Diffusional Increase and Decrease in Half-Maximal-Activity Substrate Concentrations with Two-Substrate Enzymic Reactions

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Diffusional effects on two-substrate enzymic reactions mainly depend on the relative affinities of the enzyme for its two substrates. With two substrates of widely different affinities, diffusional limitations increase and decrease the half-maximal-activity concentration of the high- and low-affinity substrate respectively.

Several studies, concerned with the heterogeneous kinetics of two-substrate enzymic reactions, have shown both increases and decreases in apparent K_m values as a result of enzyme binding (Wilson *et al.*, 1968; Smith & Lenhoff, 1974; Cho & Swaisgood, 1974; Coulet *et al.*, 1975; Barbotin, 1976; Nitisewojo & Hultin, 1976). The respective contributions of enzyme modifications and diffusional limitations on the observed kinetic variations were, however, not clearly established, although, in analogy with a single-substrate enzymic reaction, an increase in apparent K_m is usually associated with diffusional limitations and a decrease with conformational changes.

An experimental study with collagen-bound aspartate aminotransferase (Engasser *et al.*, 1977), in which the diffusion of substrates was carefully controlled, has demonstrated that diffusional effects may be quite different with a two-substrate from those with a single-substrate reaction; namely, substrate diffusional limitations manifest themselves in a decrease in the apparent affinity for one substrate but simultaneously in an increase in the apparent affinity for the other.

Since theoretical studies of diffusional effects on enzyme kinetics have been restricted to single-substrate reactions (Katchalski *et al.*, 1971; Engasser & Horvath, 1976; Goldstein, 1976), it is the aim of the present report to establish theoretically the possible diffusional influences on apparent enzymic affinities with two-substrate reactions.

Methods

The enzymes, catalysing the reaction between two substrates A and B, are bound on a membrane surface. Under these conditions, substrate concentrations are usually not uniform in the surrounding solution because of diffusional limitations. At steady state the observed rate of reaction, v , is equal to the rate of transport of the two substrates by

molecular and convective diffusion and to the rate of substrate consumption at the surface. Thus:

$$v = h_A([A] - [A]_s) = h_B([B] - [B]_s) \\ = \frac{V[A]_s[B]_s}{K_1^\dagger K_m^B + K_m^A [B]_s + K_m^B [A]_s + [A]_s [B]_s} \quad (1)$$

where h_A and h_B are the transport coefficients for A and B, $[A]$ and $[B]$ the bulk concentrations of the two substrates, and $[A]_s$ and $[B]_s$ their concentrations at the active surface, which in the presence of diffusional limitations are lower than the corresponding bulk concentrations. In eqn. (1), V represents the maximum activity, K_m^A and K_m^B the Michaelis constants for the two substrates, and $K_1^\dagger$ a fourth kinetic constant which equals zero for a Ping Pong mechanism. Eqn. (1) may be rewritten in a dimensionless form:

$$\frac{v}{V} = \frac{1}{\mu_A} \left(\frac{[A]}{K_m^A} - \frac{[A]_s}{K_m^A} \right) = \frac{1}{\mu_B} \left(\frac{[B]}{K_m^B} - \frac{[B]_s}{K_m^B} \right) \\ = \frac{\frac{[A]_s}{K_m^A} \cdot \frac{[B]_s}{K_m^B}}{\frac{K_1^\dagger}{K_m^A} + \frac{[B]_s}{K_m^B} + \frac{[A]_s}{K_m^A} + \left(\frac{[A]_s}{K_m^A} \cdot \frac{[B]_s}{K_m^B} \right)} \quad (2)$$

with

$$\mu_A = \frac{V}{h_A K_m^A} \quad (3)$$

and

$$\mu_B = \frac{V}{h_B K_m^B} \quad (4)$$

The magnitude of the two substrate moduli, μ_A and μ_B , directly reflects the importance of diffusional limitations for the two substrates.

