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The Determination of Local Reaction and Diffusion Parameters of Enzyme Membranes from Global Measurements†

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ABSTRACT: We present a study of simultaneous reaction and transport of benzoyl-L-argininamide in collodion membranes containing cross-linked papain. Two types of symmetrical membranes are investigated: homogeneous membranes and three-layer membranes in which the enzyme layers are separated by an inert region of membrane. In the case where the local substrate concentration within the pores of the membrane is much less than the Michaelis constant (K_m), the local reaction kinetics approach pseudo-first-order. In this case the ratio of the maximum reaction rate to the Michaelis constant (V_m/K_m) and the diffusion coefficient d_s of the substrate can be found from measurements of boundary flow rates and substrate concentrations. In the present case d_s can also be obtained directly by studying diffusion in reaction inhibited membranes. Consequently in the case of homogeneous membranes a self-consistent test of the data obtained with reaction can be made by comparing d_s values found with and without reaction. In the case of three-layer membranes the independent value of d_s enables one to estimate the thickness of the catalytic regions relative to the inert regions. The methods presented are nondestructive. It is shown that reaction alters the diffusion coefficient of the substrate by causing it to increase and introduces cross-diffusion coefficients between substrate and product. These membranes serve as models of facilitated transport systems.

Binding an enzyme within the pores of a membrane can markedly alter its catalytic properties (Silman and Katchalski, 1966; Goldman *et al.*, 1965; Mosbach and Mattiasson, 1970). In addition to possible changes in the intrinsic chemical rate parameters, one must also consider additional influences due to physical transport processes. The effects of the latter on reaction rate have been treated at length by chemical engineers in the analysis of porous catalysts (Thiele, 1939). In previous papers (DeSimone and Caplan, 1970, 1973) we have shown the circumstances under which enzyme membranes may serve as models for biological facilitated and active transport. In this paper we present an experimental study of symmetrical papain–collodion membranes. Our goal is to evaluate, from global measurements of substrate and product flow rates and concentrations, the intrinsic local reaction and diffusion parameters of the system. As we shall show the methods of analysis, because of their kinetic character, have the advantage of yielding results while leaving the membrane intact. By restricting the analysis to the pseudo-first-order kinetic regime we can obtain, in the case of homogeneous membranes, the substrate diffusion coefficient, d_s, as well as the pseudo-first-order rate constant, V_m/K_m, where V_m is the maximum reaction rate and K_m is the Michaelis constant for the substrate.

The membrane used in this study is the papain–collodion membrane developed by Goldman *et al.* (1965). Some of the kinetic properties of this system were examined by Goldman *et al.* (1968b). Work on related systems includes that of Sélégny *et al.* (1969) on glucose oxidase membranes, and of Meyer *et al.* (1970) who developed an alternative method to that presented here for the treatment of first-order reactions in membranes. We treat here two types of symmetrical membranes: the one-layer homogeneous membrane and the three-layer membrane. The former consists of a collodion matrix with a uniform cross-linked layer of papain throughout. The latter is a symmetrical sandwich structure of two papain–collodion layers separated by an internal section of matrix devoid of enzyme. Experimentally each membrane is treated as a "black box" even though in each case the distribution of enzyme is *a priori* known. Nevertheless in the case of three-layer membranes it is possible to establish a lower bound on the relative thickness of the enzyme layer from the results of global measurements on the intact membrane.

An important aspect of our analysis is the recognition of the useful concept of "transport" on a global scale for reacting species. The global membrane can then be characterized dynamically by both transport and reaction processes, just as each elemental membrane volume is so characterized. One's ability to formalize both processes on the local and global levels is essential for a complete characterization of the membrane.

Experimental Section

Preparation of Matrices. Collodion membranes were prepared by the method of Carr and Sollner (1944) and Gregor

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and Sollner (1946). A solution of 4% nitrocellulose in an ether–ethanol–water solvent (50:48:2, v/v) was cast in two layers over tubes rotated at 20 rpm. Immediately after casting, the tubes were placed in water. After 24 hr the membranes were removed from the tubes and stored under water at 4°. Membranes produced in this manner are typically hydrated (90% water by weight) and range from about 310 to 350 μ in thickness.

Papain Impregnation. The method of Goldman *et al.* (1968a) was followed in the preparation of papain impregnated matrices. A solution of papain (Worthington Biochemical Corp.) in 0.05 M sodium acetate buffer (pH 4) and 0.15 M sodium chloride is placed in contact with the membrane. The sorption time or the total amount of enzyme can be varied depending upon whether one- or three-layer membranes are desired. In order to prevent leaching out of papain, the enzyme is cross-linked by means of a diazo coupling reaction. The diazo precursor is 4,4′-diamino-2,2′-biphenyldisulfonic acid (Eastman Organic Chemicals).

