Journal of Supramolecular Structure
and Cellular Biochemistry 17:61-67 (1981)
Cellular Recognition 345-351

Association Dynamics and Lateral Transport in Biological Membranes

Dennis E. Koppel

Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032

A theoretical analysis is presented for the interrelated effects of lateral diffusion and a simple form of molecular association ($A + B \rightleftharpoons C$) in biological membranes. Expressions are derived for the characteristic functions measured in fluorescence redistribution after photobleaching experiments, corresponding to both the Fourier transform analysis of concentration in a plane and the normal mode analysis for a spherical surface. The results are related to the reputed binding of integral membrane proteins to submembranous cytoskeletal elements.

Key words: diffusion, fluorescence photobleaching, interactions with cytoskeleton

Considerable evidence has accumulated in recent years indicating that lateral diffusion rates of integral membrane proteins are generally controlled by interactions with submembranous cystoskeletal elements [1 – 7]. This is a result of considerable theoretical interest, in light of proposals that such interactions may act to mediate the coordination of such cell functions as cell growth, movement, and recognition [8]. Questions of possible function aside, considerable controversy exists concerning the nature of the dominant molecular interactions [5, 8 – 11]. One can envisage two basic mechanisms which might act in concert to limit integral membrane protein diffusion: (1) an indirect steric hinderance, in which a labile submembranous cytoskeletal matrix blocks the motion of transmembrane proteins projecting beyond the lipid bilayer; and (2) direct, specific, and reversible attachment of the integral membrane proteins to the matrix. We have recently developed a theoretical formulation of the first of these [5]. It is the object of this paper to present a brief treatment of the second, applicable to the types of data that one can obtain in fluorescence redistribution after photobleaching (FRAP) experiments. Analyses of similar problems have appeared previously [12, 13, 21]. This note has been prompted by recent advances in the FRAP technique [2, 14–16] which greatly facilitate the analysis.

Received April 21, 1981; accepted July 14, 1981.

0275-3723/81/1701-0061$02.50 © 1981 Alan R. Liss, Inc.

THEORY

The Problem

We consider the basic reaction of the form

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C, \tag{1}$$

where A is a fluorescently labeled integral membrane component, with diffusion coefficient D_A, which reacts with binding sites B to produce the fluorescent complex C, with diffusion coefficient D_C. In a FRAP experiment, the photobleaching pulse depletes the observable concentrations of A and C, leaving B at the average concentration $\bar{c}_B$. The parameters of association and diffusion are deduced from the kinetics of redistribution after photobleaching.

The analysis that follows will be carried through for two different geometries: a plane and the surface of a sphere of radius r. In the first case, we stipulate a periodic photobleaching pattern [14]; in the latter, an azimuthally symmetric one [16]. After photobleaching, in both cases, $c_A(x,t)$ and $c_C(x,t)$, the concentrations of A and C, respectively, evolve according to the following equations:

$$\frac{dc_A(x,t)}{dt} = D_A\nabla^2 c_A(x,t) - k_1\bar{c}_B c_A(x,t) + k_2 c_C(x,t) \tag{2a}$$

$$\frac{dc_C(x,t)}{dt} = D_C\nabla^2 c_C(x,t) + k_1\bar{c}_B c_A(x,t) - k_2 c_C(x,t), \tag{2b}$$

where x represents a Cartesian coordinate in the plane, or the cosine of the equatorial angle on the sphere. ∇^2 is the Laplacian operator in either Cartesian or spherical coordinates. In either case, Eqs 2 can be readily transformed to measureable functions of the FRAP technique [2, 14–16]. Multiplying Eqs 2 by e^{iKx}, (for the plane), or $P_l(x)$, the lth Legendre polynomial (for the sphere), and integrating over x, one obtains (after integrating by parts two times):

$$\frac{d\tilde{c}_A(q,t)}{dt} = (-D_A q^2 - k_1\bar{c}_B)\tilde{c}_A(q,t) + k_2\tilde{c}_C(q,t) \tag{3a}$$

$$\frac{d\tilde{c}_C(q,t)}{dt} = (-D_C q^2 - k_2)\tilde{c}_C(q,t) + k_1\bar{c}_B\tilde{c}_A(q,t). \tag{3b}$$

