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Topical Review

Lateral Motion of Membrane Proteins and Biological Function

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Introduction

Within the last twelve years, and especially within the last seven, there has been an explosion of research activity in the subject of lateral motion of membrane proteins. Several excellent reviews have appeared that summarize many aspects of this work (Webb, 1978; Cherry, 1979; Jacobson, 1980; Edidin, 1981; Jacobson & Wojcieszyn, 1981; Peters, 1981; Schlessinger & Elson, 1981; Webb, Barak, Tank & Wu, 1982). Attention has focused predominately upon: (i) quantitative measurement of diffusion rates for a variety of cell surface proteins, cell types, and physiological states; (ii) what these rates might imply about membrane structure and dynamics; and (iii) development of techniques for lateral mobility measurement.

Ultimately, however, the importance of lateral motion of membrane proteins hinges on its functional role in the life of a cell. Although hypotheses on this subject abound, surprisingly few have been experimentally supported. This review discusses cases where experimental evidence points toward a central biological role for lateral motion of certain proteins in cell membranes.

In the Appendix, some of the relevant mathematics of two dimensional diffusional collisional kinetics are reviewed. The results allow one to judge whether an observed biochemical reaction or clustering rate can be accounted for by lateral diffusion.

Acetylcholine Receptors and the Formation of Synapses

One prominent feature of the synaptic connection between nerves and muscles in a wide variety of species is the highly localized concentration of acetylcholine receptors (AChR) on the muscle fiber surface. *In vitro* cocultures of embryonic neurons and myotubes also can developed functional (but anatomically primitive) synapses, often concomittantly with aggregation of AChR at neuron/myotube contact points (Frank & Fischbach, 1979; Jacob & Lentz, 1979; Burrage & Lentz, 1981). Some AChR remain diffusely distributed on the surface of embryonic muscle cells *in vivo* (Braithwaite & Harris, 1979) and *in vitro* (Fischbach & Cohen, 1973; Axelrod et al., 1976*b*). At least on rat and chick myotubes in culture, these nonaggregated AChR are laterally mobile (Axelrod et al., 1976*b*; Axelrod, Ravdin & Podleski, 1978*a*).

A prime biological question is whether AChR move laterally from nonaggregated regions toward the site of a developing synapse. A major step toward answering this question is the observation that AChR on *Xenopus* myotomal muscle cells redistribute toward contacts with cocultured *Xenopus* neural tube cells (Anderson & Cohen, 1977; Anderson, Cohen & Zorychta, 1977; Cohen, 1980). This lateral redistribution appears to be neuron type specific: it does not occur if the neurons are of dorsal root or sympathetic ganglia origin (Cohen & Weldon, 1980). About 66% of nerve contacts produce AChR aggregations and the overwhelming majority of these produce electrically functional synapses (although approximately 18% of electrically functional nerve contacts show no AChR aggregations) (Anderson, Kidokoro & Gruener, 1979). It is not yet clear whether the redistributed AChR originated from areas of diffuse AChR distribution on the myotomal cell surface or from widely scattered endogenous AChR clusters which disappear at the time of neuronal contact.

One might reasonably guess that some biochemical factor released from the nerve in-

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duces AChR lateral aggregation, perhaps by trapping previously mobile AChR in the neuronal contact region. Indeed, biochemical extracts from embryonic nerves (Podleski et al., 1978; Jessell, Siegel & Fischbach, 1979) and neuronal cell conditioned medium (Bauer et al., 1980) can induce lateral redistribution of AChR into cell surface clusters and can also immobilize some laterally mobile myotube AChR (Axelrod, Bauer, Stya & Christian, 1980). Extracts of basal lamina can also induce AChR aggregation (Rubin & McMahan, 1982). Elegant *in vivo* experiments by Burden, Sargent and McMahan (1979) on muscle regenerating in an already formed basal lamina coating suggest that the basal lamina might contain molecular cues that can aggregate AChR.

Endogenous AChR clusters or "patches" develop on cultured rat, chick, and *Xenopus* embryonic muscle cells even in the absence of neural or basal laminal influence. In the case of rat, endogenous clusters are induced merely by physical contact with the substrate (Axelrod, 1980, 1981; Bloch & Geiger, 1980). These patches are composed at least partially of AChR that had laterally gathered from surrounding areas of diffuse AChR distribution (Stya & Axelrod, 1982). Even in living embryos, AChR aggregations can develop in the absence of neural contact (Braithwaite & Harris, 1979). Once gathered at the synapse, AChR are both immobile and exceedingly stable on the surface. The immobility appears likely to result from some sort of cytoplasmic anchoring, since the AChR in synapses can be mobilized by "blebbing" out the post-junctional membrane region away from the cell interior (Tank, Wu & Webb, 1982).

