KINETIC BEHAVIOR OF ENZYMES IMMOBILIZED IN ARTIFICIAL MEMBRANES[1]

W. J. Blaedel

Department of Chemistry
University of Wisconsin
Madison, Wisconsin

R. C. Boguslaski

Ames Research Laboratories
Miles Laboratories, Inc.
Elkhart, Indiana

Received March 10, 1972

SUMMARY

Steady-state flux and distribution equations are presented to show how enzymes immobilized in membranes may be used for analysis or for the study of fixed enzyme kinetics. Experimental support of the equations has been obtained with a urease-polyvinyl alcohol membrane separating two solutions.

During the past few years, there has been considerable interest in the study of insoluble enzyme derivatives.[2-6] Among these materials, enzymes fixed in or on artificial membranes have attracted considerable attention. They serve as integral components in certain analytical devices.[7,8] They have been suggested as therapeutic agents[9] and as models of biological membranes.[3,10] All of this work justifies an experimentally supported theoretical treatment of the kinetic behavior of these membrane systems in order to obtain fundamental information on the kinetic parameters of the fixed enzyme.

Recently, a few publications have described the general kinetic behavior of enzymes fixed in membranes.[11-15] However, quantitative attempts to treat these heterogeneous systems rigorously or generally, often lead to equations that cannot be solved explicitly for the parameters of interest or that cannot be easily interpreted or tested experimentally. We have derived steady-state flux and distribution equations useful in the study of fixed enzyme kinetics. Some of these expressions permit in principle the determination of the kinetic

parameters separately from the diffusional ones. Only simple, experimentally verifiable cases were considered in the derivations. Several sets of equations have been developed for several different geometries and for limiting conditions of high and low substrate concentrations.

EXPERIMENTAL PROCEDURE

One system, on which we have done considerable experimental work, consisted of an enzyme membrane sandwiched between two Plexiglas blocks with identical cavities on either side of the membrane. The block assembly and solutions were thermostated at 25°C. Equipment and procedure for measuring fluxes were similar to those used earlier for ion-exchange membranes.[16]

For the particular systems studied in this paper, Solution 1 (the donor) contained substrate at a concentration S_o and was pumped by a motor-driven syringe (Infusion pump, Model No. 975-Harvard Apparatus Company) through its cavity at a constant flow rate F_1. Solution 2 (the acceptor) was devoid of substrate and was pumped at a rate F_2. Both solutions were buffered identically, and the system was allowed to reach steady state prior to sample collection.

The following steady-state flux equations were derived for an enzyme catalyzed reaction which follows Michaelis-Menten kinetics (rate constants $\bar{K}_m$ and $\bar{V}_m$), the system being saturated with respect to substrate ($\bar{S} \gg \bar{K}_m$). Barred and unbarred quantities represent the membrane and solution phases, respectively. Subscripts 1 and 2 represent the steady-state concentrations in the two solution phases. $\bar{X}$ is the membrane thickness and the area of the membrane exposed to the solution is $\bar{A}$. $\bar{D}_s$ and $\bar{D}_p$ are the diffusion coefficients of S and P in the membrane phase, in which one-dimensional diffusion is assumed. The terms t_s and t_p refer to transport coefficients of substrate and product,[17] and δ_s and δ_p are the distribution ratios of substrate and product between the membrane and solution phases. A complete derivation of the equations which appear here will soon be published.[18]

$$\bar{S} \xrightarrow{\bar{E}} n\bar{P} \qquad (1)$$

$$\frac{P_1F_1}{\bar{A}} = -\frac{P_1 - P_2}{\frac{2}{t_p} + \frac{\bar{X}}{\delta_p\bar{D}_p}} + \frac{n\bar{V}_m\bar{X}}{2} \qquad (2)$$

$$\frac{P_2F_2}{\bar{A}} = \frac{P_1 - P_2}{\frac{2}{t_p} + \frac{\bar{X}}{\delta_p\bar{D}_p}} + \frac{n\bar{V}_m\bar{X}}{2} \qquad (3)$$

$$\frac{S_2F_2}{\bar{A}} = \frac{S_1 - S_2}{\frac{2}{t_s} + \frac{\bar{X}}{\delta_s\bar{D}_s}} - \frac{\bar{V}_m\bar{X}}{2} \qquad (4)$$

RESULTS AND DISCUSSION

Equations 2-4 were tested experimentally and verified using a urease-PVA membrane, which contained 27 mg of enzyme/cm^3 of membrane. The solutions contained 0.05M maleic acid adjusted to pH 6.5 and to an ionic strength of 0.2M with Na_2SO_4. They also contained 0.01M cysteine. Duplicate portions of each effluent solution were analyzed for ammonium ion by the Berthelot reaction and for urea by difference, via total ammonium ion, after hydrolysis with urease. Table 1 shows the steady-state S and P distributions in the donor and acceptor solutions over a substrate concentration range of 50-500 mM for equal flow rates of donor and acceptor solutions.