Determination of the Diffusion Coefficient of Benzoyl-L-argininamide. A typical membrane was placed in a diffusion chamber such as that shown schematically in Figure 1. The chambers were made of Lucite and were stirred by means of spin fins turned magnetically. The substrate selected for study was $BzArgNH_2$.[1] In the presence of an activator, such as cysteine–EDTA, papain acts on $BzArgNH_2$ to yield NH_4-BzArg. However, if the activator is withheld ammonia formation and hence reaction cannot be detected. One may therefore obtain directly the diffusion coefficient of $BzArgNH_2$. This was done by placing 4 ml of 10 mM $BzArgNH_2$ made up in phosphate buffer (pH 6.0) in one chamber while 4 ml of buffer was placed in the opposing chamber. The chambers were kept in a constant temperature enclosure (25 ± 1°), and 0.1-ml samples were withdrawn from each chamber at 5-min intervals for about 25 or 30 min. Samples withdrawn from the downstream chamber were assayed spectrophotometrically. In the phosphate buffer λ_{max} = 228 mμ and ϵ_{max} = 10,700 ± 100 cm^{-1} M^{-1} for $BzArgNH_2$.

Assay of the System with Reaction. As in the previous section a membrane was mounted between a pair of chambers. In each chamber was placed 4 ml of a solution whose composition was 0.05 M phosphate buffer (pH 6), 4 mM cysteine, 1.6 mM EDTA, and either 0, 2, 4, or 6 mM $BzArgNH_2$. The only variable component was the $BzArgNH_2$ concentration, each chamber having, in general, different initial concentrations. Experiments were done at 25 ± 1°. The chambers were stirred magnetically, and 0.1-ml samples were withdrawn from each at 5-min intervals for 25 or 30 min and assayed for ammonia by Conway microdiffusion analysis. The production of ammonia in time is a measure of the global extent of reaction.

Theoretical Analysis

Consider the following three-layer membrane, which is typical of the membranes described by Goldman *et al.* (1965, 1968a): region I, $0 \leq x \leq \delta$, enzymatically active; region II, $\delta < x < \delta'$, inert; region III, $\delta' \leq x \leq \Delta x$, enzymatically active. The membrane is symmetrical about the plane $x = \Delta x/2$ provided each region is uniform and $\delta = \Delta x - \delta'$. If $\delta = \delta' = \Delta x/2$, the general three-layer structure collapses to the case of a membrane with uniform enzyme concentration across its entire thickness, *i.e.*, a homogeneous membrane. Accordingly in our analysis we shall treat the homogeneous membrane as a special limiting case of the symmetrical three-layer membrane.

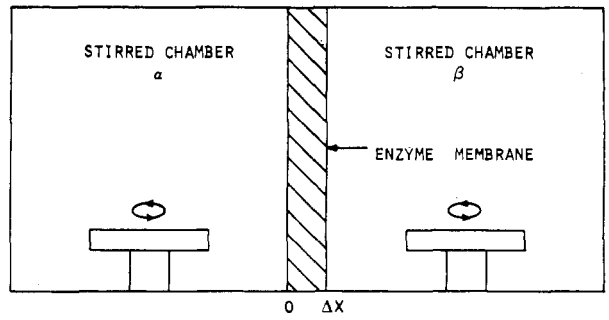

FIGURE 1: Schematic representation of an enzyme membrane between a pair of reagent chambers. Membrane area is 1.77 cm^2. Chamber volume is 4 ml.

The reaction

$$BzArgNH_2 + H_2O \xrightarrow{\text{papain}} NH_4BzArg$$

has been shown to follow a Michaelis–Menten rate law (Stockell and Smith, 1957). In general a slab membrane element in regions I and III can be characterized by the transport equation

$$J_i(x) = -d_i \frac{dc_i}{dx} \qquad (i = s, p) \tag{1}$$

and the reaction rate

$$J_r(x) = \frac{V_m}{1 + \dfrac{K_m}{c_s(x)}} \tag{2}$$

In the chemically inert region II, only the transport equations apply; consequently the flow of a species in this region is conservative. Equation 2 can be linearized provided $c_s(x)$ is everywhere much less than K_m, a condition which must apply at every local element.[2] The linearized reaction rate expression is

$$J_r(x) = k_1 c_s(x) \tag{3}$$

$$k_1 = V_m/K_m \tag{4}$$

The parameter k_1, the ratio of the maximum reaction rate, V_m, to the Michaelis constant K_m, is a pseudo-first-order rate constant. On the basis of symmetry k_1 has the same constant value in regions I and III, and is of course zero in region II.

[1] Abbreviations used are: $BzArgNH_2$, benzoyl-L-argininamide; NH_4BzArg, ammonium benzoyl-L-arginine.

[2] A more useful condition for the linearization of eq 2, applicable to homogeneous membranes, can be formulated in terms of the boundary substrate concentrations (DeSimone and Caplan, 1973)

$$\frac{c_s^3(\Delta x) - c_s^3(0)}{c_s^2(\Delta x) - c_s^2(0)} \ll 1.5K_m$$

where $c_s(\Delta x)$ and $c_s(0)$ are the boundary substrate concentrations. Should they be equal, this reduces to $c_s \ll K_m$.

