Rates of Membrane-associated Reactions: Reduction of Dimensionality Revisited

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Abstract. The hypothesis that reactions associated with intracellular membranes enjoy a kinetic advantage from a reduced dimensionality for diffusion is inconsistent with available data on lateral diffusion rates, membrane-substrate affinities, and endogenous concentrations of enzymes and their aqueous substrates.

It follows from a probability theorem by Polya that a random walker confined to one or two dimensions (1d or 2d)[1] is guaranteed to find a stationary target, wherever it is, but in three dimensions (3d) there is some chance he never will (Polya, 1921; Feller, 1957). Trurnit (1945) and Bucher (1953) suggested some time ago that the turnover numbers of certain membrane-bound enzymes might be enhanced if their aqueous substrates were to undergo guided 2d diffusion along membrane surfaces. In 1968 Adam and Delbrück put this hypothesis on quantitative grounds in proving that the diffusion-limited rate at which a surface-bound trap reacts with bulk-phase reactants can theoretically be enhanced, provided the reactants first adsorb to the surface and then diffuse within 2d before striking the trap. Echoing Dirac's (1931) reasoning that one would be surprised if nature had made no use of this possibility, they proposed that intracellular membranes enjoy a selective advantage because of an ability to adsorb and guide the diffusion of substrates to membrane-embedded enzymes. Indeed, they speculated that this advantage "contributed greatly to the evolutionary step from small, little-organized bacteria-like ancestors of cells to fully developed, internally compartmentalized unicellular organisms." A similar view is voiced by Eigen (1974), who calls reduction of dimensionality "nature's trick to overcome the barrier of diffusion control, and make multimolecular reaction processes at low concentrations more efficient." It is this device, according to Eigen, that explains the prevalence of membrane-bound enzymes in living systems.

In this paper we reappraise the dimensional reduction hypothesis of Adam and Delbrück as it pertains to reactions taking place on intracellular membranes. By dimensional reduction we refer specifically to the situation whereby a bulk phase reactant, e.g., an aqueous substrate, finds its membrane-bound target, e.g., an enzyme, not by purely 3d diffusive encounters, but by first adsorbing nonselectively to the membrane and then diffusing in 2d before reacting. We ask what magnitude of kinetic advantage this two-step searching process reasonably can be expected to afford in cellular reactions. Has natural selection actually entrained mechanisms that capitalize upon the theoretical rate advantages of the recurrent 2d random walk? In the first section we recount the Adam-Delbrück hypothesis (1968) describe Berg and Purcell's (1977) subsequent treatment of the effects of target density, and then estimate the theoretical advantages to be gained from two-step searching on intracellular membranes by using literature values of relevant parameters, e.g., diffusion coefficients, binding constants, and target densities. In the second section we offer a brief critique of experiments dealing with the effects of dimensionality on reaction kinetics in vitro. Finally, we consider other possible consequences of locating enzymes in membranes that in our opinion are more likely to influence biological reaction kinetics than are the special properties of 2d diffusion, per se.

Searching by Soluble Substrates for Membrane-bound Targets

Adam and Delbrück's Model

The gist of Polya's theorem is that on an isotropic random walk over an infinite 1d or 2d lattice there is unit probability that the walker will eventually visit every lattice point, whereas in 3d the probability is considerably less than 1 (Polya, 1921; Feller, 1957). This "critical" dependence of encounter probability on dimensionality provides a qualitative rationale for the proposal that internal membranes might function as "antennae," catching substrates and guiding their diffusion to membrane-embedded enzymes. But Polya's theorem concerns only the encounter probability, not the actual diffusion time required to encounter a target. As Adam and Delbrück

[1] *Abbreviations used in this paper:* 1d, 2d, and 3d, one-, two-, and three-dimensions (dimensional), respectively.

(1968) pointed out, the validity of their hypothesis depends crucially on the ratio of surface to bulk-phase diffusion coefficients.

To ascertain what this dependence is, they chose a model in which one perfectly absorbing target is held on a flat plate which transects the middle of a hollow sphere. Molecules within the sphere are imagined to be trapped by the target either by a direct hit from bulk solution or by lateral diffusion after first sticking to a non-target region of the plate. The average lifetime of soluble reactant molecules inside the sphere was then calculated. This led to a range of ratios of 2d to 3d translational diffusion coefficients (D_2/D_3) and diffusion space size to target size (b/a) over which combined surface and bulk-phase diffusion (two-step trapping) is favorable (Fig. 1). Notice that for a given b/a, reduction of dimensionality can either enhance or retard the search, depending on how fast the adsorbed reactant diffuses.

No data on the surface diffusion rates of membrane-bound molecules were available in 1968, yet Adam and Delbrück argued that "from our present knowledge of diffusion coefficients in liquids, gels, and solids, it appears improbable that we will encounter in biological systems any ratios D_2/D_3 much smaller than 10^{-2}." Given $D_2/D_3 = 10^{-2}$ and a target radius a of 10 Å, Adam and Delbrück (1968) noted a "transition" in kinetics when the reaction compartment is about the size of a bacterium or a eukaryotic organelle: Two-step trapping becomes favorable when the size of the diffusion space is above ~1 μm. The existence of this transition was qualitatively confirmed in recent computer simulations using lattice models of diffusion and reaction (Lee and Kozak, 1984).