It still remains to be shown directly that synaptogenesis during actual embryonic development requires or involves lateral AChR redistribution. Young and Poo (1983) have calculated that the diffusion of AChR on *Xenopus* myotomal cells (which is somewhat higher than that on rat myotubes) is at least sufficient to account for the diffusive accumulation of AChR at developing neuromuscular junctions.

Rhodopsin and Visual Transduction

The process by which photons are converted to an electrical signal in the rod photoreceptor cell of the retina is remarkable for its sensitivity; by some multi-step amplification system, a single bleached rhodopsin molecule in the rod outer segment disk triggers the flow of thousands of Na^+ ions through the rod plasma membrane. It has been proposed that one amplification step might be the diffusion-mediated collisional activation of many disk phosphodiesterase (PDE) molecules by a single laterally mobile bleached rhodopsin molecule (Yee & Liebman, 1978; Liebman & Pugh, 1979, 1981).

Liebman and coworkers base their hypothesis on observations of the fast kinetics of a light-induced PDE activity in ROS disks. The key observation is that the light-induced activity of PDE is extraordinarily high. If one assumes that one bleached rhodopsin (resulting from absorption of a single photon) activates only one neighboring PDE, the activity of that PDE would have to exceed the maximum possible rate as limited by aqueous diffusion of the substrate. In fact, Liebman and coworkers calculate that the single bleached rhodopsin must activate nearly 500 PDE molecules to account for the high PDE activity. The observed kinetic rates of PDE activity, the dependence of response rates with light intensity, the known concentrations of rhodopsin, PDE, and cofactor GTP, and the lateral diffusion rate of rhodopsin are all consistent with the hypothesis that a bleached rhodopsin successively collides with and activates many PDE's via random diffusion. A relationship between the amplified activation of PDE by bleached rhodopsin and the electrical response of the rod to light (i.e., the shutting of Na^+ channels in the plasma membrane) is currently under investigation (Hurley & Stryer, 1982).

The discovery that rhodopsin laterally diffuses in ROS disks (Liebman & Entine, 1974; Poo & Cone, 1974) was one of the early supports for the "fluid mosaic" model of membranes. The method by which it was discovered, recovery of rhodopsin light absorption into a prebleached portion of an ROS disk, was a forerunner of the fluorescence photobleaching recovery method (Peters, Peters, Tews & Bahr, 1974; Axelrod et al. 1976*a*, Edidin, Zagyanski & Lardner, 1976; Jacobson, Wu & Poste, 1976; Smith & McConnell, 1978; Wey, Cone & Edidin, 1981) that has yielded most of our subsequent measurements of protein lateral diffusion rates on other systems. The diffusion coefficient of rhodopsin in ROS disks is about 5×10^{-9} cm^2/sec. Although this diffusion rate is at least an order of magnitude faster than that of most other cell surface proteins that have been studied, the diffusion rate is about what would be expected if a rhodopsin's translational

brownian motion was rate limited by lipid bilayer viscosity (Saffman & Delbruck, 1975; Hughes, Pailthorpe, White & Sawyer, 1982).

Cell Surface Immunoglobulins and Immunological Response

Antigen binding to an immunoglobulin (Ig)-armed surface of certain cells in the immune system has long been known to trigger a number of characteristic cellular responses. Examples include: mitosis of lymphocytes (Edelman, 1974); capping of cell surface Ig on B lymphocytes (Taylor, Duffus, Raff & dePetris, 1971); release of histamine (degranulation) of mast cells and basophils (Dembo & Goldstein, 1979), and phagocytosis by macrophages (Lewis, Hafeman & McConnell, 1980). A general review and some hypotheses on the consequences of "modulation" of immune cell surface receptors by external agents is given by Edelman (1976).

In many cases, externally crosslinking of the F_c receptor for cell surface Ig is involved in triggering the cellular response (DeLisi, 1979). For F_c receptors normally scattered randomly and rather sparsely on the cell surface, crosslinking implies a lateral redistribution of receptors into aggregates. In the case of lymphocyte capping by antibodies directed against cell surface antigens, capping only occurs under crosslinking conditions sufficient to immobilize the membrane antigen (Dragsten et al., 1979).

