THEORETICAL ANALYSIS OF THE SIGNIFICANCE OF WHETHER OR NOT ENZYMES OR TRANSPORT SYSTEMS IN STRUCTURED MEDIA FOLLOW MICHAELIS-MENTEN KINETICS

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ABSTRACT Pure Michaelis-Menten enzymes have been studied (i.e., enzymes with a hyperbolic (S,V) behavior in a well-stirred solution). When such enzymes are associated with a structure in vitro, even in the simplest conceivable form (immobilization in a homogeneous gel), they can produce enzymic or transport reactions with many different kinetics (Michaelis-Menten, sigmoidal, dual-phasic, etc.). Therefore, when structured enzyme or transport processes in vivo have sigmoidal kinetics, it is not proof that the corresponding proteins are allosteric. In same manner, when the apparent kinetics are dual-phasic, it is not proof that two enzyme, or transport systems, coexist.

INTRODUCTION
Since the pioneering work of Henri (1) and of Michaelis and Menten (2), most of the theoretical models of enzyme kinetics (Michaelis-Menten, allosteric, reactions using one or several substrates, etc.) have been designed for enzymes studied in homogeneous solutions in vitro. For a review, see reference 3. However, in vivo the enzymes are often more or less tightly associated with cellular structures, especially membranes. One can thus question whether the reaction kinetics depend only on the molecular properties of the enzymes or whether they are affected by the structure of the enzyme environment as well (4–6).

As a first approach to this problem, one can consider the simple case where one or more enzymes that follow Michaelis-Menten kinetics have been inserted at random within a gel slab without altering their catalytic properties. The experimental feasibility of such a system has already been demonstrated with many different enzymes (7, 8). The general trends of the theoretical kinetic treatment have also been published previously (8–12). In the present contribution, we study a few particular cases apt to be helpful for discussing actual enzymic or transport processes in living cells.

MATHEMATICAL THEORY
One or several enzymes, Ej, catalyzing reactions

\[ S_j \xrightarrow{E_j} P_j \]  

are assumed to be purely of the Michaelis-Menten type. This means that, in solution, the rate of the enzymatic reaction, \( V_p \), is a hyperbolic function of \( S_j \) (for \( P_j = 0 \)). This is written (13)

\[ V_j = V_{mj} \gamma_j \lambda_j \]  

\[ \lambda_j = \frac{S_j}{K_{mj} + S_j} \]  

with \( V_{np}, \gamma_j, \text{ and } K_{mj} \) (assumed to be constant with respect to pH) being, respectively, the maximum rate, the pH dependence, the substrate dependence and the Michaelis constant of enzyme \( E_j \) in solution.

Now, consider a solid phase, such as an aqueous gel slab separating two solutions, \( e \) and \( i \), with enzymes \( E_j \) being distributed at random and immobilized in the gel (Fig. 1), whose thickness is \( l \). It is assumed that immobilizing enzymes \( E_j \) in the gel slab has not changed their catalytic properties, which means that the reaction catalyzed by \( E_j \) still obeys the kinetic Eqs. 2 and 3. Within the membrane, the concentration of substrate, \( S_p \), is no longer a constant, but it can be expressed (12, 14), using the law of mass conservation, by the differential equation

\[ \frac{\partial S_j}{\partial t} = D_j \frac{\partial^2 S_j}{\partial x^2} - V_j (x, t) \]  

where \( t \) is the time, \( D_j \) the diffusion coefficient of \( S_p \) and \( x \) the distance along \( l \). At the boundary of \( e \) \( x = 0 \), and at \( i \) \( x = l \).

The flux, \( J_j \), of \( S_j \) at time \( t \) and at point \( x \) in the gel slab is written (12, 14)

\[ J_j (x, t) = - D_j \left( \frac{\partial S_j}{\partial x} \right)_{x,t} \]
When there is a single enzyme, $E_j$, in the gel slab (Fig. 1a), one can drop index $j$ in all the equations. Let us introduce two simplifying assumptions: (a) the stationary state has been established

$$\frac{\partial S}{\partial t} = 0$$

at any point within the slab, and (b) the concentration of $S$ is the same in compartments $e$ and $i$,

$$S^* = S^i.$$

**General Characteristics**

**Systems without any Significant pH Effect**

**Constant $\gamma$**

Analytical solutions of the problem are easily obtained (12) in the two extreme situations when the reaction is either of zero order (enzyme saturation, $S \gg K_a$)