While one might also expect the local diffusion coefficients, d_i, to vary discontinuously across the three-layer structure, in practice no difference is observed in d_i measured in papain–collodion regions as compared with collodion matrices devoid of enzyme (*cf.* Goldman *et al.*, 1968b). Accordingly we take d_i as constant across the membrane. In order to obtain global expressions amenable to experimental test, we first must obtain the flow profile of substrate across the membrane. In the stationary state in regions I and III we have

$$\frac{dJ_s}{dx} = -J_r \tag{5}$$

Making use of eq 1, 3, and 5, we obtain

$$\frac{d^2J_s}{dx^2} - k^2J_s = 0 \tag{6}$$

$$k^2 = \frac{k_1}{d_s} \tag{7}$$

In region I, eq 6 is solved with boundary conditions (at $x = 0$)

$$J_s = J_s(0)$$

$$\left[\frac{dJ_s}{dx}\right]_0 = -k_1c_s(0)$$

In region III the boundary conditions (at $x = \Delta x$) are

$$J_s = J_s(\Delta x)$$

$$\left[\frac{dJ_s}{dx}\right]_{\Delta x} = -k_1c_s(\Delta x)$$

Since the flow through II is conservative we have

$$J_s = J_s(\delta) = J_s(\delta') = \text{const} \qquad \delta < x < \delta' \tag{8}$$

The flow profiles in regions I and III are given by

$$J_s(x) = J_s(0)\cosh kx - \frac{k_1}{k}c_s(0)\sinh kx \qquad \text{region I} \tag{9}$$

$$J_s(x) = J_s(\Delta x)\cosh k(\Delta x - x) + \frac{k_1}{k}c_s(\Delta x)\sinh k(\Delta x - x) \qquad \text{region III} \tag{10}$$

Assuming continuity of flow at $x = \delta$ and δ', eq 8–10 give

$$J_s(\delta) = \bar{J}_s\cosh k\delta + \frac{k_1}{2k}\Delta c_s \sinh k\delta \qquad \text{region II} \tag{11}$$

Here

$$\bar{J}_s = \frac{J_s(0) + J_s(\Delta x)}{2} \tag{12}$$

$$\Delta c_s = c_s(\Delta x) - c_s(0) \tag{13}$$

Since

$$J_s(\delta) - J_s(\delta') = 0$$

we can obtain with the aid of eq 9 and 10

$$J_R = K\bar{c}_s \tag{14}$$

where

$$J_R = J_s(0) - J_s(\Delta x) \tag{15}$$

$$K = \frac{k_1\Delta x}{r}G(\gamma) \tag{16}$$

$$G(\gamma) = \frac{\tanh\gamma}{\gamma} \tag{17}$$

$$\gamma = \delta\sqrt{\frac{k_1}{d_s}} \tag{18}$$

$$r = \frac{\Delta x}{2\delta} \tag{19}$$

$$\bar{c}_s = \frac{c_s(0) + c_s(\Delta x)}{2} \tag{20}$$

Equation 14 is the global expression for the reaction flow in the membrane. It is seen to be a linear function of the mean boundary substrate concentration. The dimensionless parameter γ (Thiele modulus) is determined by the characteristic reaction and diffusion parameters and the thickness of a single catalytic region δ. The parameter r, the ratio of total membrane thickness Δx to enzyme thickness, is a measure of the deviation from homogeneity. In the case of a homogeneous membrane $r = 1$ and $\delta = \Delta x/2$.

The concentration difference, Δc_s, can be obtained from

$$\Delta c_s = -\frac{1}{d_s}\int_0^{\Delta x} J_s(x)dx \tag{21}$$

The integration is of course carried out using the expression for $J_s(x)$ appropriate for each region. In this manner one may obtain

$$\bar{J}_s = -D_s\frac{\Delta c_s}{\Delta x} \tag{22}$$

where

$$D_s = d_s\frac{r(1 + (r-1)\gamma^2G)}{r - 1 + G} \tag{23}$$

Equation 22 may be considered the global analog of a transport equation for the reacting substrate. Indeed it is formally similar to Fick's law. In this case however it is the mean of the boundary flows which is proportional to the concentration difference. The significance of $\bar{J}_s$ as a "global" transport parameter has been discussed extensively by DeSimone and Caplan (1970, 1973). The mean flow $\bar{J}_s$ can be shown to be identical with the flow of substrate across a particular plane within the membrane. Such a plane (designated the "transport" plane) divides the membrane into two regions of equal chemical turnover of substrate.[3] The transport plane gives rise to a global analog of the Curie Principle; it coincides with the membrane boundaries only if the *net* chemical turnover of substrate for the entire membrane averages to zero.

It is interesting to compare the mean flow $\bar{J}_s$ across the transport plane to the flow that would occur there in the absence of reaction for the same concentration difference Δc_s. This is equivalent to comparing the global diffusion coefficient D_s to the reaction free coefficient d_s. It can be seen from eq 23 that

$$\frac{D_s}{d_s} \geq 1 \tag{24}$$

In other words the effect of reaction is in general to facilitate the passage of substrate across the transport plane. In the case of the homogeneous membrane D_s has the simple form

$$D_s = d_s \gamma \coth \gamma \tag{25}$$

which is identical with the result of Sherwood and Pigford (1952). The relationship between $\bar{J}_s$ and $J_s(\delta)$ can be obtained by substituting for Δc_s in eq 11 with the aid of eq 22 and 23, *viz.*

$$J_s(\delta) = \bar{J}_s\left[\frac{\operatorname{sech} \gamma}{1 + (r-1)\gamma^2 G}\right]$$

It can be seen that $J_s(\delta)$ can never exceed $\bar{J}_s$ and is equal to $\bar{J}_s$ only in the trivial case of no reaction or the case $\bar{J}_s = 0$, when both reservoirs are of identical composition. The global expression for the mean flow of product can be obtained by noting that the sum of the flow of substrate and product is conservative in all three regions of membrane. A convenient way of expressing this fact is

$$J_s(x) + J_p(x) = \bar{J}_s + \bar{J}_p \tag{26}$$

where $\bar{J}_p$ is the mean flow of product. Using eq 1 and the condition $\Delta c_p = 0$ we obtain

$$[\bar{J}_p]_{\Delta c_p = 0} = -d_s \frac{\Delta c_s}{\Delta x} - \bar{J}_s \tag{27}$$

