Biochem. J. (1975) **145**, 431–435
Printed in Great Britain

Electrostatic Effects on the Kinetics of Bound Enzymes

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(*Received* 24 *June* 1974)

1. The effect of the interaction between the charged matrix and substrate on the kinetic behaviour of bound enzymes was investigated theoretically. 2. A simple expression is derived for the apparent K_m. 3. The apparent K_m can only be used for the characterization of the electrostatic effect if the ionic strength does not vary with the substrate concentration. 4. The deviations from Michaelis–Menten kinetics are graphically illustrated for cases when the ionic strength varies with the substrate concentration. 5. The inhibition of the bound enzyme by a charged inhibitor at constant ionic strength is characterized by an apparent K_i. 6. When both the inhibitor concentration and the ionic strength change there is no apparent K_i, and the inhibition profile is graphically illustrated for this case. 7. Under certain conditions the electrostatic effects manifest themselves in a sigmoidal dependence of the enzyme activity on the concentration of the substrate or inhibitor.

The observed kinetic behaviour of an enzyme, which is attached to or embedded in a charged membrane, often differs from that of the same enzyme in free solution even in the absence of diffusional effects. This phenomenon can be attributed to the fact that the concentration of charged species in the microenvironment of the bound enzyme is different from that in the macroenvironment, i.e. in the external solution, owing to electrostatic interactions with the fixed charges of the membrane.

It has been shown that the difference between the internal and external pH results in a shift of the pH–activity profile of bound enzymes (McLaren & Estermann, 1957; McLaren & Babcock, 1959; Levin *et al.*, 1964). On the other hand, experimental and theoretical investigations have demonstrated that with charged substrates the electrostatic effect manifests itself in an increase or decrease in the observed K_m value for the enzymic reaction in a matrix that carries like or unlike charges respectively (Goldstein *et al.*, 1964; Hornby *et al.*, 1968; Wharton *et al.*, 1968; Katchalski *et al.*, 1971).

In the present paper we present a theoretical analysis of the effect of fixed charges on the kinetics of heterogeneous enzyme reactions with ionic substrates under a wide range of conditions, and show that the concept of an apparent K_m, which has been put forward by previous investigators, has no general applicability. In addition the effect of an ionic inhibitor on the activity of the enzyme bound to a charged membrane is examined.

Methods and Results

Theoretical model

The bound enzyme is uniformly distributed in a porous matrix with fixed ionic groups. The external solution contains the positively charged substrate, S^+, the cation, A^+, and the anion, B^-. It is assumed that there are no diffusion resistances for the substrate, so that the reaction is kinetically controlled (Engasser & Horvath, 1973). Owing to the fixed charges the concentration of the ionic species in the matrix is different from that in the exterior, and it is assumed that they are distributed between the two phases according to the Donnan equilibrium (Helfferich, 1962).

When the difference between the activity coefficients in the exterior and interior as well as the swelling pressure of the matrix are neglected, the potential difference at the membrane interface, E_D, is expressed by the ionic concentrations as

$$E_D = \frac{RT}{\mathscr{F}} \ln \frac{[S^+]}{[S^+]_o} = \frac{RT}{\mathscr{F}} \ln \frac{[A^+]}{[A^+]_o} = \frac{RT}{\mathscr{F}} \ln \frac{[B^-]_o}{[B^-]} \quad (1)$$

where $\mathscr{F}$ is the Faraday constant and the subscript $_o$ refers to the concentration inside the membrane.

Thus the electrostatic partition coefficient, Λ, is given by

$$\Lambda = \frac{[S^+]_o}{[S^+]} = \frac{[A^+]_o}{[A^+]} = \frac{[B^-]}{[B^-]_o} \quad (2)$$

and related to E_D, the Donnan potential, by

$$\Lambda = e^{-(E_D/RT)} \quad (3)$$

Electroneutrality in both the solution and the membrane requires that

$$[S^+] + [A^+] = [B^-] \quad (4)$$

and

$$[S^+]_o + [A^+]_o \pm [X^\pm] = [B^-]_o \quad (5)$$

where $[X^\pm]$ is the concentration of the fixed univalent ions in the matrix, which can be positively or negatively charged.