$$\bar{V}e(0) = -\frac{V_m \gamma l A}{2v^e}$$

or of first order ($S \ll K_a$)

$$\bar{V}e(1) = -\frac{V_m \gamma l A S^e}{2v^e} \left( e^{al} + e^{-al} - 2 \right)$$

with $al$ being a dimensionless, diffusion-reaction parameter

$$al = \sqrt{\frac{V_m \gamma l^2}{K_m D}}$$

For orders of reaction intermediate between 0 and 1 there is generally no simple analytical solution, but numerical solutions can always be obtained.

In the expression of $al$ (Eq. 11), $V_m \gamma / K_m$ is determined only by the enzymatic reaction, while $D/l^2$ depends only on the diffusion of $S$. Hence, the larger the contribution of the reaction, and the smaller that of the diffusion, the larger the value of $al$ and vice versa. Two different situations can be considered, according to the values of $al$.

When $al \ll 1$, $\bar{V}e$ can be computed for the whole range of values possible for $S^*$(i.e., from $S^* < K_m$ to $S^* > K_m$). Fig. 2 gives the result thus obtained in the usual "reciprocal" system of coordinates $(1/S^*, 1/\bar{V}e)$. The graph is linear. Moreover, when $al$ is small enough, the serial expansion to the third order of the exponential terms in Eq. 10 gives the limiting analytical expression for $\bar{V}e(1)$:

$$\bar{V}e(1) \approx -\frac{V_m \gamma l A S^e}{2v^e} = \frac{V_m S^e}{K_m}$$

with

$$\bar{V}_m = \frac{V_m \gamma l A}{2v^e}.$$

Hence,

$$\bar{V}_m = \bar{V}e(0).$$

In this particular case, the behavior of the enzyme in the gel is thus described by the Michaelis-Menten equation, with the apparent $K_m$ being

**FIGURE 1**  Schematic diagram of the immobilization of the enzymes in the gel slab: (a) monoenzymatic system with gel slab thickness $l$; (b) the spatial distribution of activity in a bi enzymatic homogeneous mixture in the gel slab is induced by the pH difference $pH^* - pH^i$. Two active layers (thickness $l$) and an inactive layer (thickness $nl$) are obtained. The concomitant functioning of both reactions catalyzed by $E_i$ and $E_j$ results in a vectorial transport of $S$.

while the rate of disappearance, $\bar{V}_j$, of $S_j$ from one of the bathing compartments (e, for instance) is written (12, 14)

$$\bar{V}_j = -\frac{A}{v^e}$$

with $A$ being the surface area of the gel slab and $v^e$ the volume of compartment $e$.

MONOENZYMATIC SYSTEMS

**FIGURE 2** Hyperbolic behavior of a monoenzymatic system at low enzyme activity ($al < 1$): the graph is linear in the reciprocal system of coordinates $1/\bar{V}e = f(1/S^*)$. 
equal to the actual $K_m$ of the enzyme in solution, and the $V_{m}$ being proportional to the $V_{m}$ of the enzyme in solution.

As $a_1$ increases, the graph of $(1/S', 1/V')$ is no longer linear. At lower $a_1$ values the limiting slope in the zero-order region is identical with that in Fig. 2 and the curve shows a monotonic upward curvature (Fig. 3b) toward the limiting slope for the first-order region given by Eq. 10. As $a_1$ is further increased, the limiting slope for the zero-order region increases and the curve shows sigmoidicity with the highest slope at the inflection point. At even higher $a_1$ values the curves show a monotonic downward curvature (Fig. 3a). Although there is a single Michaelis-Menten type enzyme present in the system, one could be tempted to interpret such kinetic data as corresponding to two different enzymatic mechanisms, one detectable at low concentrations of substrate, and the other one dominant for the high concentrations of substrate. Moreover, in this case, the apparent kinetic parameters of the enzyme in the gel no longer bear a simple relationship to the actual kinetic parameters of the enzyme in solution.

**Systems Depending on the pH**

Such a system is encountered when the enzymic reaction produces or consumes protons or hydroxyl ions, for instance

$$S \xrightarrow{E} P^- + H^+. \quad (15)$$

In this case, even if the pHs in compartments e and i are maintained constant and equal to each other, the functioning of the reaction creates a curvilinear pH-profile in the gel slab, which, in its turn, interferes with the reaction functioning (back-action effect) (12). Three different cases can now be distinguished, according to the value of $a_1$.

