Theoretical analysis of a translocation-like model with saturable kinetics

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A theoretical analysis of the initial rates of product appearance in both compartments of a specifically designed diffusion cell separated by an asymmetrical enzyme membrane is presented. Variable substrate concentrations and different substrate diffusional limitations were considered. Our analysis shows that, under specific conditions, not only a product accumulation occurs in the compartment opposite to that in which the reaction takes place, but that substrate saturable kinetics can be obtained. These product translocation-like kinetics appear similar to those observed with translocation processes reported for biological situations. For such phenomena, a key role of the diffusion layer surrounding a bioactive surface is proposed.

INTRODUCTION

Considerable advances have been made recently in the understanding of solute transport across biological membranes (West, 1983). It is now well accepted that passive transport (i.e. facilitated diffusion or not) occurs from a high electrochemical potential to a lower potential, whereas active transport occurs against an electrochemical gradient. Product accumulation in a preferential compartment generally leads to the conclusion of an active transport process.

In group-translocation processes, a substance entering a cell is converted into a product by an enzymic-like reaction. According to Peter Mitchell's (1967) theory, membrane-bound enzymes could assume a vectorial character such that the chemical modification of substrate could proceed concomitantly with transfer across the membrane, and product could be released into a different compartment from that containing substrate.

The goal of the work presented here is to show that an active product translocation effect with saturable kinetics can be observed when using an artificial porous membrane bearing an enzyme immobilized only on one side and separating two compartments of widely different volumes. Previous data obtained in our group (Bardelotti *et al.*, 1985; Maïsterrena *et al.*, 1985) allowed us to assess more precisely the role of the diffusion layer, existing close to a porous enzymic membrane, on product flux splitting and distribution in both compartments. More recently, published experimental data and mathematical analysis demonstrated that, with an asymmetrical enzyme membrane system, it is possible to concentrate the reaction product in the compartment opposite to that in which the reaction occurs (Maïsterrena *et al.*, 1986). On the basis of the results obtained with such an asymmetric system, it now appears desirable to take external substrate diffusional limitations into account. This approach allows us to calculate and compare the initial rates of product appearance in both compartments with variable substrate concentrations,

these calculations and comparisons being made for different substrate diffusional limitations.

THEORY

The scheme given in Fig. 1 (top) illustrates the situation considered here: a porous membrane bearing a one-substrate enzyme on only one side (Side II) separates two compartments of widely different volumes ($V_{II} > V_I$). It is assumed that a boundary layer of thickness δ exists only in the immediate vicinity of the enzymic surface in Compartment II. It is also assumed that the substrate concentration is equal in both compartments and remains constant with time.

When the reaction takes place, the product generated at the enzyme level diffuses both through the membrane to reach Compartment I and through the boundary layer to reach Compartment II. At steady state, the product concentrations in both compartments are expressed at any time by eqns. (1) and (2) (Maïsterrena *et al.*, 1986) (all the symbols used and their subscripts are listed and defined in Table 1):

$$P_{I,t} = \frac{r}{V_T} \left\{ t - \frac{V_{II}(V_{II}R_{\delta,p} - V_I R_{m,p})}{V_T} \left[\exp - \left(\frac{V_T t}{V_I V_{II}(R_{\delta,p} + R_{m,p})} \right) - 1 \right] \right\} \quad (1)$$

$$P_{II,t} = \frac{r}{V_T} \left\{ t + \frac{V_I(V_{II}R_{\delta,p} - V_I R_{m,p})}{V_T} \left[\exp - \left(\frac{V_T t}{V_I V_{II}(R_{\delta,p} + R_{m,p})} \right) - 1 \right] \right\} \quad (2)$$

Furthermore, the apparent bound enzyme activity, r , is dependent on the substrate diffusional limitations (Engasser & Horvath, 1976) and can be expressed as follows for a one-substrate enzyme:

$$r = \frac{r_{\max} \cdot \beta_0}{1 + \beta_0} \quad (3)$$

Symbols used: these are defined in Table 1.

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Fig. 1. Initial rate pattern obtained for variable dimensionless substrate concentrations (β) with different dimensionless external diffusion substrate moduli (μ)

The situation considered here is given at the top of the Figure. A porous membrane bearing an enzyme on only one side separates two compartments of a diffusion cell of widely different volumes: Compartment I ($V_I = 9 \text{ cm}^3$) opposite to the enzymic side and Compartment II ($V_{II} = 350 \text{ cm}^3$) adjacent to the enzymic side, where a diffusion layer of thickness δ exists. The profiles of the initial rates were obtained by using eqns. (1), (2) and (3). (See the text and Table 2 for the numerical values used in the computer calculation.) Symbols are defined in Table 1; S is substrate and E is enzyme.

where β_0 is the dimensionless substrate concentration at the enzyme level, i.e. s_0/K_m .