From eqns. (2), (4) and (5) it follows that for a given set of ionic concentrations Λ can be calculated by the second-order equation

$$[B^-]\Lambda^2 \pm [X^\pm]\Lambda - [B^-] = 0 \qquad (6)$$

When Λ is known, the substrate concentration in the membrane, $[S^+]_o$, is calculated from eqn. (2) and the rate of the enzymic reaction, v, by

$$v = \frac{V_{max.}[S^+]_o}{K_m + [S^+]_o} \qquad (7)$$

where $V_{max.}$ is the saturation rate and K_m is the Michaelis–Menten constant. The results of such calculations will be graphically presented by using the dimensionless substrate concentration in the solution, β, defined by

$$\beta = \frac{[S^+]}{K_m} \qquad (8)$$

Eqn. (6) shows that the partition coefficient, Λ, depends on the concentration of both the fixed charges, $[X^\pm]$, and the anions in the solution, $[B^-]$, which equals $[A^+]$ plus $[S^+]$ because of electroneutrality. In the following the dependence of the enzymic activity on the substrate concentration in the exterior is examined at constant values of $[B^-]$ and when $[B^-]$ varies with the substrate concentration.

Kinetics at constant ionic strength

When the concentration of the positively charged substrate is varied in the exterior, the concentration of B^- is kept constant either by adjusting the value of $[A^+]$ accordingly or by maintaining it at a sufficiently high level so that $[A^+] \gg K_m$, i.e. $[A^+]$ is always much higher than $[S^+]$. Under these conditions Λ is independent of $[S^+]$ and equal to the constant, $\Lambda^\pm$, which is given by

$$\Lambda^\pm = \frac{\pm[X^\pm] + \sqrt{[X^\pm]^2 + 4[B^-]^2}}{2[B^-]} \qquad (9)$$

As seen for the positively charged substrate, $\Lambda^\pm$ is larger and smaller than unity when X is a negative or positive ion respectively.

Since $\Lambda^\pm$ is constant at a fixed ionic strength, the dependence of the reaction rate on the macroenvironmental substrate concentration follows the Michaelis–Menten law with the same saturation rate but with an apparent K_m, K_m^{el}, which is given by

$$K_m^{el} = \frac{K_m}{\Lambda^\pm} \qquad (10)$$

and this expression is equivalent to that derived by Wharton *et al.* (1968)

Eqns. (9) and (10) show that the electrostatic effect increases or decreases the value of the apparent K_m when the membrane and substrate carry like or unlike charges respectively. In agreement with previous findings (Goldstein *et al.*, 1964), however, the effect of the fixed charge on the kinetics of bound enzymes is negligible at high ionic strength, i.e. when $[B^-] \gg [X^\pm]$. Eqn. (10) shows that under such conditions $\Lambda^\pm$ and K_m^{el} approach unity and K_m respectively.

Kinetics at changing ionic strength

When K_m and $[A^+]$ are of commensurable magnitude the ionic strength, and as a result the electrostatic partition coefficient, varies with the substrate concentration at a fixed value of $[A^+]$. Since under such conditions $\Lambda^\pm$ is a function of $[S^+]$ the reaction no longer obeys the Michaelis–Menten kinetic law, as

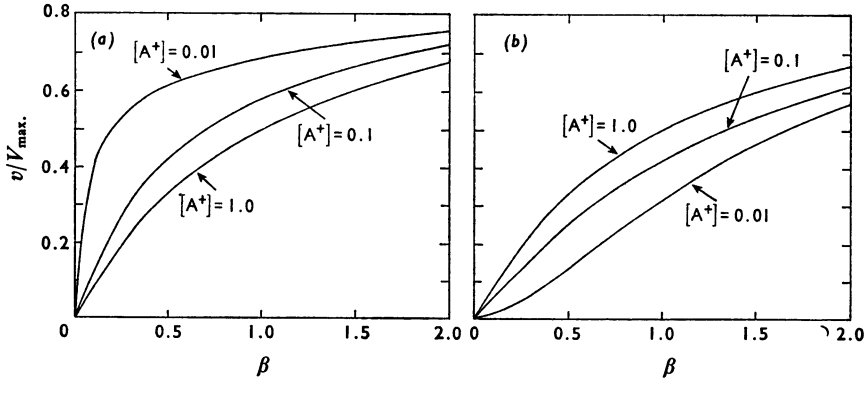

Fig. 1. *Plots of the activity of a bound enzyme against the dimensionless concentration of a cationic substrate at different ionic concentrations in the external solution* $[A^+]$

The enzymic microenvironment contains either negatively charged $[X^-]$ (*a*) or positively charged $[X^+]$ (*b*) fixed groups. The intrinsic K_m of the enzymic reaction is 5×10^{-2} M. For further details see the text.