3d and 2d Diffusion Rates In Vivo

Adam and Delbrück's guess for the lower limit to D_2/D_3 was remarkably close to actual values of D_2/D_3 observed for several diffusion probes in biological membranes, provided that a cytoplasmic viscosity significantly greater than that of pure water is assumed (Fig. 2, Table I). In Table I, experimental values of D_2 are used along with values of D_3 calculated assuming an average cytoplasmic viscosity 3.2 times that of pure water at the temperature of the diffusion measurement. The factor 3.2 is an average of 30 estimates on 14 different cell types from three kingdoms (Mastro et al., 1984; Lepock et al., 1983; Morse, 1977; Haak et al., 1976; Sachs and Latorre, 1974; Livingston et al., 1983; Burns, 1969; Lehman and Pollard, 1965; Horowitz, 1972; Hodgkin and Keynes, 1956; Horowitz and Moore, 1974; Caille and Hinke, 1974; Chang et al., 1972; Horowitz and Fenichel, 1970; Fenichel and Horowitz, 1969; Kushmerick and Podolsky, 1969). It includes eight different techniques using 19 probe molecules of ≤16,900 mol wt. Large proteins are known to diffuse through cytoplasm at rates much less than 3.2 times that of their D_3 in pure water (see, e.g., Mastro et al., 1984), but our concern here is with typical low molecular weight metabolites. Much greater cytoplasmic viscosities have also been reported even for small molecules (Keith and Snipes, 1974), it being suggested that "diffusion along the plane of the membrane or within the membrane structure may be faster and more efficient than through the aqueous protoplasm." It seems likely, however, that these very high viscosities were an artifact of the extremely hypertonic medium (~1.5 osM) in which the cells were bathed. If one includes only those estimates of viscosity based upon actual measurement of translational

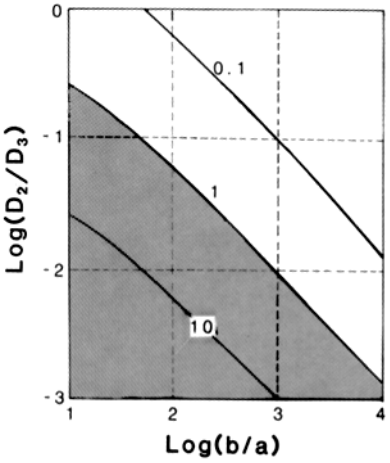

Figure 1. Criteria for an advantageous two-step search by soluble substrates for a single membrane-bound target, according to Adam and Delbrück (5; replotted from Fig. 3 in reference 1). Contours show three different values (0.1, 1.0, and 10) for the ratio of mean lifetimes of soluble reactants ($t_{2,3}/t_3$) for theoretical two-step (3d + 2d) vs. one-step (3d) searching process. D_2/D_3 is the ratio of 2d to 3d diffusion coefficient, and b and a are the linear size of the diffusion space and the target radius, respectively. Stippled region is where two-step searching (reduction of dimensionality) impedes the trapping rate. Note that for $b = 1$ μm and $a = 10$ Å, a modest 10-fold rate advantage requires a $D_2/D_3 = 0.1$. As shown in Table I, this is an unlikely ratio for biological membranes.

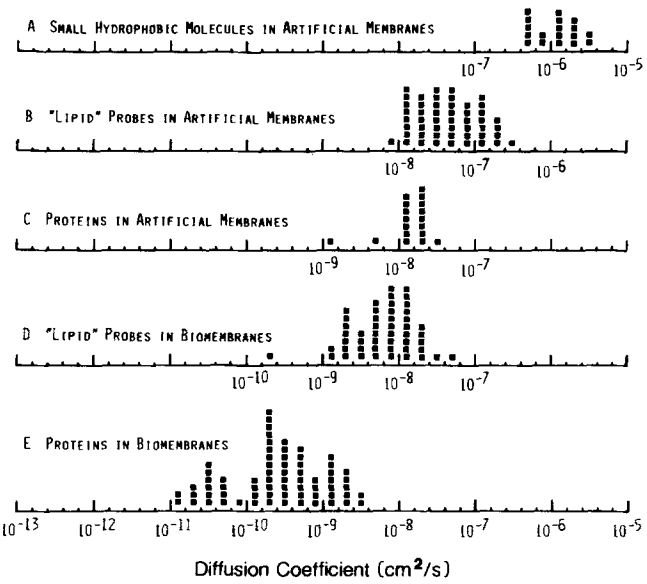

Figure 2. Histogram showing distribution of measured translational diffusion coefficients of membrane-associated molecules. Each square represents an independent measure of D; i.e., a measurement from one laboratory, for one technique, for one cell type, and usually for one temperature. All artificial membranes were in a fluid state. In *A* "small hydrophobic molecules" includes *n*-valeramide, benzene, di-*t*-butylnitroxide, tetramethylpiperidine-*N*-oxyl, lindane, and others. In *B* and *D* "lipid" includes endogenous or synthetic phospho and neutral-lipids, fluorescent derivatives of lipids and synthetic amphiphiles, pyrene, spin-labeled derivatives of phospholipids, and fatty acids. In *E*, all diffusion coefficients below 5×10^{-12} cm²/s have been disregarded, but this is not to say that proteins in biomembranes do not move more slowly than this. A full list of references is available from the authors upon request.

Table I. Comparative Diffusion Rates on Membranes and in the Bulk

Example	$\bar{T}$	$\bar{D}_2$	$\bar{D}_3$	$\overline{D_2/D_3}$
	°C	cm^2/s	cm^2/s	
A. Biological membranes				
NBD-PE‡	25	7.7×10^{-9}	7.5×10^{-7}	0.010
$DiIC_{18}$	24	9.2×10^{-9}	6.3×10^{-7}	0.015
Alp-NBD§	23	8.9×10^{-9}	9.9×10^{-7}	0.009
Cytochrome *c*	20	1.9×10^{-9}	3.1×10^{-7}	0.006
Integral proteins	25	7.2×10^{-10}	7.2×10^{-8}	0.010
All data on amphiphilic probes and pyrene	26	1.2×10^{-8}	7.1×10^{-7}	0.015
B. Artificial lipid membranes (fluid)				
Stearic acid	25	1.6×10^{-8}	4.3×10^{-6}	0.004
NBD-PE	27	5.6×10^{-8}	2.9×10^{-6}	0.019
$DiIC_{18}$	29	1.1×10^{-7}	2.5×10^{-6}	0.044
All data on amphiphilic probes and pyrene	30	8.2×10^{-8}	3.2×10^{-6}	0.029
Integral proteins	30	1.9×10^{-8}	7.9×10^{-7}	0.027
n-Valeramide	20.6	6.5×10^{-7}	7.4×10^{-6}	0.088
DTBN‖	37	1×10^{-6}	3×10^{-6}	0.33
Benzene	22	3.7×10^{-6}	9.53×10^{-6}	0.39
Lindane	37	1.2×10^{-6}	7.5×10^{-6}	0.16