When $a_1 = 1$, i.e., when $V_{m}/K_m < D/\ell^2$, the rate of proton production or consumption is very low, relative to diffusion. Hence the situation is comparable to the preceding one (as shown on Fig. 2); there is no significant pH effect. The same reasoning as above shows that in reciprocal coordinates there is again only one straight line for the whole range of substrate concentrations. The behavior of the enzyme in the gel is hyperbolic, with the apparent kinetic parameters bearing a simple relationship to the actual $K_m$ and $V_{m}$ of the enzyme in solution.

When $a_1$ is increased limiting slopes are obtained for regions $S < K_m$ and $S > K_m$, the behavior of the curve in the intermediate concentration range changes with $a_1$. When sigmoidicity is observed, the slope at the inflection point is (Fig. 4) less than that of either of the two asymptotes. When drawn in direct, or log/log coordinates, the same data give a sigmoidal graph (Fig. 5). In this case, the behavior of the enzyme in the gel seems to be interpretable as allosteric (15,16), although the enzyme is actually Michaelis-Menten type.

**TWO-ENZYME TRANSPORT SYSTEMS**

It is possible, by placing two well-chosen enzymes in the gel slab (see Fig. 1b), to obtain an active transport of $S$ from compartment e to compartment i.

**General Characteristics**

Consider two enzymes, $E_1$ and $E_2$, catalyzing two associated reactions (12, 14)

$$S + XY \xrightarrow{E_1} PX + Y \quad (16)$$

$$PX \xrightarrow{E_2} S + X. \quad (17)$$

Both these enzymes are assumed to obey hyperbolic kinetics when in solution.

These two enzymes are homogeneously distributed and immobilized in the gel slab, as above. Moreover, a pH gradient is now imposed in the gel slab by maintaining different pH values in compartments e and i (see Fig. 1b). This induces a functional asymmetry in the system. Enzymes $E_1$ and $E_2$ are chosen with their optimal pH being such that $E_1$ is active only in a layer, $l_e$, of the gel slab close to compartment e, while $E_2$ is active only in a layer, $l_i$, close to compartment i (Fig. 1b). The operation of the system then is as follows. Substrate $S$, diffusing from compartment e, is transformed into $PX$ by enzyme $E_1$ in layer $l_e$. The part of $PX$ which

**Figure 3** Example of nonhyperbolic behavior of an immobilized Michaelis-Menten type enzyme: with high values of $a_1$, in the reciprocal system of coordinates $1/V' = f(1/S')$ there are two different straight lines for the low and high values of $S'$, respectively. With $a_1 = 22(a)$ the slope of the first line is bigger than that of the second one, while the reverse is true for $a_1 = 3.7 (b)$.

**Figure 4** Nonhyperbolic behavior of an immobilized Michaelis-Menten type that induces pH variations. Three different straight lines exist, according to the level of substrate concentration, in the reciprocal system of coordinates. $a_1 = 10, \gamma = 0.51$.
FIGURE 5 Sigmoidal behavior, log (−V) vs. log S° plot, (curve S) of an immobilized Michaelis-Menten type enzyme which induces pH variations, αl = 10. γ = 0.14. Curve M depicts the kinetic behavior of the free enzyme.

diffuses towards I2 is transformed back into S by enzyme E2. This newly formed S diffuses partially towards compartment i. Depending on the concentration profiles in the gel slab, this series of events can produce an uphill transport of S, with the required energy being provided by the splitting of XY (12, 14).

For the sake of simplicity, let us assume that the two layers, I1 and I2, have the same thickness,

\[ l_1 = l_2 = \tilde{l} \]  \hspace{1cm} (18)

and that both enzymes, E1 and E2, have the same \( V_m \) and the same \( K_m \) in solution

\[ \begin{align*}
V_{m1} &= V_{m2} = V_m \\
K_{m1} &= K_{m2} = K_m.
\end{align*} \hspace{1cm} (19) \]

In each layer, the enzyme activity is also assumed to be constant with x, though, between the two enzymatically active layers, the intermediate layer, thickness \( \delta \tilde{l} \), is considered as purely diffusive, without any significant enzymatic activity (see Fig. 1b).