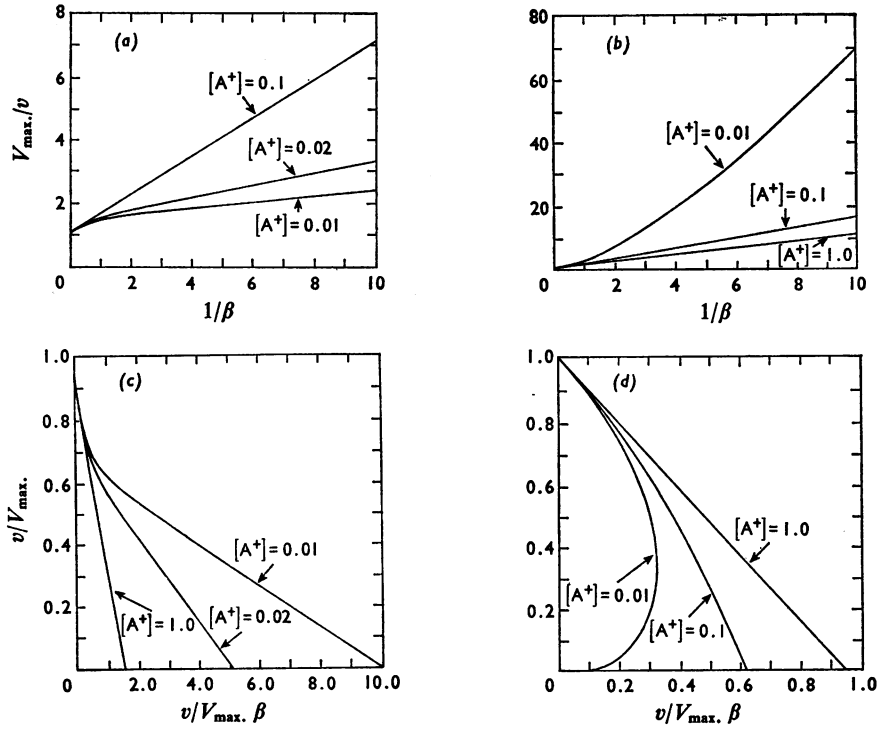

Fig. 2. *Lineweaver–Burk, (a) and (b), and Eadie–Hofstee, (c) and (d), plots for the enzymic reaction with a cationic substrate in a charged membrane at different ionic concentrations in the external solution* $[A^+]$

The intrinsic K_m value is 5×10^{-2}M. The fixed charges are negative $[X^-]$ in (*a*) and (*c*) and positive $[X^+]$ in (*b*) and (*d*) respectively.

illustrated in Figs. 1 and 2 for $K_m = 5\times10^{-2}$M and different values of $[A^+]$. Fig. 1 shows that the dependence of the enzymic activity on the macroenvironmental substrate concentration is sigmoidal when both the substrate and membrane carry like charges and $[A^+]$ is sufficiently low. The comparison of the Lineweaver–Burk- and Eadie–Hofstee-type plots (Westley, 1969) in Fig. 2 demonstrate, in agreement with previous studies on diffusion kinetics (Horvath & Engasser, 1974), that the Eadie–Hofstee-type plots are more useful for diagnosis of deviations from the Michaelis–Menten kinetics and yield characteristic curves such as for sigmoidal kinetics.

Effect of a charged inhibitor

It is assumed the charged enzymic membrane is in contact with a solution containing A^+, B^- and a positively charged non-competitive inhibitor, I^+, and the substrate concentration is high enough to obtain saturation rate. Then the reaction rate is expressed by

$$v = \frac{V_{max.}}{1+([I^+]_o/K_i)} \tag{11}$$

where $[I^+]_o$ is the inhibitor concentration in the membrane and K_i is the inhibition constant. The microenvironmental and the macroenvironmental concentrations $[I^+]$ are related by

$$[I^+]_o = \Lambda\,[I^+] \tag{12}$$

where Λ is the electrostatic partition coefficient, which is calculated from eqn. (6).

As for the positively charged substrate Λ is practically independent of $[I^+]$ when the value of $[A^+]$ is adjusted or much greater than that of K_i so that the ionic strength is constant. Then the kinetic effect of the fixed charges can be characterized by an effective inhibition constant, K_i^{el}, which is given by

$$K_i^{el} = \frac{K_i}{\Lambda^{\pm}} \tag{13}$$

where $\Lambda^{\pm}$ is determined by eqn. (9).