Another dimensionless criterion, which may be used to analyse diffusional effects with a two-substrate reaction, is the parameter $\zeta_{B/A}$, defined as the ratio of the two moduli:

$$\zeta_{B/A} = \frac{\mu_B}{\mu_A} = \frac{h_A K_m^A}{h_B K_m^B} \quad (5)$$

The transport coefficient h between a fluid and a solid surface is known to be most often proportional to $D^{2/3}$, D being the molecular diffusivity of the transported species (Bird *et al.*, 1962). As the diffusivity can be considered inversely proportional to $M^{1/2}$, the square root of the molecular weight of the species, h is approximately proportional to $M^{-1/3}$. Consequently, for two substrates with molecular weights of the same order of magnitude, the two transport coefficients h_A and h_B are not too different, and $\xi_{B/A}$ is approximately equal to the ratio of the two Michaelis constants, i.e. of the two substrate affinities. Since, according to eqn. (5), $\xi_{B/A}$ determines the relative importance of the diffusional limitations for the two substrates, the diffusion of the high-affinity substrate is expected to have the strongest rate-limiting effect. The kinetic contributions of diffusional phenomena also depend on the intrinsic rate expression, and, more precisely, as suggested by eqn. (2), on the value of the dimensionless ratio, K_1^A/K_m^A , of the kinetic parameters.

Eqn. (2) was used to calculate, first the surface concentrations $[A]_s$ and $[B]_s$ at given bulk concentrations $[A]$ and $[B]$, substrate moduli μ_A and μ_B , and kinetic constants K_m^A , K_m^B and K_1^A , then the normalized enzyme activity, v/V . Since the bound enzymes no longer show hyperbolic kinetics under diffusional limitations, the concentrations, $[A]_{0.5}$ and $[B]_{0.5}$, giving half-maximal activity, were then determined.

Results

Fig. 1 shows the ratio of the half-maximal activity substrate concentration under increasing diffusional limitations, $[A]_{0.5}$ or $[B]_{0.5}$, and the intrinsic half-maximal-activity concentration in the absence of diffusional limitations, $[A]_{0.5}^*$ or $[B]_{0.5}^*$. The ratio $[A]_{0.5}/[A]_{0.5}^*$ is calculated at a given $[B]$, the ratio $[B]_{0.5}/[B]_{0.5}^*$ at a given $[A]$ in the bulk solution. Both ratios have been determined, at given values of K_1^A/K_m^A , for different affinity ratios $\xi_{B/A}$ and at increasing values of the substrate modulus μ_A , i.e. at increasing diffusional resistances.

At a small value of K_1^A/K_m^A and a fixed substrate concentration equal to its Michaelis constant, i.e. $[A] = K_m^A$ and $[B] = K_m^B$ respectively, it is seen that when $\xi_{B/A}$ is much smaller than 1, i.e. the enzyme has a higher affinity for A than for B, diffusional limitations increase $[A]_{0.5}$ but simultaneously decrease $[B]_{0.5}$. On the contrary, when $\xi_{B/A}$ is much larger than 1, $[A]_{0.5}$ is decreased and $[B]_{0.5}$ increased by diffusional limitations. It should be noticed that the simultaneous increase and decrease in apparent affinities are of exactly the same magnitude and proportional to the departure of $\xi_{B/A}$ from 1. Moreover, when the two substrates have similar affinities, i.e. $\xi_{B/A} = 1$, no significant variation in

the half-maximal-activity concentration is observed, whatever the extent of diffusional limitations.

This interesting result, according to which diffusional limitations have exactly opposite effects on the two apparent substrate affinities or half-maximal-activity concentrations, is, however, restricted to the case of a small K_1^A , i.e. to kinetic expressions close to Ping Pong, and to fixed second substrate concentrations equal to their Michaelis constants. Diffusional effects may be different for other intrinsic kinetics and different experimental conditions. For example, Fig. 1 shows that, when $K_1^A = 10K_m^A$, diffusion can still yield opposite effects on $[A]_{0.5}$ and $[B]_{0.5}$, but only at very high or very low $\xi_{B/A}$. When $\xi_{B/A}$ is close to 1, on the contrary, both $[A]_{0.5}$ and $[B]_{0.5}$ are decreased. On the other hand, in the case of a small K_1^A but fixed substrate concentrations different from their Michaelis constants, namely $[A] = 10K_m^A$ and $[B] = 10K_m^B$, diffusional effects on $[A]_{0.5}$ and $[B]_{0.5}$ are opposite only with very large or very low affinity ratios; but with two substrates of comparable Michaelis constants both $[A]_{0.5}$ and $[B]_{0.5}$ then increase in the presence of diffusional limitations.