For all but the integral proteins in biomembranes, the values of D_2/D_3 were calculated for each experimental measurement and then averaged, rather than formed by the ratio of the average D values.

* Values of D_2 taken from data given in Fig. 2. D_3 for comparison with D_2 in biomembranes was calculated assuming the cytoplasmic viscosity (η) is 3.2 times that of pure water at the same temperature. In the calculation of D_3 values for comparison with D_2 values in artificial lipid membranes, the viscosity of pure water was assumed. The Stokes-Einstein relation $D_3 = kT/6\pi\eta R$, where k is Boltzmann's constant, was used to calculate hypothetical D_3 values for integral proteins in reconstructed lipid membranes; the effective hydrodynamic radius R was approximated as the radius of a sphere for each protein, assuming a specific volume of 0.73 g/cm³. For lipids, lipid analogues, and small hydrophobic molecules D_3 was calculated from the molar volume, as outlined in Othmer and Thakar (1953).

‡ NBD-PE, nitrobenzoxadiazole-phosphatidylethanolamine.

§ Alp-NBD, 7-(2-allylphenoxy)-2,2-dimethyl-6-hydroxy-1-(7-NBD)-1,4-diazaheptane; it is a fluorescent β-adrenoceptor blocker.

‖ DTBN, di-*t*-butylnitroxide.

diffusion rates for small molecules, the increase relative to pure water is closer to 2.5; hence the predicted D_2/D_3 values would be even lower than those in Table I.

One might suppose that molecules ionically adsorbed to membranes could zip along much faster than integral proteins or lipids, but direct photobleaching measurements have not substantiated this idea. Cytochrome *c* diffuses as fast as 10^{-9} cm²/s on purified inner mitochondrial membranes at intermediate ionic strengths (Gupte et al., 1983), yet this is still nearly 10^3-fold slower than its diffusion in aqueous solution (Setlow and Pollard, 1962). Indeed, in intact (and coupled) mitochondria cytochrome *c* diffusion is not much faster than 10^{-10} cm²/s (Maniara et al., 1984). It is conceivable that small molecules that partition into the bilayer could diffuse faster than those that adsorb strictly at the interface, but this is not a universal finding. The nonspecifically "bound" fraction of a small (441 mol wt) fluorescent β-adrenoreceptor blocker, for example, diffuses at 8.9×10^{-9} cm²/s in membranes of Chang liver cells, but this is about 500 times slower than is its predicted diffusion in water (Henis et al., 1982). On the other hand, there is evidence that lateral diffusion of some small molecules in pure lipid bilayers is nearly as fast as in water, presumably because of an ability to dissolve in and sneak through the central, most disordered region of the membrane (Lange et al., 1974; Dix et al., 1978; Omann and Lakowicz, 1982; see Fig. 2). Most biological substrates, coenzymes, and effectors are simply too bulky or too polar to allow such behavior. Except for these small hydrophobic molecules, then, comparison of the available data on D_2/D_3 with the contours in Fig. 1 lends no credence to the speculation that biological reactions going on within a space of ~1 μm enjoy a large kinetic advantage from reduction of dimensionality. Whereas the ratios D_2/D_3 are borderline for rate advantages inside organelles or bacteria, they are consistent with profitable operation of two-step searching within a diffusion space of, say, 10 μm. Other factors must also be considered, however, to evaluate this advantage.

Theoretical Effects of Target Density

Reaction of soluble substrates with real membrane-bound "targets" differs in at least one important respect from the Adam-Delbrück model: The target density in biological systems is usually much greater than one per organelle. In 1977 Berg and Purcell considered the effect of target surface density on the reaction rate, assuming that the membrane either (*a*) perfectly reflects or (*b*) partially adsorbs substrate molecules that contact it in a nontarget area. They found that in case a the steady state diffusive flux of reactant should saturate when a small fraction of the total surface area is covered with perfect traps. As they pointed out, having once reached the surface, a molecule in random motion is likely to have multiple collisions with it before escaping. The probability (P) it will strike a trap once it has hit the surface is therefore much greater than the ratio of trap area to total surface area:

$$P = Ns/Ns + \pi a, \quad (1)$$

where N is the number of traps (radius s) on the surface of a sphere with radius a ($a \gg s$). (P is related to the reactive flux J by $P = J/J_{max}$, where J_{max} is the flux to a completely absorbing sphere). Shoup and Szabo (1982) have confirmed this result using completely independent means. To reiterate, diffusional target searching at an inert (nonadsorbing) surface is far more effective than bullet-shooting analogies would predict.