The reasoning is comparable to that with the single-enzyme system. Numerical values can be computed in all the cases, while analytical solutions can be calculated only in the two extreme situations of zero-order and first-order reactions.

Symmetrical Compartments, e and i

In this first approach, we start with identical compartments, e and i; i.e., at time zero

\[ S^* = S^* = S \]  \hspace{1cm} (21)

For the initial rate of transport, \( \bar{V} \), the analytical solutions are (14).

\[ \bar{V}(0) = V_m \gamma \frac{A \tilde{l}}{n + 2} \]  \hspace{1cm} (22)

with

\[ \bar{V}'(1) = V_m \gamma \frac{A l S}{\sqrt{\alpha l}} \]

\[ = \frac{2 - 2 \cosh (\alpha l)}{2 \sinh (\alpha l) \cosh (\alpha l) + \alpha l [n \cosh (\alpha l) + 1]} \hspace{1cm} (23) \]

Three cases can again be identified.

When \( \alpha l < 1 \), whether or not there is a pH feedback, the plot of the reciprocal of the rate of transport, 1/\( \bar{V} \), with respect to the reciprocal of the concentration of substrate in the bathing compartments, 1/S, is linear. The transport process thus appears as a single Michaelis-Menten type process. Moreover, in this case, the apparent \( K_m \) of the transport process is shown to be equal to the actual \( K_m \) of the enzymes in solution (14, 17).

When \( \alpha l \) is not too different from 1, whether or not there is a pH feedback, two different asymptotes are found, corresponding to the extreme situations of the first-order (S \( \ll \) \( K_m \)) and the zero-order (S \( \gg \) \( K_m \)) reactions. These two extreme straight lines are connected by a monotonic curve. The graph is thus comparable to that already given in Fig. 3, except that the ordinates refer now to rates of transport instead of reaction rates. Although there is a single pair of enzymes with a single value for their \( K_m \) and for their \( V_m \), it seems that two transport processes are present. The first one, with a low apparent \( K_m \) and \( V_m \), would be important at the low concentrations of substrate, while the second one, with high apparent \( K_m \) and \( V_m \), would play the major role for large values of S. Moreover, these apparent \( K_m \) and \( V_m \) of transport no longer bear a simple relationship to the actual kinetic parameters of the enzymes in solution.

When \( \alpha l \gg 1 \), one finds again two different linear asymptotes in reciprocal coordinates, corresponding to S \( \ll \) \( K_m \) (first-order reaction) and S \( \gg \) \( K_m \) (zero-order reaction), respectively. Moreover, in the case where a pH feedback exists (protons produced or consumed in reactions (16, 17), (a) the connecting curve between both straight lines can become sigmoidal, and (b) the apparent \( V_m \) for S \( \ll \) \( K_m \) can be either larger or smaller than the apparent \( V_m \) for S \( \gg \) \( K_m \). The more the enzyme with the high optimal pH tends to increase the pH, and the more the enzyme with the low optimal pH tends to decrease the pH, the larger the effect.

Nonsymmetrical Compartments, e and i

Let us consider a situation where the splitting of XY pushes S from e to i, while the concentrations of S are S* in compartment e and zero in compartment i. In such a case, both the reactions and the diffusion contribute to the transport of S from e to i. Let \( J_S \) be the total flux of S, and \( J_D \) the purely diffusive flux. For instance, \( J_S \) would be obtained with \( XY = 0 \). The ratio of \( J_S \) to \( J_D \) is given (17) by

\[ \frac{J_S}{J_D} = 1 + \frac{(1 + n) (\alpha l)^2 K_m}{(2 n + 1)^2 S^*}. \]  \hspace{1cm} (25)

When \( V_m \gamma /K_m \ll D/\tilde{l}^2 \) (hence \( \alpha l < 1 \)) and \( K_m/S^* \ll 1 \) (zero-order reaction), it is clear that

\[ J_S = J_D. \hspace{1cm} (26) \]

The contribution of the reaction is then negligible, and the transport of S is purely diffusive. Conversely, when both \( V_m \gamma /K_m \gg D/\tilde{l}^2 \) (hence \( \alpha l > 1 \))
and \( K_m / S' \gg 1 \) (first-order reaction), one obtains
\[
J_S \gg J_D
\]
and the transport of \( S \) then depends only on the reactions (active transport).