Nevertheless, rigid crosslinking of F_c receptors does not seem to be required in all cases of immune cell triggering. In the case of a macrophage attacking a target membrane (a model of a target bacterium), increasing the lateral mobility of an antigen on the target membrane increases its binding rate to the macrophase (Lewis et al., 1980). In recent studies (Balakrishnan, Hsu, Cooper & McConnell, 1982; Weis, Balakrishnan, Smith & McConnell, 1982), target vesicles composed of phospholipids mixed with a haptenic dinitrophenylated phospholipid were found to trigger serotonin release from rat basophil leukemia cells. Surprisingly, the triggering of serotonin release was effective for both freely mobile, nonaggregated lipid haptens in "fluid" membranes (i.e., those composed of lipids whose phase transition temperature was below ambient temperature) and also for "solid" membranes. Since the haptenic groups are small and monovalent, rigid crosslinking of the basophil's F_c receptors by the fluid membrane target could not have occurred. The authors therefore hypothesize a generalization of the "requirement" for F_c crosslinking. As basophil F_c receptors laterally diffuse to the regions of contact with a target membrane, they become trapped by binding to the hapten in the target membrane. If one then postulates a dynamic equilibrium between monomeric and aggregated F_c receptors on the basophil surface, the increased local F_c receptor concentration shifts the equilibrium toward F_c receptor aggregation which then (somehow) triggers degranulation.

The mechanisms by which membrane protein microaggregation might trigger biological events in various cell types are not yet known and may well involve complex connections with other cellular structures. In a simpler view, microaggregation might slow lateral or rotational diffusion (the latter being relatively more sensitive to the size of the molecular aggregate (*see* Saffman & Delbruck, 1975; Koppel, Sheetz & Schindler, 1981; Koppel, 1981; Hughes et al., 1982), thereby affecting the rate of some surface biochemical reaction critical to triggering.

β-Catecholamine Receptors and Response to Hormones

In a wide variety of cells, hormones bind to cell surface receptors and somehow stimulate a dramatic increase in the activity of the membrane-bound enzyme adenyl cyclase. Based on enzyme kinetic measurements, Tolkovsky and Levitzki (1978) have hypothesized that a β-receptor with its bound hormone laterally diffuses into successive collisions with laterally mobile adenyl cyclase molecules and, in the presence of GTP, thereby activates the adenyl cyclase. The adenyl cyclase subsequently returns to its inactive state when GTP is hydrolyzed. Evidence for the lateral diffusion/activation hypothesis includes the observation that, in the presence of a nonhydrolyzable GTP analog, a reduction in the number of functioning β-receptors by inactivation causes no change in the maximal level of adenyl cyclase activity but only a reduction in its rate of activation.

Other lines of evidence also support this model with varying degrees of strength. Inhibiting the lateral diffusion of membrane proteins inhibits hormone-dependent cyclase activity (Atlas, Volsky & Levitzki, 1980). The temperature-dependence curve of cyclase activity exhibits "kinks", which have been interpreted to correspond to "transitions" in the membrane

lipids (Hanski, Rimon & Levitzki, 1979; Rimon, Hanski & Levitzki, 1980; Bakardjeva et al., 1981). [Note, however, that no kinks or discontinuities have been observed in the lateral mobility *vs.* temperature curves of certain lipid probes (Thompson & Axelrod, 1980; Axelrod, Wight, Webb & Horwitz, 1978*b*) or proteins (Axelrod et al., 1978*b*) in animal cell membranes; presumably, these kinks are suppressed by the cell's high cholesterol content]. Also, variations in the cyclase activity upon manipulation of membrane lipid content have been interpreted as arising from measured changes in lipid microviscosity (Rimon, Hanski, Braun & Levitzki, 1978; Hirata, Strittmatter & Axelrod, 1979; Rimon et al., 1980; Bakardjieva et al., 1981). [Note, however, that changes in lipid microviscosity, as measured by the *rotational* mobility of a fluorescent lipid probe, do not necessarily correspond to changes in the *lateral* mobility of the lipid probe (Kleinfeld et al., 1982), nor to changes in the lateral mobility of proteins imbedded in the lipid matrix and (perhaps) attached to other cellular structures].