For case b, Berg and Purcell (1977) first considered the case of an adsorbing surface with a single trap and obtained the same 2d mean lifetime (t_{2d}) as did Adam and Delbrück (1968). For the situation of multiple traps, they divided the total membrane area into cells, each containing a single trap, and found t_{2d} by solving a 2d diffusion equation for each cell. The steady state flux (J_2) of adsorbed substrates into traps on the outer surface of a sphere was then expressed as the ratio m/t_{2d}, where m is the average number of adsorbed substrate molecules. This equation is valid when the reactive flux is small as compared with the maximal flux, i.e., the flux onto a perfectly absorbing sphere. It implicitly assumes that equilibration of nonspecifically bound substrates with bulk phase substrates is rapid as compared with the rate of absorption by traps. Berg and Purcell put m in terms of an energy of adsorption, but it is convenient for our purposes to have m in terms of the empirical parameters K_d or K_v, i.e., the equilibrium dissociation constant or the volume partition coefficient. With these substitutions, the Berg-Purcell criterion

Table II. Theoretical Rate Advantage from Two-Step Searching by Aqueous Reactants for Hypothetical Membrane-bound Targets

Reactant	Membrane	K_{eq}*	c_{aq}‡	D_2/D_3§	Advantage‖	Reference
		mol/liter	*mol/liter*			
Apolipoprotein C-III	Egg PC	4.2×10^{-7}	$\geq 3.5 \times 10^{-4}$	*0.11*	No	Träuble et al., 1974; Vaz et al., 1979
			10^{-12}–3.5×10^{-4}	*0.11*	Yes (1–30×)	
Cytochrome *c*	Soy PC	5.5×10^{-4}	10^{-3}–10^{-12}	*0.006*	No	Gupte et al., 1983; Setlow and Pollard, 1962; Cannon and Erman, 1980
$ACTH_{1-24}$	PA/PC (10:90)	1.7×10^{-5}	$\geq 10^{-4}$	0.034	No	Gysin and Schwyzer, 1984
			10^{-12}–10^{-4}	0.034	Yes (1–7×)	
Factor X (+2 mM Ca)	PS/PC (16:84)	3×10^{-7}	$\geq 1.3 \times 10^{-4}$	0.034	No	Nelsestuen, 1978
			10^{-12}–1.3×10^{-4}	0.034	Yes (1–14×)	
Prothrombin (+2 mM Ca)	PS/PC (16:84)	1×10^{-6}	$\geq 6 \times 10^{-5}$	0.034	No	Nelsestuen, 1978
			10^{-12}–6×10^{-5}	0.034	Yes (1–14×)	
Ovalbumin	HFL cell PM	1.2×10^{-4}	$\geq 3.6 \times 10^{-3}$	*0.017*	No	Krumins and Stotzky, 1980; Burghardt and Axelrod, 1981
			10^{-12}–3.6×10^{-3}	*0.017*	Yes (1–30×)	
Lysozyme	HFL cell PM	2.5×10^{-5}	$\geq 10^{-2}$	0.017	No	Krumins and Stotzky, 1980
			10^{-12}–10^{-2}	0.017	Yes (1–20×)	
n-Valeramide	Egg PC	*1.2*		*0.32*	No	Lange et al., 1974
TEMPO	DPPC	*20*		*0.31*	Yes (7×)	Dix et al., 1978
Lindane	DOPC	*1.3 × 10⁵*		*0.51*	Yes (31×)	Omann and Lakowicz, 1982

TEMPO, tetramethyl piperidine-*N*-oxyl; PC, phosphatidylcholine; DPPC, dipalmitoyl PC; DOPC, dioleoyl PC; PA, phosphatidic acid; PS, phosphatidylserine; PM, plasma membrane.

* K_{eq} refers to equilibrium dissociation constant, K_d, for nonspecific membrane-reactant binding, or to the volume partition coefficient, K_v. K_d in units of molarity and the italicized K_v values are dimensionless. $K_v \approx K_w \approx 0.0199\ K_x$, where K_x is the mole fraction-based partition coefficient and K_w is the weight-based partition coefficient.

‡ c_{aq} is the concentration of the reactant in the aqueous phase. J_2/J_3 is assumed independent of c_{aq} for those reactants characterized by a partition coefficient.

§ D_2/D_3 is the ratio of 2d to 3d translational diffusion coefficients. Italic values indicate that D_2 is known and D_3 is either known or calculated as in Table I (assumes a cytoplasmic viscosity 3.2 times that of pure water at the same temperature). Values not in italics are hypothetical for illustration.

‖ Theoretical rate advantage is determined by evaluation of J_2/J_3 in Eq. 2. We have assumed a dilute target density $\rho = 10^9/cm^2$ (see text for details). The organelle radius $a = 0.5\ \mu m$ and the effective reaction radius $s = 12\text{Å} + r$, where r is the radius of reactant and 12 Å is the radius of target. For proteins, r is taken from the literature or estimated from the protein's molecular weight, a specific volume of 0.7, and the assumption of a spherical shape; r for ACTH is estimated from amino acid composition (Gysin and Schwyzer, 1984), molar volumes of amino acid residues, and the assumption of a spherical shape. Values of ρ_o (in molecules per square centimeter) are taken from binding curves in the literature. Values of r (in Angstroms) and ρ_o (number per square centimeter) used in calculations are: apoliprotein C-III (22, 1.52×10^{12}); cytochrome *c* (15, 1.11×10^{13}); $ACTH_{1-24}$ (9.6, 1.19×10^{12}); Factor X (25.7, 1.99×10^{12}); prothrombin (27.5, 1.44×10^{12}); ovalbumin (23, 1.06×10^{14}); lysozyme (15, 3.15×10^{14}); *n*-valeramide (3.8); TEMPO (3.2); lindane (4.7). An area per lipid molecule of 60 Å² was assumed.

for a favorable two-step search becomes (see Table II):

$$\frac{J_2}{J_3} \approx \left(\frac{\pi}{1.1s}\right)\frac{D_2}{D_3}\left(\frac{\rho_o}{K_d + c_{aq}}\right)\left(\ln\frac{1.2}{4\pi\rho s^2}\right)^{-1} \qquad (2a)$$

or

$$\frac{J_2}{J_3} \approx \left(\frac{1.4 \times 10^{-6}\ \text{cm}}{s}\right)\frac{D_2}{D_3}\left(\frac{K_v}{\ln(1.2/4\pi\rho s^2)}\right). \qquad (2b)$$

D_2/D_3 and s are as defined above, ρ is the surface density of targets in molecules per square centimeter, c_{aq} is the concentration of the reactant in the aqueous phase in molecules per cubic centimeter, K_d is the equilibrium dissociation constant in molecules per cubic centimeter, and ρ_o is the surface density (number per square centimeter) of the nonspecifically adsorbed reactant when c_{aq} is much greater than K_d. K_v is the partition coefficient expressed as the ratio of molarity in the membrane to that in bulk solution. The ratio J_2/J_3 must be greater than 1 for two-step searching to confer an advantage.