With the aid of eq 22 and 23 this becomes

$$[\bar{J}_p]_{\Delta c_p = 0} = -D_{ps} \frac{\Delta c_s}{\Delta x} \tag{28}$$

where

$$D_{ps} = d_s - D_s = d_s\left[\frac{G - 1 - r(r-1)\gamma^2 G}{r - 1 + G}\right] \tag{29}$$

From eq 29 it is clear that the reaction induced cross-diffusion coefficient D_{ps} is either zero or negative.

[3] $\bar{J}_s$ can also be considered as a weighted average of $J_s(x)$ across the membrane. It can be shown that

$$\bar{J}_s = \frac{\int_0^{\Delta x} J_s(x) J_r(x) dx}{\int_0^{\Delta x} J_r(x) dx}$$

where

$$J_r(x)/\int_0^{\Delta x} J_r(x) dx$$

is the distribution of chemical sources and sinks.

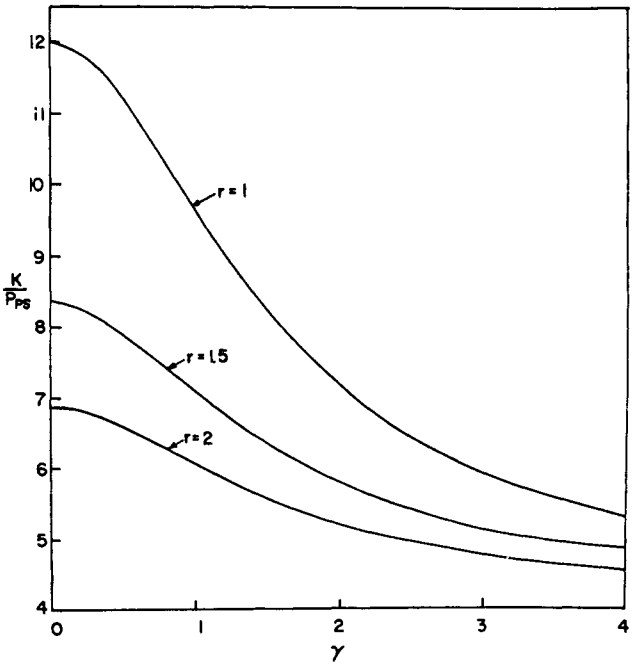

FIGURE 2: The global ratio K/P_{ps} as a function of γ for a symmetrical three-layer membrane. Here $r = \Delta x/2\delta$ and $\gamma = \delta(k_1/d_s)^{1/2}$, where δ is the thickness of a single catalytic region. All curves approach 4 asymptotically. For $r = 1$ the membrane is homogeneous.

Equations 14, 22, and 28 constitute the basic global relations for the general symmetrical three-layer membrane restricted to the pseudo-first-order kinetic regime. In principle D_s, D_{ps}, and K may be obtained from experiment. Each is a function of the dimensionless parameters r and γ, but none is a function of these groupings only. However ratios of global coefficients such as K/P_s, K/P_{ps}, or P_{ps}/P_s *do* depend on r and γ only, where we have introduced the permeabilities $P_s = D_s/\Delta x$ and $P_{ps} = -D_{ps}/\Delta x$. It is for this reason that at least two global parameters must be determined. For the system at hand assay of product is more convenient that that of substrate. As a consequence we have chosen K/P_{ps}. From eq 16 and 29 this quantity is

$$\frac{K}{P_{ps}} = \frac{4\gamma^2 G r(r - 1 + G)}{1 - G + r(r-1)\gamma^2 G} \tag{30}$$

Figure 2 shows K/P_{ps} as a function of γ for three values of r. For $r = 1$, the homogeneous case is represented. As r increases the maximum attainable value of K/P_{ps} decreases. Consequently it is possible to obtain at least an upper bound on r when the precise value is unknown, and therefore an estimate of the lower limit of the thickness of the enzyme layer.

Equation 30 is fundamental to the analysis. If r is known, the appropriate value of the Thiele modulus can be obtained from eq 30 using the value of K/P_{ps} obtained experimentally. In this case both k_1 and d_s may then be calculated. Using eq 16, we can write

$$k_1 = \frac{Kr\gamma \coth \gamma}{\Delta x} \tag{31}$$

and with eq 18 and 19 this gives

$$d_s = \frac{K\Delta x \coth \gamma}{4r\gamma} \tag{32}$$

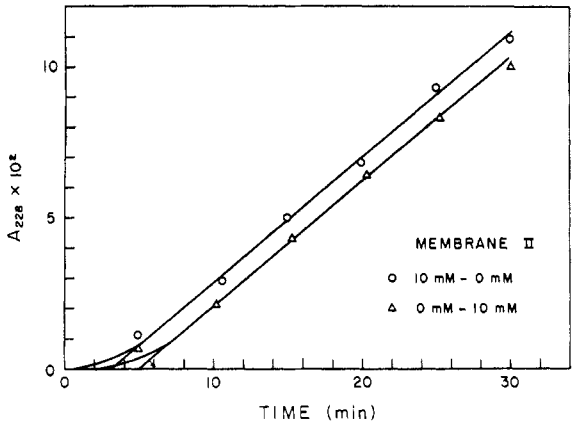

FIGURE 3: The time course of the assay of the downstream chamber in $BzArgNH_2$ diffusion experiments for membrane II showing the same steady-state response for both initial gradient directions.