In this notation, $\tilde{c}_A(q,t)$ is the generalized transform function,

$$\tilde{c}_A(q,t) \equiv \begin{cases} \int c_A(x,t)e^{iKx}dx \text{ (for the plane)} \\ \int c_A(x,t)P_l(x)dx \text{ (for the sphere)}; \end{cases} \tag{4}$$

and

$$q^2 = \begin{cases} K^2 & \text{(for the plane)} \\ l(l+1)/r^2 & \text{(for the sphere)}. \end{cases} \tag{5}$$

Recent advances in the FRAP technique, incorporating laser scanning [15, 16] and image intensification [2, 14], have made it possible to analyze data directly in transform space [2, 16]. We then need only to solve Eqs 3 for the time dependence of the combined transform function of the fluorescent components:

$$\tilde{c}(q,t) \equiv \tilde{c}_A(q,t) + \tilde{c}_C(q,t). \tag{6}$$

If it is desired, a particular form of $c(x,0)$ can be specified and incorporated into the calculated values of $\tilde{c}(q,0)$. The particular solution for $c(x,t)$ in coordinate space can then be derived from $\tilde{c}(q,t)$, in the usual way, with the appropriate inverse transform.

The Solution

Fortunately, the solution of Eqs 3 has already been derived in a different context [17, 18]. Equations formally identical to these apply to an analogous problem for coherent, "quasi-elastic" laser light scattering, where $\tilde{c}(q,t)$ would then represent the time correlation function of light scattered by spontaneous concentration fluctuations, with scattering vector q [19]. In our case, after appropriate changes in notation, we then have [17, 18]:

$$\tilde{c}(q,t) = a_+ e^{-\Gamma_+ t} + a_- e^{-\Gamma_- t}, \tag{7a}$$

where

$$a_\pm = \frac{1}{2}[1 \pm (-1)(R - \frac{D_-}{D_+}\frac{c_-}{c_+}) / (R^2 - 2R\frac{D_-}{D_+}\frac{c_-}{c_+} + \frac{D_-^2}{D_+^2})^{0.5}] \tag{7b}$$

$$\Gamma_\pm = D_+ q^2 [1 + R \pm (R^2 - 2R\frac{D_-}{D_+}\frac{c_-}{c_+} + \frac{D_-^2}{D_+^2})^{0.5}] \tag{7c}$$

with

$$D_\pm = \frac{1}{2}(D_A \pm D_C), \tag{7d}$$

$$R = \frac{k_1 \bar{c}_B + k_2}{2 D_+ q^2}, \tag{7e}$$

and

$$c_\pm = \frac{1}{2}(\bar{c}_A \pm \bar{c}_C), \tag{7f}$$

so that

$$\frac{c_-}{c_+} = \frac{1-\bar{c}_B K'_{eq}}{1+\bar{c}_B K'_{eq}}. \tag{7g}$$

Note that the effects of reaction kinetics are all contained in the parameter R (Eq 7e), which is an expression of the ratio of the reaction rate to a diffusion rate. Interestingly, however, $\bar{\Gamma}$, the average relaxation rate, is totally independent of R. For all values of R,

$$\bar{\Gamma} \equiv a_+\Gamma_+ + a_-\Gamma_- = \frac{\bar{c}_A D_A + \bar{c}_C D_C}{\bar{c}_A + \bar{c}_C} q^2, \tag{8}$$

a simple average diffusion rate weighted by the equilibrium concentrations. $\bar{\Gamma}$ can be obtained from the initial slope of the data [19, 20];

$$\bar{\Gamma} = -\lim_{t\to 0} \frac{d\ln \mathscr{C}(q,t)}{dt}. \tag{9}$$

The general form of $\mathscr{C}(q,t)$, beyond its initial slope, *is* affected by the value of R. Consider two limiting cases. In the "diffusion limit" [13], as $R\to\infty$, we have, from Eqs 7,

$$\lim_{R\to\infty} a_+ = 0 \tag{10a}$$

$$\lim_{R\to\infty} \Gamma_- = \bar{\Gamma} \tag{10b}$$

As expected, one sees in this limit a single time-averaged diffusing component. Jähnig [10] analyzed the effects of binding on diffusion, but only in this diffusion limit.