Nonspecific Membrane-Substrate Affinities

If one imagines that the bulk (cytosolic) concentration of a substrate is kept at a steady level by synthesis and consumption, then diffusion from the cytosol to the outer surface of an imaginary 1-μm-diam organelle may be modeled by Eq. 1. In Table II we have plugged values from the literature of the parameters into Eqs. 2a and 2b to get an idea of how many molecules might fit the criteria required for an advantageous two-step searching process on intracellular membranes. We do not imply that all of these molecules are found intracellularly, nor that they are actual substrates of membrane-bound enzymes; we use them just to fathom the range of affinities and D values expected for nonselective binding. There is an obvious lack in the literature of complete data on individual biochemical systems; until these data become available one is limited to such an approach. As in Table I, the D_3 values pertain to a cytoplasmic viscosity 3.2 times that of pure water. Evidently, possession of a moderate affinity for membranes is no guarantee that a substrate will find its enzyme faster with the aid of surface diffusion than without, even when that diffusion is nearly as rapid as in the bulk. This is in sharp contrast to the assertion of Richter and Eigen that "Whenever there exists a nonspecific affinity of the membrane for the respective substrate molecules, a rate enhancement may be expected due to diffusion on that membrane" (Richter and Eigen, 1974).[2]

Successful application of dimensional reduction requires a

[2] Taken out of the context of Richter and Eigen (1974), this statement may be superficially interpreted to mean that given prior adsorption, the reaction rate is faster with surface diffusion of the adsorbate than without—a statement that is obviously true. But in fact the rate enhancement referred to was specifically for two-step vs. one-step mechanisms.

nontrivial membrane-substrate affinity. Note the overtly hydrophilic nature of most of the substrates listed in Table III. Intuition tells us that those aqueous substrates that have a marked affinity for membranes will be the exception. But one predicts them to be the rule if, indeed, the reason that "in nature so many enzymes are bound to membranes" is that such localization permits a rate enhancement by guided diffusion (Eigen, 1974). Potential substrates most likely to exhibit strong affinity without suffering a large drop in their diffusion rates are small amphiphiles and small hydrophobic

Table III. Estimated Concentrations of Membrane-associated Enzymes and Their Aqueous Substrates

Enzyme/protein*	Membrane‡	ρ	Substrate(s)§	K_m (c_o)	Notes
		No./cm²		*mmol/liter*	
Phosphate translocator	CE (spinach)	9×10^{11}	P_i, 3PGA	2.5 (*7–10*), 0.72 (*2.2*)	
P-450c (dioxin-induced)	ER (rab. liver)	6.5×10^{11}			a
NADH dehydrogenase	PM (*E. coli*)	6×10^{11}	NADH	0.05	b, c
ATP-ADP translocator	IMM (rat liver)	3×10^{11}	ATP, ADP	0.15, 0.01	d
$P\text{-}450_{IIIb}$ (uninduced)	ER (rab. liver)	2.7×10^{11}	Aminopyrine	~1	a
Ca-Mg ATPase	SR (avn. leg mus.)	$1.6\text{–}3.0 \times 10^{11}$	ATP	5	e
Glycerol-3-P dehydrogenase	PM (*E. coli*)	1.9×10^{11}	Glycerol-3-P		f, g
Na-K ATPase	PM (avian hrt.)	$1.6\text{–}1.9 \times 10^{11}$	ATP		
Ferredoxin-$NADP^+$ reductase	CT (spinach)	$>1.6 \times 10^{11}$	Ferredoxin, $NADP^+$	(*0.2*), (*0.2*)	
H^+ ATPase	IMM (rat hrt.)	1.4×10^{11}	ATP	*1.32*	h
D-Amino acid dehydrogenase	PM (*E. coli*)	1.2×10^{11}	D-Amino acids	3–40	b
Ca-Mg ATPase	PM (*E. coli*)	$0.6\text{–}1.5 \times 10^{11}$	ATP, ADP, P_i	(*7.9*) (*1.04*) (*7.9*)	b
Dopamine-β-hydroxylase	CGM (bovine)	8.9×10^{10}	Ascorbate	(*11.9*)	h
			Dopamine	4 (*3.8*)	
SFV spike glycoproteins	GC (BHK cells)	7.5×10^{10}			
HMG CoA reductase	ER (UT-1 cells)	7×10^{10}	HMG CoA	0.02	i
α2-6 Sialyltransferase	GC (rat liver)	6.5×10^{10}	CMP-NeuNAc	(*0.04*)	j
Epoxide hydolase	ER (rat liver)	$1.1\text{–}5.9 \times 10^{10}$	Octene-1,2-oxide	0.014	a, k
H^+ ATPase	CT (spinach)	$>2.5\text{–}5.5 \times 10^{10}$	P_i, ADP	(*7*)	l
Succinate dehydrogenase	IMM (rat)	5.3×10^{10}	Succinate	1.3	
Cyclic GMP phosphodiesterase	ROS (bovine)	$1.4\text{–}7.2 \times 10^{10}$	cGMP	0.15	m
Pyridine nucleotide transhydrogenase	IMM (cow hrt.)	$1.3\text{–}6.7 \times 10^{10}$	NAD^+, NADPH	0.03, 0.02	n
Glycerol-3-P acyl transferase	PM (*E. coli*)	2.9×10^{10}	Glycerol-3-P	0.15	f
D-Lactate dehydrogenase	PM (*E. coli*)	$2.5\text{–}3.3 \times 10^{10}$	D-Lactate	0.6–0.9	b
NADH dehydrogenase	IMM (rat)	2.8×10^{10}	NADH		
NADPH P-450 reductase	ER (rat liver)	$1.4\text{–}2.0 \times 10^{10}$	NADPH	0.006 (*0.14*)	a
N-acetylglucosaminyl transferase I	GC (pig liver)	1.6×10^{10}	UDP-GlNAc	(*1.4*)	j, o
Glucose-6-PO_4 translocase	ER (rat liver)	1.6×10^{10}	Glucose-6-P	(*0.09–1.3*)	
NADH cytochrome b_5 reductase	ER (calf liver)	1.5×10^{10}	NADH	0.006 (*0.12–0.36*)	a
N-Acetylglucosaminyl transferase I	GC (rab. liver)	1.3×10^{10}	Oligosaccharides	0.5–4	e, j
Galactosyltransferase	GC (HeLa cells)	$>10^{10}$	UDP-Gal	(*0.16*)	p
UDP-glucuronyl-transferase (uninduced)	ER (rat liver)	10^{10}	UDP-glucuronic acid	5.4	a
SFV spike glycoproteins	ER (BHK cells)	9×10^9			
UDP-galactose:LPS galactosyltransferase	PM (*S. typhimurium*)	$>8 \times 10^9$	UDP-Gal	0.074	q
Carnitine palmitoyl-transferase	IMM (cow hrt.)	5.2×10^9	Carnitine	0.2–2.9 (*2.5–4.8*)	r
Phosphatidylinositol synthase	ER (yeast)	4.8×10^9	Inositol	(*0.1*)	a
Phosphatidylserine synthase	ER (yeast)	3.6×10^9	Serine	0.58	a
α2-3 Sialyltransferase	GC (rat liver)	1.5×10^9	CMP-NeuNAc	(*0.04*)	j