The lateral mobility of a fluorescent antagonist specifically bound to β-receptors on Chang liver cells has been measured by fluorescence photobleaching to be $D=1\times10^{-10}$ cm^2/sec, roughly typical of many membrane proteins; diffusion of the probe nonspecifically dissolved in the lipid bilayer was two orders of magnitude higher (Bakardjieva et al., 1981), typical of membrane lipid diffusion. The Saffman and Delbruck (1975) theoretical model of protein Brownian motion in membranes predicts a much smaller difference between lipid and protein diffusion rates. The slow diffusion of β-receptors is consistent with models in which β-receptor lateral motion is rate limited by interprotein aggregation or by anchoring to exoskeletal or cytoskeletal structures, rather than by lipid viscosity. Theoretical models have been devised for protein diffusion retarded either by steric hindrance in a labile matrix (Koppel et al., 1981) or by association/dissociation dynamics with immobile structures (Koppel, 1982).

Ligand Binding: Receptor Down-Regulation

The binding of certain ligands to cell surface receptors can induce rapid changes in the rate of uptake of metabolites and an increase in the synthesis rate of metabolic products. In the case of insulin, at least, the rapid response is triggered by microaggregation of insulin receptors (Kahn, Baird, Jarrett & Flier, 1978), perhaps via a lateral diffusion-dependent crosslinking analogous to the F_c receptor behavior discussed earlier. The response of cells to the hormone lutropin may also be triggered by ligand-induced microaggregation of receptors (Amsterdam, Berkowitz, Nimrod & Kohen, 1980).

But the longer term fate of bound-up cell surface receptors has also been given considerable attention. The agonist appears to speed the removal of its own receptors from the surface by a lateral motion-dependent mechanism. By this phenomenon, the cell can regulate its overall sensitivity to agonists according to agonist concentration.

Electron microscopic studies show that receptors for insulin, epidermal growth factor (EGF), α_2-macroglobulin (α_2M), and other agonists are initially diffusely distributed on the cell surface (Pastan & Willingham, 1981). Within a few minutes after exposure to agonist, the bound-up receptors are seen aggregated in "coated pits" (small membrane invaginations cytoplasmically coated with a protein called clathyrin). These coated pits somehow (perhaps by pinching off) lead to the formation of cytoplasmic vesicles containing the receptors. The vesicles then proceed to the Golgi region. In general, crosslinking of cell surface receptors often leads to an increased rate of receptor internalization (*see* Axelrod, 1980, and references therein), although the role of coated pits is not established in all cases.

Viewed in a fluorescence microscope with fluorescent agonists, initial diffuse surface labeling of insulin, EGF, and α_2M is followed by clustering. By the time clustering is seen, EGF receptors are already internalized (Yarden, Gabbay & Schlessinger, 1981; Hillman & Schlessinger, 1982). Visible clusters apparently form well after aggregation at surface coated pits.

Since the receptors for insulin, EGF Schlessinger et al., 1978) and α_2M (Maxfield et al., 1981) are known to be laterally mobile (at a rate of 3×10^{-10} to 9×10^{-10} cm^2/sec), it is reasonable to speculate that transport to the coated pits occurs by lateral diffusion. Employing a quantitative theory related to that in the Appendix of this review, Goldstein, Wofsy and Bell (1981) have calculated that random diffusion can indeed account for the observed rate of transport to coated pits.

Bioenergetics and Cytochrome Chemistry

In the mitochondrial membrane, electrons are transported from cytochrome reductase (the cytochrome bc_1 complex) to cytochrome c and then to cytochrome oxidase. Speck, Ferguson-

Miller, Osheroff and Margoliash (1979) and Hackenbrock (1981) have suggested that these cytochromes may be independent entities, free to diffuse in the plane of the membrane and only able to transfer electrons during two-dimensional collisions. Indirect kinetic evidence for this conclusion is the observed decrease in electron transfer activity as the cytochromes are "diluted" in the membrane by phospholipid enrichment (Schneider, Lemasters, Höchli & Hackenbrock, 1980). In addition, various cytochromes have been observed to be rotationally mobile in intact or model membranes (Vanderkooi et al., 1982; Kawato et al., 1982), and interactions among cytochromes appear dependent on the state of "fluidity" of phospholipids (Strittmatter & Rogers, 1975). Furthermore, the cytochromes appear structurally independent in some experiments: "patches" of the reductase, induced by specific antibody crosslinking, are not always coincident with antibody-induced patches of the oxidase (Hoechli, Hoechli & Hackenbrock, 1982).