An intermediate situation exists when \( V_m^o / K_m \) and \( D/\ell^2 \) are of the same order of magnitude (\( \alpha l = 1 \)). In such a case, the process can still be purely diffusive for \( S' = K_m \) (zero-order reaction), although it is active for \( S' < K_m \) (first-order reaction). Hence, the distinction between active and passive mechanisms might not be always as clear-cut as it is generally considered to be.

**CONCLUSIONS**

Embedding the simplest hyperbolic enzymes in a gel slab is enough to confer quite unexpected kinetic properties. These enzymes are then able to catalyze not only scalar processes but also vectorial ones, including the uphill transport of a substrate between two compartments. Moreover, a single Michaelis-Menten enzyme (or a pair of complementary Michaelis-Menten enzymes, acting as a single transport system) can then be made to behave like a dual enzymatic (or transport) mechanism, or like an allosteric process. The larger the term characteristic of the reaction \( (V_m^o / K_m) \) compared with that of the substrate diffusion \( (D/\ell^2) \), the more pronounced these effects. Introducing a pH feedback also tends to increase these effects. The reason for enzymes behaving differently according to whether they are in solution or immobilized in a gel slab is as follows. In solution, under the usual conditions of experiment, the system can be considered to be perfectly homogeneous. In the gel slab, on the contrary, gradient profiles of pH and of substrate concentration appear, and these become steeper as the diffusive substrate supply and proton clearance are overcome by the reaction velocity. The local conditions of enzyme activity thus differ from point to point in the gel slab, and they also differ from those existing in the solutions in contact with it.

Our results are also a warning for those working on membrane-bound enzymes or on cellular transport systems. In reciprocal coordinates, their experimental data are often better fitted by two straight lines (one for the high, and one for the low concentrations) than by a single line. Following the original proposal of Epstein and Hagen (18, 19), most authors interpret such results as revealing the presence of two different membrane mechanisms of reaction or transport, with the apparent \( K_m \) and \( V_m \) of the membrane processes being equated to the actual \( K_m \) and \( V_m \) of the membrane bound enzyme or carrier. In like manner, sigmoidal experimental curves, in direct coordinates, have been taken as suggesting an allosteric character of the membrane-bound active proteins (20). Our present contribution shows that such interpretations, however popular they are, can be completely erroneous. Indeed, in a structured system, the apparent kinetic parameters are generally quite different from the actual molecular parameters of the catalyzing proteins, a biphasic kinetic curve may correspond to a single mechanism, and a sigmoidal kinetic curve may be obtained with a perfectly hyperbolic enzyme. An example of this has already been reported in an actual biological system, and an alternative interpretation to allosteric interaction has been given for glucose induced secretion of insulin (5).

Limiting values of \( \alpha l \) can be estimated by considering the ranges of variation of the parameters as they occur in real biological systems. \( K_m \) is generally between \( 10^{-2} \) and \( 10^{-8} \) M, while \( V_m = k_1 [E] \) can be considered as being between \( 10^{-7} \) and \( 10^{-2} \) mol s\(^{-1}\) cm\(^{-2}\), for \( [E] \) generally in the range of \( 10^{-7} \) to \( 10^{-6} \) M and \( k_1 \) ranging from 1 to \( 10^4 \) s\(^{-1}\). \( D \) is most often on the order of \( 10^{-5} \) cm\(^2\) s\(^{-1}\); \( l \) can be estimated to lie between \( 10^{-4} \) cm (thickness of a bilayer membrane) and \( 10^{-2} \) cm, or even more, when taking into account possible diffusion barriers in an intact organism (5); \( \gamma \) is smaller than 1; we assigned a value of 1 in the present calculation. Given these ranges of the parameters, the extremes of variation for \( \alpha l \) turn out to be \( 10^{-6} < \alpha l < 3 \times 10^{1} \). Hence, the full range of possibilities considered in this paper are within the range of biological possibilities. We agree that differences exist between a living membrane and a thick homogeneous gel slab with enzymes immobilized in it. However, this does not rule out our above assertions for at least two reasons: (a) even a real membrane is accompanied by diffusion layers where concentration profiles, similar to those in the gel slab, will develop, and (b) the real membranes being much more highly structured than our gel slabs, it is likely that the effects of the structure on the kinetic processes will be even more numerous and diverse than they are in the gel.

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