* Lateral densities of italicized proteins are taken directly from literature, others we calculated from primary data. The original references are available upon request from us. SFV, Semliki Forest virus. LPS, lipopolysaccharide.

‡ CT, chloroplast thylakoids; CE, chloroplast inner envelope; SR, sarcoplasmic reticulum; PM, plasma membrane; IMM, inner mitochondrial membrane; ER, endoplasmic reticulum; GC, Golgi complex; CGM, chromaffin granule membrane; ROS, rod outer segment (disk membrane); hrt., heart; rab., rabbit; avn., avian; mus., muscle; *E. coli, Escherichia coli*; *S. typhimurium, Salmonella typhimurium*; BHK, baby hamster kidney.

§ P_i, inorganic P; 3PGA, 3-phosphoglyceric acid; HMG, hydroxymethylglutaryl; CoA, coenzyme A. NeuNAc, *N*-acetylneuraminic acid; GlcNAc, *N*-acetylglucosamine.

(*a*) Calculated from moles enzyme per milligram total microsomal protein, assuming a membrane area of 3.7×10^3 cm² per mg total membrane protein. Derived assuming that all the proteins are cylinders 60 Å high, with an average molecular weight of 45,000, and an amino acid composition equal to average of 10 isolated membrane proteins. We also assumed a 1:1 weight ratio of lipid to protein in the ER (and microsomes), an average molecular weight for the lipids of 770, and an average area per lipid molecule of 60 Å². (*b*) Assumes a plasma membrane area of ~500 cm²/mg total membrane protein (outer and inner membranes). (*c*) Assumes that NADH dehydrogenase constitutes 2% of total membrane protein. (*d*) 1.5–2.0 mol/mol cytochrome $a \times (1.88 \times 10^{11}$ cytochrome a/cm²). (*e*) ATP is bulk concentration in human skeletal muscle. (*f*) Assumes a surface area per cell of 4.8×10^{-8} cm² (from electron micrographs of *E. coli* spheroplasts). (*g*) Assumes 9,000 molecules/cell. (*h*) ATP is bulk concentration in rat liver. (*i*) This cell line has 200–600 times more enzyme than the progenitor Chinese hamster ovary line, but it is present in a greatly proliferated ER, so the lateral densities may be comparable. (*j*) Calculated from stereological data on liver tissue, and assuming a parenchymal density of 1.06 gm/ml. CMP-NeuNAc is bulk cellular concentration uncorrected for compartmentation. (*k*) Two different estimates based upon purification table and immunochemical quantitation. (*l*) Assumes an average of 4.75×10^{13} chlorophylls/cm² membrane, and uniform distribution of the ATPase. The ATPase is known to be highly nonuniform in distribution, making this a lower bound. (*m*) Assumes 3400 Å²/rhodopsin and 40–70 rhodopsin/phosphodiesterase. (*n*) Assumes 1.7×10^3 cm² membrane area per mg total protein in bovine submitochondrial particles. (*o*) Endogenous GlcNAc calculated from 2 nmol/10^6 HeLa cells, and 1.47×10^{-12} liters/cell. (*p*) From direct counting of antibody-coated gold particles in thin section electron micrographs. (*q*) Assumes a maximum of 500 cm² of plasmalemma area per mg of supernate protein. (*r*) Assumes 0.34 g protein/cm³ of mitochondrial volume and ~40 m² of inner membrane surface area per cm³ of heart mitochondrion.

molecules, e.g., the endogenous and xenobiotic substrates for microsomal cytochrome P450's. These molecules spend a vast majority of their time in the membrane, and it is not too surprising that enzymes that act on them are also membrane bound. To argue that things are this way because of kinetic advantages due to 2d diffusion would be superfluous. Furthermore, the actual translation of small, rapidly diffusing molecules within the hydrophobic core may deviate enough from 2d to negate the theoretical rate advantages of the 2d random walk.