Several groups are currently measuring the lateral diffusion rates of cytochromes (Sowers & Hackenbrock, 1981; Hochman, Schindler, Lee & Ferguson-Miller, 1982; Sowers et al., 1982) to see if lateral diffusion is fast enough to account for the observed electron transfer rates or if instead the cytochromes must remain together in a (temporary) complex at least long enough to accomplish the transfer of several electrons. By using Eq. (3) of the Appendix, we can calculate what the minimum lateral diffusion coefficients must be to account for the known turnover rates. For example, the cytochrome bc_1 complex has a turnover time of 8.6 msec (Hackenbrock, 1981). If we interpret this figure as the average time between collisions of cytochrome c's with a particular bc_1 complex, and if we assume a cytochrome c concentration of 1.5×10^{11} molecules/cm^2 in mitochondrial membrane, then the reactants must have a $D \geqq 3 \times 10^{-10}$ cm^2/sec where D is the sum of cytochrome c and cytochrome bc_1 complex diffusion coefficients.

Lateral diffusion also has been implicated in a photosensitive bioenergetic system. Based on an analysis of reaction kinetics, Takano, Takahashi and Asada (1982) propose that the reduction of the photoxidized photosystem I reaction center (P-700+) by plastocyanin in spinach thylakoid membrane is mediated by plastocyanin diffusion in two dimensions.

Cell-Cell Contact Specificity

During development, cells presumably express some sort of directionality that enables them to associate in a spatially nonrandom manner. This tropicity is perhaps correlated with a nonrandom distribution of cell surface proteins that mediate cell-cell contact specializations. Chow and Poo (1982) have experimentally demonstrated that certain specific cell surface glycoproteins laterally migrate to regions of cell-cell contact, at which they become trapped. The authors speculate that such contact-induced redistribution of all surface receptors might serve both to populate contact regions with functionally appropriate membrane proteins and simultaneously to deplete other membrane regions so that superfluous contacts cannot be established.

At cell-cell contacts, specialized junctions can form that contain channels for the interchange of ions or molecules between neighboring cells. Loewenstein (1981) has hypothesized that membrane "protochannels" on a cell surface can laterally diffuse toward a contact region where they couple with a protochannel from the apposing cell. This inter-cell molecular coupling might serve three simultaneous functions: (i) formation of a complete functional channel between the two cells; (ii) trapping of the protochannels so that they accumulate at the contact rather than diffuse away to noncontact regions; and (iii) stabilization of the bond between the cells.

Viral Infection of Cells

Two events in the infection of cells by Semliki Forest virus particles depend on lateral transport along the cell membrane (Simons, Garoff & Helenius, 1982). The first event is the virus particle's motion down the shaft of a cellular microvillus with which it made initial contact, on its path toward entrapment by a cell surface coated pit. The second lateral motion event is the selective aggregation of newly synthesized viral glycoproteins from their random sites of incorporation into the plasma membrane toward a viral nucleocapsid attached to the inner surface of the host membrane during the final stages of viral assembly just before budding from the cell.

The two-dimensional membrane biochemistry involved in the assembly of a variety of membrane proteins has been reviewed recently by Lodish et al. (1981).

Reaction Rate Enhancement by Surface Diffusion

Another sort of two-dimensional diffusion in biology takes place *on* rather than *in* membranes. Adam and Delbruck (1968) have shown