At steady state, the enzymic rate and the substrate flux toward the enzymic membrane are equal. If β is the dimensionless substrate concentration for the bulk solution, i.e. s/K_m , it becomes:

$$\frac{r_{\max} \cdot \beta_0}{1 + \beta_0} = \frac{K_m}{R_{\delta, s}} (\beta - \beta_0) \quad (4)$$

where $R_{\delta, s}$ is the boundary-layer resistance for the substrate, equal to $\delta/(A \cdot D_s)$.

Eqn. (4) can be also expressed by the following equation:

$$\frac{\beta_0}{1 + \beta_0} = \frac{1}{\mu} (\beta - \beta_0) \quad (5)$$

where μ is the dimensionless external diffusion modulus for substrate, i.e. $r_{\max} \cdot R_{\delta, s}/K_m$. The roots of eqn. (5) can be expressed as follows:

$$\beta_0 = \frac{-(\mu + 1 - \beta) \pm \sqrt{(\mu + 1 - \beta)^2 + 4\beta}}{2} \quad (6)$$

When substituting into eqn. (3) the β_0 value given by eqn. (6), it becomes:

$$r = \frac{-r_{\max}[(\mu + 1 - \beta) \pm \sqrt{(\mu + 1 - \beta)^2 + 4\beta}]}{2 - [(\mu + 1 - \beta) \pm \sqrt{(\mu + 1 - \beta)^2 + 4\beta}]} \quad (7)$$

Only the positive root of eqn. (6) was introduced in eqns. (1) and (2) for the calculus of $P_{I, t}$ and $P_{II, t}$ versus time. A similar equation was proposed some years ago (Hill & Whittingham, 1957) and used more recently (Walker *et al.*, 1979) in the study of the effects of unstirred layers on amine transport in charophyte cells.

Invertase (EC 3.2.1.26) was chosen as the enzyme model. Different situations were explored and computerization performed. The parameters then introduced in eqns. (1), (2) and (3) were as follows: a total liquid volume (V_T) of 359 cm^3 with the volume of Compartment I (V_I) 9 cm^3 and that of Compartment II (V_{II}) 350 cm^3 ; a membrane porosity (A_w/A) of 0.205; a membrane thickness (d) of 0.012 cm ; an enzymic area (A) of $\Pi \text{ cm}^2$; an intrinsic activity of the bound enzyme ($r_{\max}$) of $0.2 \text{ mmol} \cdot \text{min}^{-1}$ for $\Pi \text{ cm}^2$; a Michaelis constant (K_m) of 9 mM ; a substrate diffusivity (i.e. sucrose diffusivity, D_s) of $3.12 \times 10^{-4} \text{ cm}^2 \cdot \text{min}^{-1}$; and a product diffusivity (i.e. glucose diffusivity, D_p) of $4.04 \times 10^{-4} \text{ cm}^2 \cdot \text{min}^{-1}$. The choice of these values was made in agreement with previously reported experimental data (Maïsterrena *et al.*, 1985). Parameters with variable values introduced in eqns. (1), (2) and (3) are given in Table 2.

Five cases were considered for computerization, allowing the calculation of the product concentrations (P_I and P_{II}) in Compartments I and II at any time and then the initial rates ($r'_I = dP_I/dt$ and $r'_{II} = dP_{II}/dt$).

RESULTS AND DISCUSSION

(a) Study of the initial rate of product appearance versus variable substrate concentration for different substrate moduli

Compartmentalized system. The initial rates in both compartments, r'_I and r'_{II} , were plotted against variable dimensionless substrate concentrations, β , ranging from 0 to 10. Fig. 1 shows the initial rates (expressed in product concentration per time unit obtained in both compartments) versus variable dimensionless substrate concentrations for the five cases considered in Table 2. When one first analyses the rates obtained in the compartment opposite to that in which the reaction occurs, substrate saturable kinetics are observed, and the greater the substrate modulus, μ (i.e. the greater the boundary layer thickness in Compartment II), the higher the initial rate in Compartment I. Now let us consider Compartment II, where the enzymic reaction occurs.