Perhaps the most serious consequence of promiscuous bonding is that, by definition, membranes throughout the cell will sequester substrates, diverting them from membranes containing the specific target enzymes. No such problems exist for nonselective trapping of air-borne pheromones by antennae of *Bombyx mori* (Adam and Delbrück, 1968), but for intracellular reactions in eukaryotes this concern is real: With a modest partition coefficient of 300 and a membrane thickness of 50 Å, stereologic analyses show that in a typical eukaryotic cell 95% of the substrate would be membrane bound, most of it to the wrong membrane (Buschmann and Manke, 1981; Blouin et al., 1977; Griffiths et al., 1984). Unless the enzymes that made the substrates were themselves localized near a particular set of target membranes, reduction of dimensionality could be quite deleterious.

Substrate Concentrations In Vivo

Adam and Delbrück (1968) specified that reduction of dimensionality would be most useful where "small numbers of molecules and their diffusion are involved," and this fact is evident in Eq. 2a and also in Table II, where most entries show a cross-over from impediment to advantage as the bulk concentration of reactants is lowered. Note, however, that the intracellular concentrations of aqueous metabolites acted on by membranous enzymes are often closer to milli- than to nanomolar (Table III) and cannot automatically be considered "small numbers." (We have used K_m values as crude indicators of substrate levels when the latter were hard to find or equivocal, and have not corrected for depletion due to specific binding).

Actual Target Densities In Vivo

For those molecules in Table II that could experience a rate enhancement by guided diffusion, we must ask how realistic our dilute target approximation is. Eq. 2 is valid only for reactive fluxes that are small as compared with the maximal possible flux ($J_{3,max}$) reaching the surface, so for Table II we have assumed a target density ($\rho = 10^9$ cm^{-2}), giving $J_3/J_{3,max} = 0.03$ for a reaction radius $s = 17$ Å. Because of the saturation behavior noted above it is only when the lateral density of target enzymes is very low ($P \ll 1$) that two-step searching can yield a rate advantage.

What is known about the lateral densities of membranous enzymes? Are they low enough for two-step searching to confer an advantage? The densities listed in Table III span three orders of magnitude, yet the density assumed in our calculations for Table II is beneath the low end of the spectrum. In fact, 84% of the proteins are present at a density of at least 10^{10}/cm^2, which means that for all these the maximum possible enhancement according to the steady state model is $\leq 3\times$, under ideal conditions of K_d, D_2/D_3, and c_{aq}. Although the numbers in Table III are inexact, they were obtained by a variety of methods for organisms in four separate kingdoms; most are better than order-of-magnitude estimates. Thus it appears that the frequency of enzymes that may benefit appreciably from two-step searching on intracellular membranes is low.[3] It would be surprising if a non-steady state calculation analogous to that of Adam and Delbrück (1968) would lead to a conclusion drastically different from that reached here, although such a calculation has yet to be performed (but see Torney et al., 1985).

What Happens in the Absence of Surface Diffusion?

Without surface diffusion, when one reactant in an elementary bimolecular reaction is removed from the bulk and placed on a surface, the rate constant (in 3d units) can only go down (Astumian and Schelly, 1984). Schurr (1970) also concluded that in the absence of surface diffusion (and neglecting any ability of the membrane to concentrate substrate), when a single type of enzyme is immobilized V_{max} can only go down or remain the same, and the reverse is true for K_m. This potential for slowing reactions by embedding enzymes in membranes makes the possibility seem even more remote that membrane-enzyme association was entrained primarily because of a kinetic advantage due to dimensional reduction (Eigen, 1974).

Experimental Systems

Redox Reactions at Surfaces

Most discussions on the role of dimensional reduction in biology and chemistry have been speculative or theoretical in nature, but some quantitative experiments have been done. It is known that disproportionation of certain radical ions occurs at a rate 10^2–10^3 times greater on micelle surfaces than in the bulk (Frank et al., 1976; Henglein and Proske, 1978). Photooxidation of cytochromes *c* and c_2 by bacterial photosynthetic reaction centers incorporated into phospholipid vesicles can be eight times faster when the cytochromes are adsorbed to the membrane than when they are diffusing in the bulk (Overfield and Wraight, 1980). These rate enhancements have been attributed to a reduced dimensionality for diffusion, although a simple calculation shows that the effective 3d concentrations of the adsorbed reactants could have contributed substantially to the observed rate enhancements. Note also that the maximal eightfold enhancement observed for bacterial reaction centers may bear little relevance to photosynthetic function in *Rhodopseudomonas spheroides*, since it occurs only at an ionic strength well above that of the periplasmic space, which contains cytochrome c_2 (Overfield and Wraight, 1980). At "physiological" ionic strength the reaction of adsorbed cytochromes is retarded (≥10 times) as compared with that of bulk-dissolved cytochromes.

[3] A few entries are for plasma membrane enzymes of procaryotes and one is for a mammalian plasma membrane enzyme. It is interesting that the ρ values for these systems fall in the same range as those for enzymes of the intracellular organelles of eukaryotes. Because the dimensions of most bacteria are also comparable to those of eukaryotic organelles, one is tempted to suggest that two-step searching is unlikely to be important in either system. But the main reason for including these values is to broaden the scope of the Table, which is interesting outside the immediate context of reduction of dimensionality.