mathematically that nonspecific adsorption of a bulk-dissolved ligand to a cell surface, followed by two-dimensional surface diffusion (i.e., a "two-step" process) can lead to much greater collision rates with specific receptor targets than would simple three-dimensional diffusion from the bulk (i.e., a "one-step" process). Berg and Purcell (1977) have provided expressions for the binding energy of nonspecific adsorption required for such rate enhancement to be effective. Based on these hypotheses, various authors have proposed biologically functional roles for surface diffusion of a nonspecific adsorbate: transport of energy, oxygen, fatty acid residues, and cytochrome *c* along mitochondrial membranes (Archakov, Karyakin & Skulachev, 1975; Roberts & Hess, 1977; Bakeeva, Chentsov & Skulachev, 1978); surface migration of viruses on a cell surface before finding an injection site (Bayer & Starkey, 1972; Wong, Bayer & Litwin, 1978; Heller & Braun, 1979); facilitated solute diffusion through the plasmodesmata between plant cells (Gunning & Robards, 1976); increased sensitivity of olefactory sensors (Adam & Delbruck, 1968; Kasang, 1973). transport of palmityl CoA to membrane-bound enzymes at which phospholipids are synthesized (Sumper & Träuble, 1973); facilitated assembly of viruses on cytoplasmic membranes (Caliguiri & Tamm, 1970); binding of neurotransmitters to surface-bound receptors or esterases (Belleau, 1971; Kaufmann, 1977); and gliding of bacteria along solid surfaces (Humphrey, Dickson & Marshall, 1979). In most of these cases, involvement of two-dimensional surface diffusion is only hypothesized, or at best, inferred indirectly from reaction kinetics results. However, one direct experimental observation of surface diffusion on a solid surface (Burghardt & Axelrod, 1981) shows that bovine serum albumin reversibly adsorbed to glass can laterally diffuse $>1\,\mu m$ before desorption, with a diffusion coefficient of $5\times10^{-9}\,cm^2/sec$. Bimolecular surface diffusion and adsorption/desorption kinetics can be observed experimentally by selective fluoresence excitation of adsorbed species via total internal reflection combined with fluorescence photobleaching recovery or fluorescence correlation spectroscopy (Hirschfeld & Block, 1977; Thompson, Burghardt & Axelrod, 1981).

Summary

In the last decade, the lateral mobility of many membrane proteins was clearly established and versatile means to measure the rate of motion were developed. Future advances will demonstrate (i) instances in which lateral mobility is centrally involved in biological function and (ii) whether the functional mobility is random diffusion or directed flow.

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Appendix

Diffusional Collision Kinetics in Two Dimensions

A biomolecular chemical reaction is said to be "diffusion limited" when every random diffusional encounter between reactants leads to the formation of a reaction complex (i.e., the immediate pre-reaction state). Conversely, if almost every diffusional encounter is unsuccessful at forming a complex (either because one of the reactants is still in a complex from a previous collision or because the probability of complex formation is much less than unity), the reaction is termed "reaction limited."

If the measured reaction rate is slower than or equal to the theoretical diffusion limited rate, we can conclude only that the reaction *could* be dependent upon diffusion. If the measured rate is faster than the diffusion limited rate, the reaction *must* be mediated by some mechanism other than random isotropic diffusion; e.g., permanently formed complexes or encounters by some sort of directed flow. Analogous considerations apply to irreversible aggregation of cell surface proteins at a local "trap" region. Aggregation slower than the diffusion limit *may* depend upon diffusion; faster aggregation *requires* some other mechanism.

In this appendix, expressions are presented for the two-dimensional diffusion limited rates for (i) reversible reactions; and (ii) irreversible aggregation into a surface trap. Numerous theoretical studies on aspects of two-dimensional reaction kinetics have appeared (Adam & Delbruck, 1968; Eigen, 1974; Naqvi, 1974; Owen, 1975; Berg & Purcell, 1977; Hardt, 1979, 1981; Naqvi, 1979; Naqvi, Waldenstrom & Mark, 1979; Weaver, 1979, 1983; DeLisi, 1980; Eldridge, 1980; Pedersen, 1980; Prager & Frisch, 1980; Szabo, Schulten & Schulten, 1980; Chao, Young & Poo, 1981; Goldstein et al., 1981; Waldenstrom, Naqvi & Mork, 1981; Shoup & Szabo, 1982).

(1) Reversible Reactions

Consider the reversible reaction between two molecules B and C, both diffusing in the same two-dimensional surface:

$$B+C \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} B\sim C \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} BC.$$

$B \sim C$ symbolizes a pre-reaction complex such that the centers of Band C are just within some characteristic reaction distance a of each other. BC symbolizes the reaction product. In steady state such that $d(B \sim C)/dt = 0$, the overall reaction

$$B + C \underset{k_r}{\overset{k_f}{\rightleftarrows}} BC$$

has the effective rates (Eigen, 1974):

$$k_f = \frac{k_{12} k_{23}}{k_{21} + k_{23}}$$
$$k_r = \frac{k_{32} k_{21}}{k_{21} + k_{23}}. \quad (1)$$

In the diffusion limit, $k_{21} \ll k_{23}$, so that

$$k_f = k_{12}$$
$$k_r = k_{21} \frac{k_{32}}{k_{23}}. \quad (2)$$

We here wish to obtain an expression for k_{12} in terms of the diffusion coefficients D_B and D_C for the two reactants. With no loss of generality, think of the B molecules as "targets" of the C molecules. Rate k_{12} can be understood as the number of collisions per unit time engaged in by a single C molecule per unit concentration of target molecules B. We will now assume as a simplifying approximation that the targets B are uniformly rather than randomly distributed so that each B is at the center of a circle of radius b containing no other B target. (The assumption of a circular rather than a polygonal domain for each B is also an approximation.) Each target B has a radius a equal to the characteristic reaction distance for $B \sim C$ complex formation. In reality, both B and C diffuse, but mathematically we can hold B fixed and assign C the diffusion coefficient $D_B + D_C$ (Eldridge, 1980). (In two-dimensional problems this step is only an approximation: *see* Hardt, 1979).