From Table 1, it is clear that P_1 is equal to P_2 within experimental error and independent of the substrate concentration in the range 50-500 mM in accord with Equations 2 and 3 when $F_1 = F_2$. It is notable that the product concentrati are equal on both sides of the membrane even though substrate enters on one side

TABLE 1

Steady-State Distribution of Substrate and Product for a Urease-PVA Membrane Separating Two Solutions[a]

Initial Urea Concentration in Donor S_o, mM	P_1 (mM)	P_2 (mM)	S_1 (mM)	S_2 (mM)	Material Balance on Ure Micromoles per Minute	
					In[b]	Out[c]
50.2	3.35	3.85	40.3	6.22	301	301
76.5	3.70	3.85	64.4	10.6	459	473
100.0	3.85	3.85	81.3	12.6	600	586
188.5	3.70	4.04	161.6	28.5	1131	1164
245.0	3.85	3.85	202.2	37.1	1470	1459
400.0	3.85	3.85	344.1	60.3	2400	2451
514.0	4.04	3.85	414.9	78.6	3084	2985
50.2	3.35	3.20	42.1	7.56	301	317
Average	3.71	3.78				

a. Donor and acceptor flow rates equal at 0.60 ml per minute.

b. Calculated as S_o x 0.60 micromoles per minute.

c. Calculated as $(S_1 + S_2 + P_1/2 + P_2/2)$ x 0.60 micromoles per minute.

of the membrane and even though S_1 is over five-fold greater than S_2. The equality of product concentrations on both sides of the membrane indicates that the membrane is saturated with respect to substrate and also that the enzyme is uniformly distributed with respect to depth in the membrane. A plot of S_2 against S_1-S_2 yields a straight line with a negative intercept on the S_2 axis, which supports the correctness of Equation 4.

Equations 2 and 3 were also tested at unequal flow rates in which F_2 ranges from 0.6 to 2.8 ml/min. with F_1 constant at 0.6 ml/min.; S_o was 200 mM. Plots of P_1F_1 and P_2F_2 against P_1-P_2 gave straight lines in accord with Equations 2 and 3. However, the membranes contained some anion and cation exchange capacity, so the

P_1F_1 and P_2F_2 intercepts of the two plots were significantly different, in contradiction to Equations 2 and 3, and the slopes of the two plots were not exactly equal and opposite.

As far as estimating $\bar{V}_m$ from the variable flow rate data is concerned, the best way is to sum Equations 2 and 3 to obtain

$$P_1F_1 + P_2F_2 = n\bar{V}_m \bar{X} \bar{A} \qquad (5)$$

For a given membrane, the sum of the product fluxes in both solutions should be constant and equal to $n\bar{V}_m\bar{X} \bar{A}$. Five membranes were prepared, characterized, and mean values of $n\bar{V}_m\bar{X} \bar{A}$ for each determined from seven pairs of product flux measurements. The precision of the measurements was acceptable; the 90% confidence interval for the mean was always less than $\pm 5\%$. An absolute value for $\bar{V}_m$ could not be calculated because $\bar{X}$ and $\bar{A}$ were not measured.

Additional work was done to establish that the membrane was a symmetrical one and that there was no leakage of solution across the membrane or loss of enzyme from the membrane. The stability of the enzyme in the membrane is apparent from a comparison of the first and last runs of Table 1, which were made over 6 hours apart.

The flux Equations 2-4, strongly supported by the preceding experimental work, offer a way of finding $\bar{V}_m$ for the fixed enzyme because this kinetic parameter is nicely separated from the other transport parameters. Thus, $\bar{V}_m$ may be calculated from P_1 and P_2 values if only three other quantities are known, F_1 or F_2 and $\bar{A}$ and $\bar{X}$. Other as yet untested equations for the system in which the substrate concentration is low, in principle, permit $\bar{K}_m$ to be calculated if the other transport parameters are measured. Precise determination of the rate constants for the fixed enzyme from transport measurements awaits the precise measurement of these other parameters.

REFERENCES

1. This work was presented in part on December 3, 1970 in the Symposium on Kinetic Methods of Analysis, Southeast-Southwest Regional Meeting, A.C.S., New Orleans, La.

2. Manecke, G., Naturewissenschaften, 51, 25 (1964).
3. Silman, I., and E. Katchalski, Ann. Rev. Biochem., 35, 873 (1966).
4. Rechnitz, G. A., Anal. Chem., 41, 109A (1969).
5. Guilbault, G. G., Anal. Chem., 42, 334R (1970).
6. Kay, G., Process Biochemistry, 3, 36 (1968).
7. Updike, S. J., and G. P. Hicks, Nature, 214, 986 (1967).
8. Guilbault, G. G., and J. G. Montalvo, J. Amer. Chem. Soc., 92, 2533 (1970).
9. Henahan, J. F., Chem. Eng. News, 49, 42 (1971).
10. Goldman, R., O. Kedem, I. H. Silman, S. R. Caplan, and E. Katchalski, Biochem., 7, 486 (1968).
11. Goldman, R., O. Kedem, and E. Katchalski, Biochem., 7, 4518 (1968).
12. Goldman, R., O. Kedem, and E. Katchalski, Biochem., 10, 165 (1971).
13. Sundaram, P. V., H. Tweedale, and K. J. Laidler, Cand. J. Chem., 48, 1498 (1970).
14. Selegny, E., G. Broun, J. Geffroy, and D. Thomas, J. Chim. Phys., 66, 391 (1969).
15. Kasche, V., H. Lundqvist, and R. Bergman, Biochem. Biophys. Res. Commun., 45, 615 (1971).
16. Blaedel, W. J., T. J. Haupert, and M. A. Evenson, Anal. Chem., 41, 583 (1969).
17. Treybal, R. E., "Mass Transfer Operations," McGraw-Hill, N.Y., second edition, 1968.
18. Blaedel, W. J., R. C. Boguslaski, and T. R. Kissel, Anal. Chem., in press.