Table 1. Definitions of symbols used and their subscripts

(a) Symbol	Definition	Units
A	Membrane area	cm^2
A_w	Effective membrane pore area	cm^2
D	Diffusion coefficient	$\text{cm}^2 \cdot \text{min}^{-1}$
d	Membrane thickness	cm
K_m	Michaelis constant	mM
P	Product concentration	mM
R	Diffusion resistance	$\text{min} \cdot \text{cm}^{-3}$
$r_{\text{max.}}$	Intrinsic rate of the bound enzyme	$\text{mmol} \cdot \text{min}^{-1}$
r	Initial rate of the bound enzyme	$\text{mmol} \cdot \text{min}^{-1}$
r'	Initial rate of the bound enzyme	$\mu\text{M} \cdot \text{min}^{-1}$
s	Substrate concentration	mM
t	Time	min
V	Volume compartment	cm^3
V_T	Total liquid volume of both compartments	cm^3
(b) Greek letter	Definition	Units
β	Dimensionless substrate concentration, i.e. s/K_m	—
δ	Boundary layer thickness	cm
μ	Dimensionless external diffusion modulus for substrate, i.e. $r_{\text{max.}} \cdot R_{\delta,s}/K_m$	—
(c) Subscript	Definition	
O	Membrane level	
I	Compartment I	
II	Compartment II	
δ	Relative to the boundary layer	
m	Relative to the membrane	
p	Relative to the product	
s	Relative to the substrate	
T	Relative to the total volume	

Table 2. Selected values introduced in eqns. (1), (2) and (3) for computerization

δ is the thickness of the boundary layer, μ represents the external substrate diffusion modulus, i.e. $r_{\text{max.}} \cdot R_{\delta,s}/K_m$; $R_{\delta,s}$ is the substrate-diffusion-layer resistance, i.e. $\delta/(A \cdot D_s)$, and $R_{\delta,p}$ is the product-diffusion-layer resistance, i.e. $\delta/(A \cdot D_p)$

Case	δ (cm)	μ (dimensionless)	$R_{\delta,s}$ ($\text{min} \cdot \text{cm}^{-3}$)	$R_{\delta,p}$ ($\text{min} \cdot \text{cm}^{-3}$)
1	0	0	0	0
2	0.02	0.45	20.6	15.9
3	0.04	0.91	41.1	31.8
4	0.1	2.28	102.7	79.6
5	1	22.80	1026.0	795.8

Substrate saturable kinetics are also obtained in the five cases considered, but it can be seen that the rates are far lower than those obtained in Compartment I. Furthermore, what must be underlined in this case is the inversion of the effect obtained in Compartment I: namely the thinner the boundary layer, the higher the rate in Compartment II.

The most important effect observed is the difference among the rates obtained in both compartments for the same substrate-modulus value. For example, with $\mu = 22.8$ and $\beta = 10$, we observe an initial rate in Compartment I equal to $15.5 \mu\text{M} \cdot \text{min}^{-1}$, and only $0.025 \mu\text{M} \cdot \text{min}^{-1}$ in Compartment II. So r_I is about 600 times higher than r_{II} . On the other hand, with the same β -values but for a negligible boundary layer ($\mu \approx 0$), the rate is $0.025 \mu\text{M} \cdot \text{min}^{-1}$ in Compartment I, whereas a rate of $0.55 \mu\text{M} \cdot \text{min}^{-1}$ is observed in Compartment II, giving an r_I/r_{II} ratio equal to 0.045.

Comparison with an 'enzyme-reflecting system'. When looking at the compartment in which the reaction occurs it is important to compare the case of a compartmentalized system such as that described here (Fig. 2a) with what can be called an 'enzyme-reflecting system', i.e. involving an impervious enzyme membrane (Fig. 2b). The simulation of the reflecting case described in Fig. 2(b) was performed with the previously given parameters using only eqn. (3). To express the enzymic rate in terms of product concentration per time unit, a total volume (V_T) of 359 cm^3 , corresponding to $V_I + V_{II}$, was used.

Fig. 2(b) shows that the effects of the diffusional limitations on the apparent enzymic rate are far less pronounced than those obtained in a compartmentalized enzyme system (Fig. 2a).

Fig. 2. Comparison of the initial enzymic rates observed in a compartmentalized enzyme membrane system and a reflecting enzyme system

Profiles of the initial enzymic rates versus variable dimensionless substrate concentrations in Compartment II obtained by using eqns. (2) and (3) are given in Fig. 2(a) for comparison with Fig. 2(b), which shows the enzymic-rate profiles obtained in a reflecting system by using eqn. (3). The values introduced in the different equations are those given in the text. For symbols, see Table 1. S is substrate and E is enzyme.

Fig. 3. Initial enzymic rate profiles plotted versus the external diffusion substrate modulus for three selected dimensionless substrate concentrations

For symbols, see Table 1.

Furthermore, what is of considerable importance is the fact that, in a reflecting system, the effects of the diffusional limitations on the enzymic rate vanish for high substrate concentrations, whereas in a compartmentalized system the effects are greatly amplified and maintained, whatever the substrate concentration.

(b) Study of the initial rate versus the external substrate diffusional limitations

Fig. 3 shows the effect of the external diffusional limitations for values of the substrate modulus ranging

from 0 to 10 and for three different dimensionless substrate concentrations ($\beta = 0.1, 1$ and 10) either on the overall initial rate, r_T , or on the rate of product appearance in Compartments I and II, i.e. r_I and r_{II} (expressed here for a better understanding of product quantity per time unit). In order to have a better comparison between the three cases here considered, the same scale has been chosen for Figs 3(a), 3(b) and 3(c).