Symmetry indicates there would be no net diffusive flow of C at the midlines between uniformly spaced B targets, so we may consider only a single circular domain with a perfectly reflecting boundary at radius b. Molecule C is now placed at a random location in the circle and allowed to diffuse. Rate k_{12} is then the reciprocal of the mean time before a first collision of C with B, divided by the concentration of B which in molecules per area is just $1/\pi b^2$. For low concentrations of B (i.e., $a \ll b$), we get (Berg & Purcell, 1977):

$$k_{12} = \frac{2\pi(D_B + D_C)}{\ln \frac{b}{a} - \frac{3}{4}} \quad (3)$$

where $b = \{\pi[B]\}^{-1/2}$ and $[B]$ is the concentration of B in molecules/cm^2. Hardt (1979) has derived a somewhat more general (but still approximate) expression which reduces to Eq. (3) for cases in which the concentrations of B and C are within an order of magnitude of each other.

(2) Irreversible Trapping by a Target

Expressions have been derived for the time course of accumulation of molecules diffusing into a circular trap on a spherical surface as a function of time (Chao et al., 1981). Here we show original analogous results for the geometry discussed in subsection *(1)* above: a uniformly distributed two-dimensional array of targets (or traps) of radius a, each centered in a circular domain of area πb^2, in an initially homogeneous distribution of diffusing molecules C. For irreversible trapping, we make the targets become perfect absorbers starting at time $t = 0$, so that at $t = \infty$, all the C molecules are absorbed in the traps.