In the "reaction limit" ($R\to 0$), Eqs 7 reduce to:

$$\lim_{R\to 0} a_+ = \frac{\bar{c}_A}{\bar{c}_A + \bar{c}_C} \tag{11a}$$

$$\lim_{R\to 0} a_- = \frac{\bar{c}_C}{\bar{c}_A + \bar{c}_C} \tag{11a}$$

$$\lim_{R \to 0} \Gamma_+ = D_A q^2 \tag{11c}$$

$$\lim_{R \to 0} \Gamma_- = D_C q^2. \tag{11d}$$

One simply sees, in this case, the two separate diffusing components. If we consider the case of $R << 1$, but specify further that $D_C << D_A$, then Γ_- to first order in R, becomes

$$\Gamma_- \cong D_C q^2 + k_2. \tag{12}$$

Figure 1 presents semi-log plots of $\tilde{c}(q,t)$ covering the whole range from the diffusion limit ($R \to \infty$, Eqs 10) to the reaction limit ($R \to 0$, Eqs 11), for the particular case $\bar{c}_A = \bar{c}_C$, $D_C << D_A$.

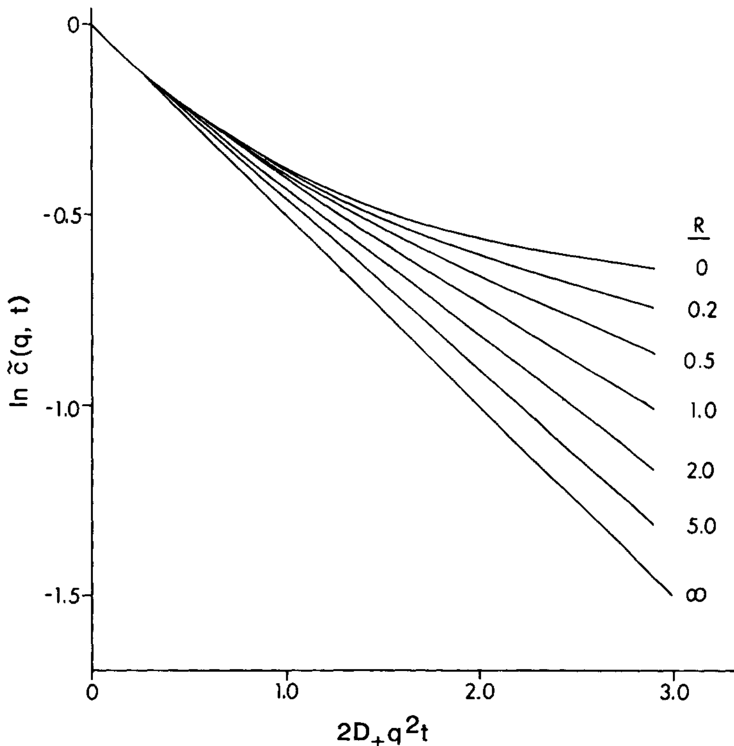

Fig. 1. Semi-log plots of $\tilde{c}(q,t)$ for $\bar{c}_A = \bar{c}_C$, $D_C << D_A$, demonstrating the transition between the reaction limit ($R << 1$) and the diffusion limit ($R >> 1$). Note that all curves have initial slope $-\bar{\Gamma}$.

DISCUSSION

Applying Eqs 7 to the parameters derived from a single FRAP experiment, one is faced, in general, with three equations with four unknowns. Under favorable circumstances, however, one should be able to sort things out by varying the magnitude of q^2 (and hence R; see Eq 7e) in a systematic way. This can be accomplished by changing the periodicity (and hence K; see Eqs 4 and 5) of pattern bleaching in the plane; or the order of the Legendre polynomial (ie, l; see Eqs 4 and 5) used in the normal mode analysis on the sphere.