If r is not known, eq 30 permits one to express r as a function of γ for fixed values of K/P_{ps}, *viz.*

$$r = \frac{\left(1 + \frac{G}{F-1}\right) + \sqrt{\left(1 + \frac{G}{F-1}\right)^2 - \frac{4F(1-G)}{(F-1)\gamma^2 G}}}{2} \quad (33)$$

where $F = K/4P_{ps}$. Substituting eq 33 into 32 gives d_s as a function of γ only. If d_s is known from independent measurements, γ and r may then be calculated.

Results

In this section the results of experiments on two membranes are given. In the case of the homogeneous membrane (I), the ratio of membrane thickness to catalytic thickness is known *a priori*. Consequently the substrate diffusion coefficient can be calculated along with the pseudo-first-order rate constant. In the case of the three-layer membrane (II), the independently determined value of d_s is used to estimate both r and k_1.

The diffusion coefficient of $BzArgNH_2$ in both membranes was determined as described earlier. In order to investigate possible dependence of the diffusion coefficient on the direction of the $BzArgNH_2$ gradient, determinations were made with the gradient set up initially in opposite directions. The results are shown in Figure 3 and Table I. No variation in d_s with reversal of gradient could be detected in either case. The assay of the chemically active system was performed also as described earlier. Typically the flow of product (NH_4BzArg) at each membrane boundary was determined by the change in concentration of product with time in each reservoir for a variety of substrate concentrations. This is illustrated for membrane II in Figure 4.

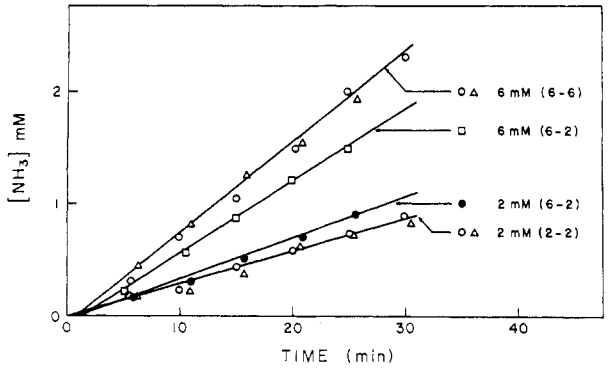

FIGURE 4: Product assay in reaction–diffusion studies on membrane II. The initial concentration of $BzArgNH_2$ in the assayed chamber is indicated. The numbers beside it in parentheses indicate its relation to the $BzArgNH_2$ concentration in the opposing chamber.

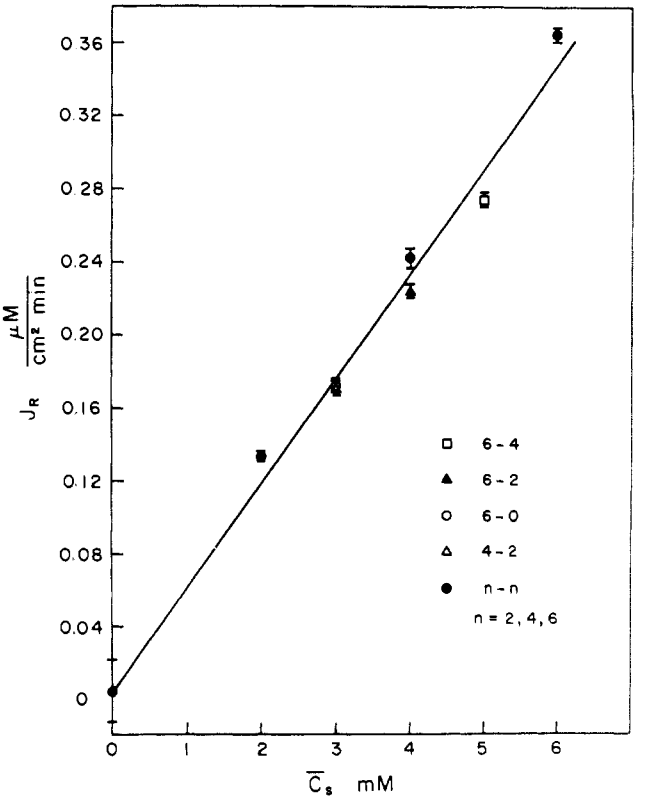

FIGURE 5: The global reaction rate J_R plotted as a function of the mean substrate concentration, $\bar{c}_s$, for membrane II. The slope gives the global reaction coefficient K. The error bars refer to standard deviations.

In order to test eq 14, the global reaction rate J_R, calculated as the difference in boundary product flow rates, was plotted as a function of the mean of the concentrations of substrate in both reservoirs. The relation was linear up to a mean concentration of 6 mM $BzArgNH_2$. The global reaction coefficient K was obtained from the slope of the least-squares line. The results for membrane II are shown in Figure 5. In order to test eq 28, the mean flow of product, $[J_p]_{\Delta c_p = 0}$, was computed and plotted as a function of the concentration difference of substrate across the membrane. The slope of the least-squares line is P_{ps}, the cross permeability coefficient between product and substrate. This is shown for membrane II in Figure 6.