Bacteriophage Infection

It has been argued that in certain bacterial species lipopolysaccharide mediates an initial "nonspecific" adsorption of bacteriophage, which then facilitates binding of the phage to other specific surface receptors via an Adam-Delbrück two-step process (Heller and Braun, 1979; Wong et al., 1978). This argument is questionable on several counts: (*a*) The data on infection of *Salmonella anatum* by ϵ_{15} (Heller and Braun, 1979) fit a two-step better than a one-step search, but this two-step search is slower than the one-step. (*b*) Interaction of phage with lipopolysaccharide is a highly specific event (Lindberg, 1973), hardly the nonspecific adsorption envisioned by Adam and Delbrück (1968): Slight mutations in the lipopolysaccharide structure of *Escherichia coli* F completely reverse the tail fiber-mediated enhancement in the rate of T5 phage adsorption reported by Heller and Braun (1979). (*c*) Since the enhancement observed with the latter system is mediated by phage tail fibers, one has to be sure that they alone are not increasing the capture cross-section, but no relevant calculations were performed. Phage-bacterium binding can be nearly as rapid as the theoretical diffusion-controlled rate even at very low receptor densities, and a hyperbolic dependence of rate constant on receptor density is observed upon systematic manipulation of the latter (Schwartz, 1976). It is interesting that these findings are exactly those predicted by Berg and Purcell (Eq. 1) for simple one-step (3d) searching.

Fatty Acid Biosynthesis

Sumper and Träuble (1973) proposed that in yeast, fatty acyl-coenzyme A is released from the fatty acid synthetase complex only upon collision of fatty acid synthetase complex with an acceptor membrane, whereupon it diffuses laterally until it finds an enzyme that processes it. This is certainly an appealing mechanism, since free fatty acids and fatty acyl-coenzyme A bind to and inhibit some soluble enzymes. But in attributing a kinetic advantage to this hypothetical scheme, the authors did not consider the saturation effects of target density discussed in the previous section, i.e., they used the bullet-shooting analogy for diffusion. If one modifies their calculated 3d mean lifetime with the probability factor of Berg and Purcell (1977) the purported rate advantage drops to nearly zero.

Polypeptide Hormone Binding

Several polypeptide hormones such as glucagon, ACTH, calcitonin, and β-endorphin have amphiphilic structures that are essential for biological activity (Kaiser and Kézdy, 1984). Gysin and Schwyzer (1984) suggest that this amphiphilicity accelerates binding of the hormones to membrane-associated receptors by promoting two-step searching. The K_d for nonspecific binding of ACTH to negatively charged lipid vesicles is fairly low (10^{-6} M) (Gysin and Schwyzer, 1984), but the important 2d diffusion coefficient has not been measured. In any case, two-step searching via nonspecific binding of hormones to membranes seems counterproductive, since these hormones exist at extremely low concentration (10^{-9}–10^{-12} M) (Tepperman, 1980), and the relatively high nonspecific affinity required for an advantageous 2d search would tie up precious hormone in nontarget tissues throughout the body. References to further discussion on possible biological applications of dimensional reduction can be found in the review by Axelrod (1983).

Other Considerations of Membrane-associated Reactions

In this paper we have focused on just one aspect of the kinetics of membrane-associated reactions, namely, potential rate enhancements arising solely from the recurrent nature of the 2d random walk. However, localization of enzymes in membranes could conceivably yield several benefits that are independent of surface diffusion, kinetic and otherwise. Reduced transient times have been observed in artificial immobilized multienzyme systems (Mosbach, 1976; Welch and Gaertner, 1975), and may facilitate rapid adjustment to differing metabolic rates in vivo (Gaertner, 1978). In some sequential reactions even the steady state rates might be increased by proximity of the enzymes or diffusional resistances (unstirred layers) near the membrane surface (Gaertner, 1978). The local compartmentation of intermediates responsible for these effects also lowers the bulk substrate concentration necessary to sustain a given steady state rate. Atkinson (1969) has emphasized the finite solvent capacity of intracellular water and suggests that devices that maintain a low bulk concentration of substrates are favored by natural selection. "Channeling" of common intermediates through anabolic or catabolic pathways is often considered a function of multienzyme assemblies, be they on the membrane or elsewhere (Welch, 1977; Freedman, 1981). Even for a single enzyme, partitioning of substrates into or on the membrane could yield higher effective concentrations, and maintain orientations favorable for reaction (Fendler and Fendler, 1975; Nesheim et al., 1981). Increased sensitivity to small changes in the concentrations of substrate, product, and effector molecules opens new possibilities for regulation mechanisms with immobilized enzymes (Engasser and Horvath, 1976; Lecoq et al., 1975). Most of these potential advantages have yet to be demonstrated for multienzyme systems held together only by a phospholipid bilayer; but in comparison with these and other very obvious nonkinetic factors, the contribution that dimensional reduction, i.e., two-step searching, makes to any "selective advantages" of intracellular membranes would seem to pale.

Conclusions

The theoretical predictions of Adam and Delbrück (1968) have been tested in model 2d systems. As predicted, dimensional reduction or two-step searching can enhance as well as retard reaction rates. It remains to be seen whether the rate enhancements actually derive from the special properties of 2d diffusion and reaction or from other sources such as increased local reactant concentrations. The hypothesis that dimensional reduction was entrained by natural selection demands a delicate balance between two seemingly opposing factors: rapid 2d diffusion and strong nonspecific attraction. Examination of available data on diffusion coefficients and binding constants, scarce and imprecise as they may be, suggests that this balance is not generally met. Even when it is met, theory predicts diminishing returns from dimensional reduction as the target density and bulk phase substrate concentration are increased. From estimates of the lateral densities of several membranous enzymes and from the endogenous levels of their substrates, it appears that the maximum rate advantage to be expected from two-step searching on intracellular membranes is in most cases small to nil. Thus, the teleological notion that internal membranes may

somehow have "capitalized" on Polya's theorem remains a cure in search of a disease.

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