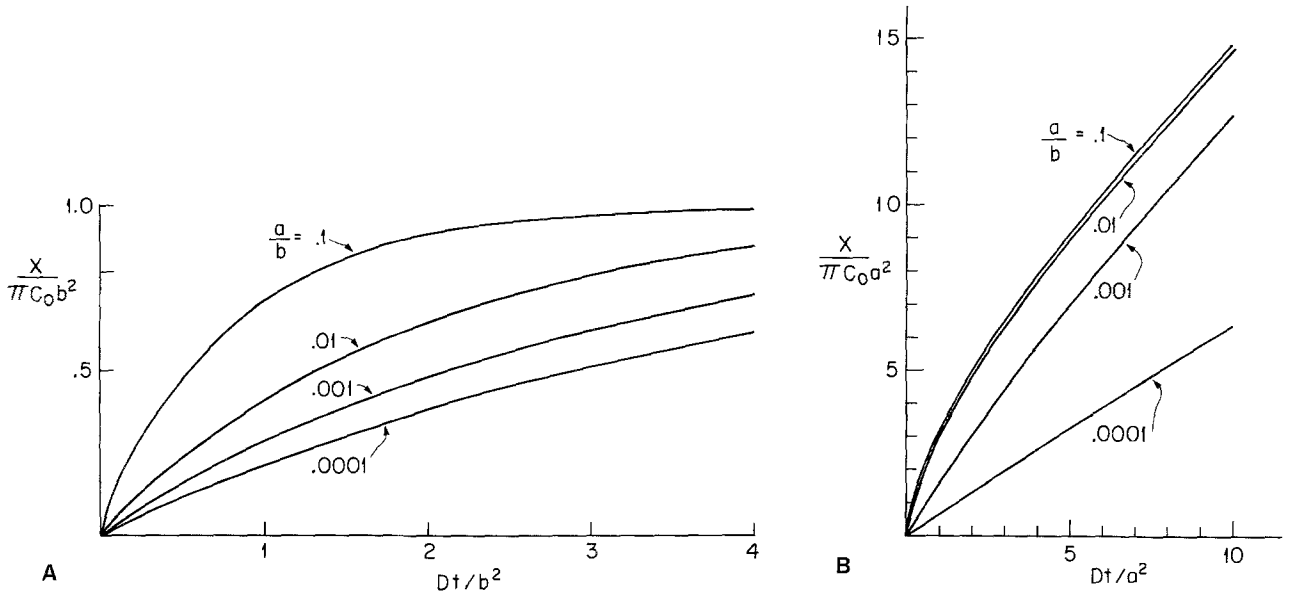

Fig. 1. (*A*): The amount of two-dimensional diffusion-limited irreversible accumulation X into a trap of radius a from a region of radius b with reflecting walls, expressed as a fraction of maximal accumulation ($X/\pi C_0 b^2$), versus time t expressed as the dimensionless variable (Dt/b^2), for various ratios a/b. (*B*): Same as *A* except that accumulation is expressed the ratio $X/\pi C_0 a^2$ and t as the dimensionless variable Dt/a^2. This figure emphasizes the early time behavior, much before maximal accumulation is approached

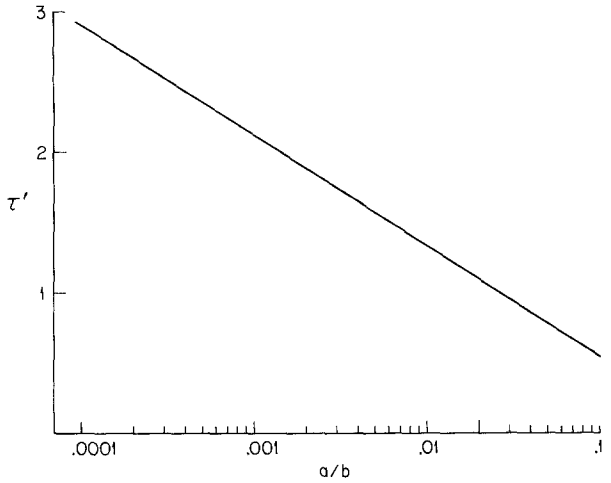

Fig. 2. Characteristic time $t'_{1/2}$ for half-maximal accumulation in a trap of radius a from a region of radius b, expressed as the dimensionless variable $Dt'_{1/2}/b^2 \equiv \tau'$, versus a/b

Starting with a general solution of the diffusion equation in cylindrical coordinates with general boundary conditions (Crank, 1975, p. 86), one can express the number $X(t)$ of C molecules absorbed in each trap as an infinite series:

$$X(t) = 4\pi C_0 \sum_{n=1}^{\infty} \frac{(1-e^{-D\alpha_n^2 t})}{\alpha_n^2} \left[\frac{J_0^2(a\alpha_n)}{J_1^2(b\alpha_n)} - 1\right]^{-1} \quad (4)$$

where C_0 = initial concentration of C at $t=0$, in molecules/cm^2; $D = D_B + D_C$; J_0 and J_1 are zero and first order Bessel functions of the first kind; and α_n are the positive roots of the equation:

$$J_0(a\alpha)\, Y_1(b\alpha) - J_1(b\alpha)\, Y_0(a\alpha) = 0$$

where Y_0 and Y_1 are zero and first order Bessel functions of the second kind.

Figure 1*A* is a plot of $X/\pi C_0 b^2$ (i.e., the fraction of maximal accumulation) for various values of a/b *vs.* the unitless time variable Dt/b^2. Figure 1*B* is a plot of $X/\pi C_0 a^2$ (i.e., the ratio of the number of molecules accumulated in a trap to the number in an equally sized nontrap area) *vs.* the unitless time variable Dt/a^2.

An appropriate "characteristic time" for accumulation into an array of traps may be defined in two different ways: (i) the unitless time $\tau' \equiv Dt'_{\frac{1}{2}}/b_2$ required for half-maximal accumulation in a trap $[X(t'_{\frac{1}{2}}) = (1/2)\pi C_0 b^2$; or (ii) the unitless time $\tau''_n \equiv Dt''_n/b^2$ required for accumulation of a multiple n of the number of C molecules expected in an equally sized nontrap area $[X(t''_n) = n\pi C_0 a^2]$. Figure 2 shows how τ' varies with a/b. τ' is on the order of 1.5 for a rather wide range of a/b from 10^{-4} to 10^{-1}. For a given a and for $a/b < 0.1$, characteristic times τ''_n are virtually independent of b for $n < 10$. These characteristic times occur very early in the accumulation process when each trap accumulates molecules as if it were the only trap present (*see* Crank, 1975, p. 87). We find $\tau''_1 = 0.15$ and $\tau''_{10} = 5.6$.

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