Profiles of r_T and r_{II} always decrease for increasing values of μ , whereas the r_I profiles first increase and, after reaching a maximum, decrease. The maximal initial rate,

r_I , is obtained for values of μ which are different in the three cases. For increasing substrate concentrations, the optimum substrate modulus shifts to higher values; this optimum μ -value corresponds to the highest initial rate which can be reached in Compartment I. Considering first-order enzyme kinetics, i.e. when $\beta = 0.1$ (see Fig. 3a) and for high values of μ , we notice that the initial rate in Compartment I (r_I) is approximately equal to the overall initial rate (r_T). In this case, the quasi-totality of the product reaches Compartment I.

It must also be pointed out that, as seen in Figs 3(a), 3(b) and 3(c), the intercept of the r_I and r_{II} profiles are obtained for the same μ -value (equal to 1.4). This corresponds to the equality of the product membrane resistance and product diffusion-layer resistance, $R_{m,p}$ and $R_{d,p}$ respectively, only in this case the initial enzymic rates are the same in both compartments, whatever the substrate concentration.

Conclusions

Up to now, most of the studies dealing with enzymes attached to a solid surface in contact with a substrate solution have been performed taking into account the rates of transport of substrate and product to and from the enzymic surface. In such a 'reflecting system', the effect of the external diffusional limitations for the substrate tends to lower the apparent enzyme activity because of the substrate depletion in the microenvironment. In the work reported here, the case of a non-impervious surface has been considered, leading to the asymmetric distribution on both sides of the membrane of the product generated at the enzyme level.

Results previously reported using such a membrane separating two compartments of a specially designed diffusion cell had shown that, for a fixed substrate concentration, a product accumulation could be obtained in the compartment opposite to that in which the reaction occurred (Maïsterrena *et al.*, 1986).

In the present work the theoretical approach used allowed us to show that substrate saturable kinetics could be obtained (Figs 1 and 2). Thus it appears that, under specific conditions, an enzyme bound on to the surface of a porous membrane exhibits product-translocation-like kinetics and then can play the role of a translocase.

So far, when an unstirred layer was taken into account for the study of transport systems, this layer was considered as an additional resistance to the membrane resistance itself. In our case it must be pointed out that the product to be transported is generated at the active-membrane/unstirred-layer interface. This may be

considered as a situation closer to the situation which may exist in biological systems.

In recent work concerning membrane-linked free-energy transduction (Westerhoff *et al.*, 1984), the authors pointed out that "...proton diffusion through aqueous phases is too rapid to allow for the steady state proton electrochemical potential to be significantly heterogeneous." The authors concluded that "...rather than trying to formulate mechanisms for rapid surface conduction, one should try to envisage ways in which equilibration of protons on the membrane's surface with the bulk aqueous phase can be appreciably retarded."

In our opinion the present work may tentatively provide an explanation: by keeping the proton from moving too rapidly into the bulk aqueous phase, the unstirred layer existing close to the membrane surface may maintain a significant electrochemical gradient.

In conclusion, our results demonstrate that the boundary layer existing in the immediate vicinity of a porous enzymic surface should be taken into account, not only as a substrate diffusional barrier, but essentially as a new compartment in which the high product concentration level governs its own distribution on both sides of the membrane. The existence of this new compartment may be of general significance in numerous biological situations and may tentatively explain group-translocation processes.

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REFERENCES

- Bardeletti, G., Maïsterrena, B. & Coulet, P. R. (1985) *J. Membr. Sci.* **24**, 285–296
- Engasser, J. M. & Horvath, Cs. (1976) in *Applied Biochemistry and Bioengineering* (Wingard, L. B., Jr., Katchalski-Katzir, E. & Goldstein, L., eds.), vol. 1, p. 127, Academic Press, New York
- Hill, R., & Whittingham, C. A. (1957) *Photosynthesis*, Methuen, London
- Maïsterrena, B., Bardeletti, G. & Coulet, P. R. (1985) *J. Membr. Sci.* **22**, 175–186
- Maïsterrena, B., Blum, L. J., Bardeletti, G. & Coulet, P. R. (1986) *Biochem. J.* **235**, 693–698
- Mitchell, P. (1967) *Adv. Enzymol. Relat. Areas Mol. Biol.* **29**, 33
- Walker, N. A., Beilby, M. J. & Smith, F. A. (1979) *J. Membr. Biol.* **49**, 21–55
- West, I. C. (1983) *The Biochemistry of Membrane Transport*, Chapman and Hall, London and New York
- Westerhoff, H. V., Melandri, B. A., Venturoli, G., Azzone, G. F. & Kell, D. B. (1984) *Biochim. Biophys. Acta* **768**, 257–292

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