TABLE I: Diffusion Coefficient of $BzArgNH_2$.

Membrane	[$BzArgNH_2$], mM α[a]	β[a]	d_s (mean ±SD), $cm^2\ sec^{-1} \times 10^6$
I	10	0	2.7 ± 0.1
	0	10	2.6 ± 0.1
II	10	0	2.3 ± 0.1
	0	10	2.3 ± 0.1

[a] Refer to Figure 1. $\Delta x(I) = 350\ \mu$; $\Delta x(II) = 333\ \mu$.

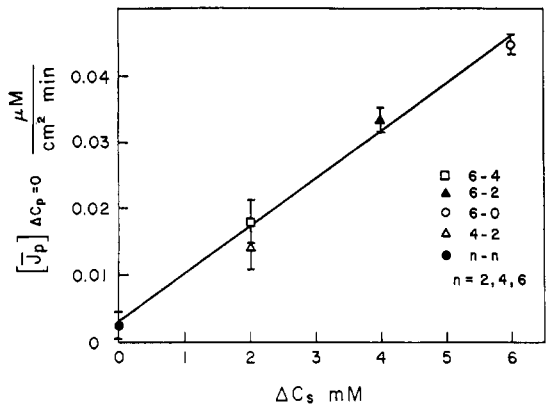

FIGURE 6: The mean flow of product plotted as a function of the concentration difference of substrate for membrane II. The slope gives the cross coefficient P_{ps}. The error bars refer to standard deviations.

Table II summarizes the data obtained for each membrane. The error in K and P_{ps} is reported as the standard deviation from the mean least-squares slope.

In the case of membrane I, K/P_{ps} uniquely determines γ, or more precisely (given the inherent experimental uncertainty) a range of possible γ values is defined. Derived values of γ, k_1, and d_s are displayed in Table III for membrane I. The rows marked $+\sigma$ and $-\sigma$ are based on a value of γ associated with K/P_{ps} at the upper and lower limits of a single standard deviation. The best agreement between the calculated and measured values of d_s occurs at the $+\sigma$ level. In the case of membrane II the ratio K/P_{ps} can be used to determine the maximum domain of r. As a reasonable estimate of the lowest value of K/P_{ps}, we shall take the mean value reduced by two standard deviations from the mean, 6.47. The maximum value of r occurs in the limit as γ approaches zero when K/P_{ps} has its minimum possible value. Making use of eq 33 we find

$$1 < r \lesssim 2.2 \qquad (34)$$

Accordingly the minimum thickness of enzyme is estimated as $2\delta \simeq 152\ \mu$. Of course the limit as γ approaches zero implies an arbitrarily large value of d_s. Therefore in most practical situations this limit will not be approached.

In order to arrive at estimates of the local parameters of membrane II, we seek for a given value of K/P_{ps}, the values of r and γ for which the calculated and measured values of d_s differ least. Equations 33, 32, and 31 are accordingly utilized to construct Table IV. The parameters are calculated for the mean value of K/P_{ps} and for values at one and two standard deviations lower than the mean. (In using eq 32 and 31 the mean value of K was adopted.) The best agreement between the measured and calculated values of d_s occurs at the -2σ level of K/P_{ps}. Indeed the best agreement occurs for $r = 1$, which corresponds to a homogeneous membrane. However, microscopic examination of cross sections of membrane II shows the lower limit of r to be about 1.2. For values of r between 1 and 1.2, k_1 is not appreciably altered for a given value of K/P_{ps}. Consequently there is practically no error here in selecting $r = 1$ over $r = 1.2$, as far as k_1 is concerned.

It may be noted that even though the three-layer structure of membrane II is considered, the values of k_1 for membranes I and II are significantly different. The most likely reason is that the concentration of active enzyme is different in each case. There is inevitably some inactivation of the enzyme during the sorption and cross-linking processes. If we assume that the local turnover number and Michaelis constant are the same for each membrane (but not necessarily the same as reported for free solution), the ratio of k_1 values corresponds to the ratio of active enzyme in one membrane relative to the other. In this case k_1 for membrane II relative to I is about 0.38. In other words the concentration of active enzyme in membrane II is only about 40% of that in I.

In their analysis of papain–collodion membranes, Goldman *et al.* (1968b) used an indirect method for obtaining the Thiele modulus for $BzArgNH_2$. Their method depends upon the availability of a "slow" substrate for papain, and involves a number of assumptions. It is assumed that $G(\gamma)$ (in our notation) is unity for the "slow" substrate. It is further assumed that the values of Michaelis constants and the turnover numbers which are applicable to catalysis in free solution apply locally within the membrane. If each of these assumptions is valid, then the active enzyme concentration for a given membrane can be calculated directly from reaction rate measurements on the "slow" substrate. Once the active enzyme concentration is found, the value of the Thiele modulus can be calculated for faster substrates such as $BzArgNH_2$, again assuming that solution parameters can be used in

TABLE II: Experimentally Determined Global Parameters (mean ± SD).