With few exceptions, FRAP measurements of membrane protein diffusion have generally yielded two component recoveries (eg, [4, 6, 9, 12]), with the slower component deemed "immobile" on the time scale of the experiment ($\sim 10^3$sec). In the context of the binding model, one would thus be near the reaction limit ($R << 1$, with $D_C << D_A$), with species C presumably corresponding to complexes with cytoskeletal elements.

Recent experiments (Koppel and Sheetz, unpublished data), following the redistribution of glycoproteins in erythrocyte membranes over a time span of several hours, have shown that the slow component observed in this system [4] is not truly immobile. In the binding model, there are two parallel processes contributing to Γ_- in this limit (see Eq 12): the diffusion of complex C, and the dissociation of A from C. Experiments are underway designed to determine the relative contributions of these processes by measuring Γ_- for several values of q.

The "fast" component observed for membrane protein redistribution is still generally much slower, relative to measured values of phospholipid diffusion, than one would predict for free protein diffusion (for discussion, see Ref [5]). This might be caused by another protein binding reaction, observed this time in the diffusion limit. For erythrocyte membranes, however, additional evidence [5, 9] indicates that an indirect steric hinderance by the submembranous matrix may be the dominant factor in this case.

It should be emphasized, finally, that, with the possible exception of the erythrocyte membrane [1, 3–5], the specific interactions responsible for retarding membrane protein mobility are currently unknown. It is hoped, however, that the analysis presented in this paper will prove useful for the future evaluation of general molecular associations in membranes.

ACKNOWLEDGMENTS

This work was supported by NIH Grants GM 23585 and GM 28250.

REFERENCES

1. Fowler, V, Bennett, V: J Supramol Struct 8:215, 1978.
2. Smith, BA, Clark, WR, McConnell, HM: Proc Natl Acad Sci USA 76:5641, 1979.
3. Sheetz, MP, Schindler, M, Koppel, DE: Nature (London) 285:510, 1980.
4. Golan, DE, Veatch, W: Proc Natl Acad Sci USA 77:2537, 1980.
5. Koppel, DE, Sheetz, MP, Schindler, M: Proc Natl Acad Sci USA 78:3576, 1981.
6. Henis, YI, Elson, EL: Proc Natl Acad Sci USA 78:1072, 1981.
7. Webb, WW, Barak, LS, Tank, DW, Wu, E-S: J Supramol Struct Cell Biochem Suppl 5:264, 1981.
8. Edelman, GM: Science 192:218, 1976.
9. Cherry, RJ: Biochim Biophys Acta 559:289, 1979.
10. Jähnig, F: Nature (London) 289:694, 1981.
11. Koppel, DE, Osborn, MJ, Schindler, M: Nature (London) 289:696, 1981.

12. Elson, EL, Schlessinger, J, Koppel, DE, Axelrod D, Webb, WW: In Marchesi VT (ed): "Membranes and Neoplasia: New Approaches and Strategies." New York: AR Liss, 1976, pp 137–147.
13. Thompson, NL, Burghardt, TP, Axelrod, D: Biophys J 33:435, 1981.
14. Smith, BA, McConnell, HM: Proc Natl Acad Sci USA 75:2759, 1978.
15. Koppel, DE: Biophys J 28:281, 1979.
16. Koppel, DE, Sheetz, MP, Schindler, M: Biophys J 30:187, 1980.
17. Bloomfield, VA, Benbasat, JA: Macromolecules 4:609, 1971.
18. Ford, NC Jr: Chem Scripta 2:193, 1972.
19. Pusey, PN, Koppel, DE, Schaefer, DW, Camerini-Otero, RD, Koenig, SH: Biochemistry 13:952, 1974.
20. Koppel, DE: J Chem Phys 57:4814, 1972.
21. Elson EL, Reidler JA: J Supramol Struct 12:481, 1979.