Discussion

According to this quantitative analysis, with two-substrate enzymic reactions the half-maximal-activity substrate concentration is not only increased, as with single-substrate reactions, but may also be decreased by diffusional limitations. It is shown that diffusional kinetic modifications depend on the magnitude of diffusional limitations, the intrinsic kinetic rate expression and the relative affinity of the two substrates, as well as the value of the fixed concentration of the second substrate. More precisely, with two substrates of very different Michaelis constants, diffusional interferences always result in an increase and a decrease in the half-maximal-activity concentrations of the high- and low-affinity substrates respectively. With two substrates of similar Michaelis constant, on the contrary, diffusion has generally a much smaller effect on the half-maximal-activity concentrations, which can then vary in similar or opposite directions.

These theoretical findings are partly verified by the experimental results obtained with aspartate aminotransferase bound at the surface of collagen membranes (Engasser *et al.*, 1977). Owing to the great difference in the Michaelis constants of the two substrates oxaloacetate and glutamate ($K_m^{\text{Glu}}/K_m^{\text{OxAc}} = 100$), diffusional limitations for the high-affinity substrate oxaloacetate were indeed found to increase the oxaloacetate concentration for half-maximal activity, but simultaneously to decrease the glutamate concentration for half-maximal activity.

As bound-enzyme kinetics are usually determined

Fig. 1. Effect of diffusional limitations on the half-maximal-activity concentrations of the two substrates

The two half-maximal-activity concentrations, $[A]_{0.5}$ and $[B]_{0.5}$, are normalized to the intrinsic half-saturation concentrations in the absence of diffusional limitations, $[A]_{0.5}^*$ and $[B]_{0.5}^*$. The different graphs illustrate the influence of the magnitude of diffusional limitations represented by μ_A , of the parameter $\zeta_{B/A} \approx K_m^A/K_m^B$, of the normalized fixed substrate concentration $[B]/K_m^B$ or $[A]/K_m^A$, and of the ratio of the kinetic constants, K_1^A/K_m^A , on the apparent half-maximal-activity substrate concentrations. (a) $[B]/K_m^B = 1$, $K_1^A/K_m^A = 0.1$; (b) $[A]/K_m^A = 1$, $K_1^A/K_m^A = 0.1$; (c) $[B]/K_m^B = 10$, $K_1^A/K_m^A = 0.1$; (d) $[A]/K_m^A = 10$, $K_1^A/K_m^A = 0.1$; (e) $[B]/K_m^B = 1$, $K_1^A/K_m^A = 10$; (f) $[A]/K_m^A = 1$, $K_1^A/K_m^A = 10$.

by varying substrate concentrations around their respective Michaelis constants, diffusional effects established in this study are expected to account for many of the kinetic differences previously reported when comparing the kinetic behaviour of enzymes in soluble and immobilized form.

References

- Barbotin, J. N. (1976) *FEBS Lett.* **72**, 93–97
- Bird, R. B., Stewart, W. E. & Lightfoot, E. D. (1960) *Transport Phenomena*, pp. 396–405, Wiley, New York
- Cho, I. C. & Swaisgood, H. (1974) *Biochim. Biophys. Acta* **334**, 243–256
- Coulet, P. R., Godinot, C. & Gautheron, D. C. (1975) *Biochim. Biophys. Acta* **391**, 272–281
- Engasser, J. M. & Horvath, C. (1976) in *Applied Biochemistry and Bioengineering* (Wingard, L. B., Katchalski, E. & Goldstein, L., eds.), vol. 1, pp. 127–220, Academic Press, New York
- Engasser, J. M., Coulet, P. R. & Gautheron, D. C. (1977) *J. Biol. Chem.* **252**, 7919–7922
- Goldstein, L. (1976) *Methods Enzymol.* **44**, 397–443
- Katchalski, E., Silman, I. & Goldman, R. (1971) *Adv. Enzymol. Relat. Areas Mol. Biol.* **34**, 445–536
- Nitisewojo, P. & Hultin, H. O. (1976) *Eur. J. Biochem.* **67**, 87–94
- Smith, N. L. & Lenhoff, H. M. (1974) *Anal. Biochem.* **61**, 392–415
- Wilson, R. J., Kay, G. & Lilly, M. D. (1968) *Biochem. J.* **108**, 845–853