$z = a \sin kx , \qquad \text{(3a)}$$

where a is the amplitude and k is the wave vector ($k = 2\pi/\ell$). Then the length of the cross section along x of one period is according to Eq. 2c

$$L = \int_0^{\ell} [1 + (ak)^2 \cos^2 kx]^{1/2}\, dx$$
$$= (1/k) \int_0^{2\pi} [1 + (ak)^2 \cos^2 u]^{1/2}\, du , \qquad \text{(3b)}$$

where $u = kx$. It then follows that

$$D_{slow}/D_{fast} = (4\pi^2) \bigg/ \left\{\int_0^{2\pi} [1 + (ak)^2 \cos^2 u]^{1/2} du\right\}^2$$
$$= \pi^2/(4[1 + (ak)^2]\, E^2\{ak/[1 + (ak)^2]^{1/2}\}) , \qquad \text{(3c)}$$

where E is a complete elliptic integral of the second kind. (Notice that our $E(z)$ corresponds to $E(z^2)$ of reference 13.) From this relation (Eq. 3c) the dependence of the anisotropy, D_{slow}/D_{fast} on the surface wavelength, ℓ, and on the amplitude, a, can be calculated. The results are given in Fig. 1 and in Table I. We see from Fig. 1 that the dependence of the anisotropy on the amplitude (for fixed wavelength) looks hyperbolic. Also, it is clearly illustrated in this figure that the dependence of the amplitude on the wavelength is linear (for a given anisotropy), for example, for an anisotropy 0.25 and for a corrugated surface height of ridges = $0.4 \times \ell$.

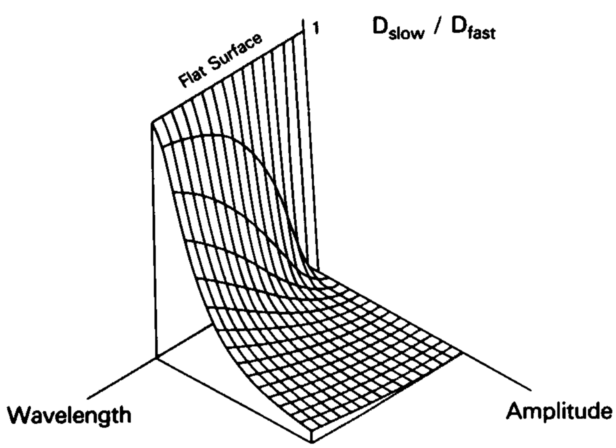

FIGURE 1 The dependence of diffusion anisotropy, D_{slow}/D_{fast}, on the amplitude and wavelength of the corrugated surface—$z = a \sin kx$. D_{slow} is the diffusion coefficient along the corrugated axis, x and D_{fast} is the diffusion constant along the noncorrugated axis, y. The amplitude of the corrugation is a, and its wave vector (2π/wavelength) is k.

TABLE I
THE DEPENDENCE OF D_{slow}/D_{fast} ON a, THE AMPLITUDE OF THE CORRUGATION FOR THE SURFACE $z = a \sin kx$

ak	D_{slow}/D_{fast}
0.0	1.000
0.1	0.995
0.2	0.980
0.3	0.957
0.4	0.927
0.5	0.890
0.6	0.850
0.7	0.807
0.8	0.763
0.9	0.719
1.0	0.676
2.0	0.353
3.0	0.202
4.0	0.127
5.0	0.086
6.0	0.062
7.0	0.047
8.0	0.036
9.0	0.029
10.0	0.023

The result of Eq. 3c can be easily generalized to any periodic function that can be described by the equation

$$z(x) = a\, f(kx) . \qquad \text{(3d)}$$

Here a is the amplitude, k is $2\pi/\ell$, where ℓ is the wavelength. It is easy to show that

$$L = \int_0^{\ell} \{1 + [akf'(kx)]^2\}^{1/2}\, dx$$
$$= (1/k) \int_0^{2\pi} \{1 + [akf'(u)]^2\}^{1/2}\, du \qquad \text{(3e)}$$

and therefore

$$D_{slow}/D_{fast} = 4\pi^2 \bigg/ \left(\int_0^{2\pi} \{1 + [akf'(u)]^2\}^{1/2}\, du\right)^2 . \qquad \text{(3f)}$$

Of course when $Ak \rightarrow 0$, $D_{slow}/D_{fast} \rightarrow 1$, and when $Ak \rightarrow \infty$, $D_{slow}/D_{fast} \rightarrow 0$. For a fixed value of k, D_{slow}/D_{fast} decreases hyperbolically when a increases. Therefore, Fig. 1 describes qualitatively any surface described by Eq. 3d.

DISCUSSION

A central question in this work is the influence of nonplanarity on diffusion anisotropy. Given the results of "Large Scale Diffusion on a Planar Periodic One-Dimensional

Manifold" and "Geometrical Factor of Anisotropy," we can answer this question and see, for example, what part of the anisotropy observed by Smith et al. (7) can be explained by nonplanarity.

Smith et al. (7) obtained for the ratio of the diffusion coefficients in the perpendicular and the parallel directions, D_{slow}/D_{fast}, the value of 0.27. From relation 2h it follows that if the whole anisotropy is due to nonplanarity, the length of the membrane in the corrugated direction (x in Fig. 1) has to be about twice as long as in the noncorrugated one (y in Fig. 1). If the surface in the corrugated direction can be described by a simple sin kx function, then, as mentioned in the previous section, the height of the ridges should be 0.4 times the wavelength. This means, for example, that for an amplitude $a = 0.04$ μm, the wavelength ℓ should be 0.1 μm and for $a = 0.08$ μm, $\ell = 0.2$ μm. Under the usually employed experimental conditions in electron microscopy, there is no evidence for such pronounced corrugation (14 and Triche, T., private communication).

Another possible source of anisotropy is that the surface is smooth, but is curved in the slow direction like an arc. In order to produce an anisotropy of 0.25, for example, the length of the arc has to be about twice as long per unit increment in the x direction as in the fast direction. If the arc can be represented by a half circle with a diameter of 10 μm, D_{slow}/D_{fast} is only 0.4.

From these considerations it is clear that corrugation alone cannot explain the amount of diffusion anisotropy obtained by Smith et al (7). Thus, in addition to possible corrugation perpendicular to the direction of the stress fibers, there should be contributions from other effects, mentioned by Smith et al. (7), i.e., some of the diffusing proteins might be excluded from elongated regions that are above the stress fibers due to interactions with cytoplasmic structures, or due to variations in lipid composition.

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