Membrane	K, cm min^{-1} × 10^2	P_{ps}, cm min^{-1} × 10^2	K/P_{ps}
I	7.97 ± 0.47	1.37 ± 0.12	5.82 ± 0.62
II	5.72 ± 0.43	0.72 ± 0.04	7.97 ± 0.75

TABLE III: Derived Local Parameters for Membrane I (See Text).

	γ	k_1, sec^{-1}	d_s, cm² sec^{-1} × 10^6	d_s,[a] cm² sec^{-1} × 10^6	D_s/d_s
$-\sigma$	2.49	0.096	4.7		2.53
mean	3.13	0.12	3.7	2.7 ± 0.1	3.14
$+\sigma$	4.32	0.16	2.7		4.32

[a] Value for reaction-free case.

TABLE IV: Calculated Parameters for Membrane II (See Text).

	K/P_{ps}	γ (Trial Values)	r	d_s, cm² sec^{-1} × 10^6	k_1, sec^{-1}	D_s/d_s
Mean	7.97	1.3	1.2	6.3	0.052	1.70
		1.5	1.1	5.6	0.053	1.78
		1.7	1.0	5.1	0.052	1.82
$-\sigma$	7.22	1.5	1.2	4.5	0.053	1.90
		1.6	1.1	4.2	0.050	1.87
		1.9	1.0	4.0	0.053	1.99
-2σ	6.47	1.5	1.4	3.5	0.057	2.16
		2.0	1.2	2.9	0.061	2.45
		2.5	1.0	2.7	0.061	2.54

the defining equation (*e.g.*, our eq 18). From the data reported by Goldman *et al.* (1968b) a value of 0.39 sec^{-1} can be calculated for k_1. This value is assumed to apply to all membranes; consequently the values of γ reported by these authors vary only with the thickness of the catalytic region. They range from 0.88 to 8.5. Although one would not expect perfect agreement between k_1 values obtained by Goldman *et al.* (1968b) and those reported herein, they are certainly mutually consistent. However, it should be noted that our more direct approach eliminates the need for a "slow" substrate and many of the assumptions central to the analysis of Goldman *et al.*

As indicated earlier the mean flow $\bar{J}_s$ is equal in value to the flow of substrate within the membrane across a transport plane which divides the membrane into two regions of equal chemical turnover of substrate. In the presence of reaction this flow is facilitated compared to the reaction free case. The ratio D_s/d_s is a measure of the degree of facilitation. The facilitation ratio is reported for both membranes in Tables III and IV. It is clear that the qualitative effect in all cases is the augmentation of flow by reaction. The effect is quite general and is independent of detailed molecular mechanism.

Discussion

The methods outlined above for determining the local reaction and diffusion parameters of an enzyme membrane can be utilized in principle whenever experimental access to the pseudo-first-order regime is possible. Sélégny *et al.* (1968) have shown that a study of the zero-order limit can yield V_m. Consequently a study of both limits can give K_m uniquely. It should be clear that because of diffusion V_m and K_m cannot be obtained generally by the usual Lineweaver–Burk analysis. The methods described here can be used to obtain estimates of the substrate diffusion coefficient as well as the reaction rate constant provided the thickness of the enzyme regions is known relative to the overall thickness of the membrane. This is particularly useful in such cases where it is either impractical or even impossible to obtain substrate diffusion measurements without reaction occuring. If r, the ratio of membrane thickness to enzyme thickness, is not known, an independent estimate of the substrate diffusion coefficient permits the calculation of both r and the Thiele modulus γ. The ratio K/P_{ps} provides an upper bound or r even in the absence of diffusion data.

In the diffusion limited case ($\gamma \gg 4$) the method for determining γ from a knowledge of K/P_{ps} becomes insensitive because the curves in Figure 2 become increasingly flatter as γ increases. In such cases k_1 can still be found provided d_s can be measured independently (DeSimone and Caplan, 1973). In the case of homogeneous membranes it can be shown that

$$KP_s = k_1 d_s \tag{35}$$

Equation 35 is also asymptotically valid (as $\gamma \to \infty$) in the case of three-layer membranes and may be used practically for $\gamma > 4$. It may be noted that all curves K/P_{ps} converge for sufficiently large γ irrespective of the value of r. This reflects the fact that in diffusion limited cases most of the reaction is confined to the outer surfaces of the membrane and hence the internal structure of the membrane does not affect the overall dynamics.

The chief advantage of our approach, as compared with that described by Meyer *et al.* (1970), is that a single membrane configuration is required rather than the two configurations suggested by these authors, *i.e.*, experiments with both surfaces in contact with reservoirs and additional experiments with only one side exposed. As mentioned earlier our method does not rely on the availability of "slow" substrates, nor does it involve assumptions concerning the applicability of solution rate parameters to the local environment of the membrane.

In our treatment of symmetrical membranes we have shown that reaction may facilitate flow. However, active transport cannot be modeled in such membranes. Suitably asymmetrical structures, on the other hand, do permit various aspects of active transport to be modeled (Blumenthal *et al.*, 1967; Bunow, 1970; Broun *et al.*, 1972).

Symbols

$c_i(x)$ concentration of species i at a given point x
c_i arithmetic mean of the boundary concentrations of species i
D_{ps} global cross-diffusion coefficient between the product and substrate
D_s global diffusion coefficient of substrate
d_i local diffusion coefficient of species i
G catalyst effectiveness factor $\tanh \gamma/\gamma$
$J_i(x)$ flow of species i at a given point x within the membrane
$\bar{J}_i$ arithmetic mean of the boundary flows of species i
J_R global reaction flow for the membrane
$J_r(x)$ local reaction flow at a given point x within the membrane
K global reaction coefficient
K_m Michaelis constant for the substrate $BzArgNH_2$
k reciprocal characteristic length $(k_1/d_s)^{1/2}$
k_1 pseudo-first-order rate constant V_m/K_m
P_{ps} cross-permeability of product $-D_{ps}/\Delta x$
P_s permeability of substrate $D_s/\Delta x$
p product (NH_4BzArg)
r ratio of membrane thickness to total enzyme thickness $\Delta x/2\delta$
s substrate ($BzArgNH_2$)
V_m maximum local reaction rate, proportional to the concentration of enzyme
x membrane normal coordinate
α, β reservoir designations
γ Thiele modulus $\delta(k_1/d_s)^{1/2}$
Δc_i difference in concentration between the reservoirs for species i
Δx membrane thicknesss
δ thickness of a single enzyme layer in a symmetrical three-layer membrane
ϵ_{max} maximum extinction coefficient of $BzArgNH_2$
λ_{max} wavelength of maximum extinction
σ unit standard deviation

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Spectral Changes on Binding of Oligosaccharides to Murine Immunoglobulin A Myeloma Proteins†

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ABSTRACT: Six mouse myeloma proteins with binding activity directed against multiple $\beta(1\rightarrow6)$-linked D-galactopyranose units have been isolated from ascites on a Sepharose–bovine serum albumin–*p*-azophenyl β-D-thiogalactopyranoside column. The proteins gave single bands on immunoelectrophoresis against rabbit anti-whole mouse serum (BALB/c). The changes in fluorescence properties of the six proteins on binding with $\beta(1\rightarrow6)$-linked oligosaccharides of galactopyranose were investigated. The intensities of fluorescence of two IgA proteins, J-539 and X-24, were increased on binding with the tri- and tetrasaccharide. The fluorescence properties of three other proteins, S-10, T-191, and J-1, were not significantly affected by these oligosaccharides. The fluorescence of protein X-44 was quenched upon binding $\beta(1\rightarrow6)$-galactotriose and -tetraose. From these changes in fluorescence, binding constants for proteins J-539 and X-24 and their Fab′ fragments with the tri- and tetrasaccharide have been calculated. Strong evidence for the presence of tryptophan in or near to the active sites is presented.

Myeloma proteins[1] are homogeneous immunoglobulins produced by neoplastic plasma cells. A number of myeloma proteins have been shown to specifically bind antigens and by many immunochemical parameters to resemble homogeneous antibodies. The specificities of antigen binding myeloma proteins from mice are usually detected by screening sera against polyvalent antigens by double diffusion in agar gel. In an earlier paper (Potter *et al.*, 1972) we reported finding two IgA myeloma immunoglobulins with specificities directed against $\beta(1\rightarrow6)$-linked D-galactopyranosyl moieties. We now wish to report three more IgA myeloma immunogloublins with the same antigalactan specificity (proteins X-24, X-44, and J-1). A sixth IgA myeloma protein (J-539) previously reported (Sher and Tarikas, 1971) and shown to bind proteins substituted with β-D-galactopyranosyl units has also been investigated.

These six myeloma proteins of the IgA class are all precipitable by larchwood arabinogalactan. The general structure of this polysaccharide is fairly well known (Aspinall, 1970). It consists of backbone of $(1\rightarrow3)$-linked anhydrogalactose units, with short side chains of $\beta(1\rightarrow6)$-linked galactopyranosyl moieties. Single side groups of arabinose also occur. All immunoglobulins described here had their active sites directed toward the β-galactosyl groups. In studies to be reported elsewhere we have found that these six myeloma proteins have different idiotypic antigenic determinants indicating that they are structurally different (Rudikoff *et al.*, 1973[2]). This group of proteins therefore provides a unique set of homogeneous immunoglobulins, structurally diverse, but all directed toward an extremely simple carbohydrate determinant involving sequences of only one neutral monosaccharide moiety and only one type of linkage. We wish to describe a study of the changes in the fluorescence of these immunoglobulins following their binding with ligand. Since the inhibiting oligosaccharides[3] (Potter *et al.*, 1972) that were available were of a series Gal_2, Gal_3, and Gal_4, and since these oligosaccharides do not absorb in the ultraviolet (uv) spectrum, they are particularly suitable for the study of these spectral changes induced in the native protein on binding.

Materials and Methods

The plasmacytomas which produced the myeloma proteins used in the present study arose in BALB/c mice following the intraperitoneal injection of mineral oil or pristane (Potter

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[1] Nomenclature and abbreviations are those recommended by The World Health Organization. All immunoglobulins are designated by the first letter and number of the parent tumor.

[2] Rudikoff, S., Mushinski, E. B., Potter, M., Glaudemans, C. P. J., and Jolley, M. E., manuscript in preparation.

[3] Abbreviations used are: Gal_2, D-galactopyranosyl-$\beta(1\rightarrow6)$-D-galactose; Gal_3, corresponding $\beta(1\rightarrow6)$-galactotriose; Gal_4, corresponding $\beta(1\rightarrow6)$-galactotetraose; APTG, *p*-aminophenyl β-D-thiogalactopyranoside.