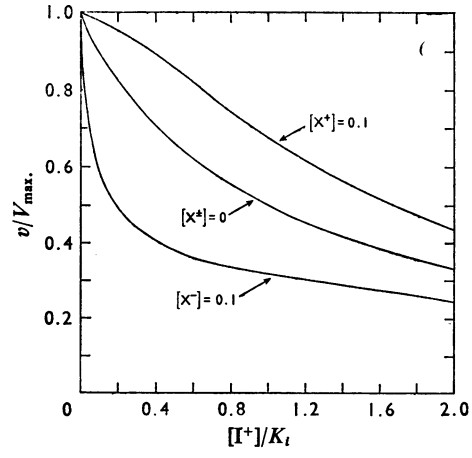

Fig. 3. *Plots of the activity of a bound enzyme against the dimensionless concentration of cationic inhibitor in the macroenvironment* $[I^+]/K_I$

The value of the inhibition constant, K_I, is 5×10^{-2}M. The enzymic microenvironment is either neutral, $[X^\pm]=0$, or contains fixed positive charges $[X^+]$ or negative charges $[X^-]$. $[A^+]=0.01$.

The kinetic behaviour of bound enzymes, however, is more complex when K_I and $[A^+]$ are the same order of magnitude, as shown in Fig. 3, which illustrates the dependence of the observed enzyme activity in a neutral, a positively and a negatively charged membrane on the macroenvironmental inhibitor concentration for the particular case of $K_I=5\times10^{-2}$M and $[A^+]=10^{-2}$M. The effect of the charged inhibitor is greatly modified by the fixed charges and the sensitivity of the enzyme to the inhibitor depends on the signs of the respective charges.

Discussion

In all experimental and theoretical studies so far the effect of the interaction between an ionic substrate and a charged matrix on the kinetics of bound enzymes has been characterized by an apparent K_m. The present work, however, shows that the effect of the charged enzymic microenvironment is more complex and the notion of an apparent K_m is only applicable if the ionic strength does not change with the substrate concentration.

As shown in Figs. 1 and 2 Michaelis–Menten kinetics are not obtained in the presence of significant electrostatic effects when the ionic strength varies with the substrate concentration. Under certain conditions sigmoidal kinetics are observed for substrate and matrix with like charge; deviations from Michaelis–Menten kinetics due to electrostatic interactions are illustrated by familiar linearized plots. The departure from straight lines is more pronounced on Eadie–Hofstee plots than on the commonly used Lineweaver–Burk plots.

In previously published experiments the ionic strength was maintained constant either by adjusting the salt concentration in the reaction mixture (Goldstein *et al.*, 1964; Wharton *et al.*, 1968) or by working at salt concentrations much higher than the substrate concentration (Hornby *et al.*, 1966); therefore the data indeed yielded an apparent K_m value. In agreement with earlier findings our study also predicts that the electrostatic effect vanishes at sufficiently high ionic strength with respect to the concentration of fixed charges.

The non-uniform distribution of a charged effector between the enzymic micro- and macro-environment due to fixed ionic groups has also been investigated in the literature. The inhibition of immobilized trypsin by soya-bean trypsin inhibitor is, for instance, dependent on the nature of the fixed charges according to Levin *et al.* (1964). On the other hand the pH–activity profile of many enzymes has shifted on immobilization on an electrically charged support (McLaren & Esterman, 1957; Goldstein *et al.*, 1964). The usual bell-shaped pH profile of the enzymic activity is the result of an activation and inhibition of the enzyme by H^+ ions at concentrations lower and higher than that at the pH optimum respectively. The shape of the activity profile is then characterized by the corresponding activation and inhibition constants, which are usually much smaller than the total ionic concentration of the solution. Therefore the ionic strength remains practically constant when the H^+ concentration is varied in the pH domain around the activation and the inhibition constants. Under such conditions both constants are modified by the same factor to yield the corresponding apparent activation and inhibition constants according to eqn. (13). Thus the pH–activity profile is shifted owing to electrostatic effects, but the shape of the curve is not affected.

It is expected that the results of this study are of interest in the design of kinetic experiments with bound enzymes and the interpretation of the data. It should be kept in mind, however, that diffusion resistances can also affect the results obtained with heterogeneous enzyme systems (Engasser & Horvath, 1974). Although the intrusion of diffusion alone has been extensively investigated, the combined effect of electrostatic and transport phenomena has been treated only for an enzymic surface reaction (Shuler *et al.*, 1972). Nevertheless, most aspects of the kinetic behaviour of bound enzymes can be inferred when the separate effects of the two phenomena are considered together. Owing to the polyelectrolyte nature of cellular membranes the results of the present study can also give insight into the behaviour of bound

enzymes in the cellular milieu. For instance, the sigmoidal dependence of the activity of bound enzymes on the substrate or inhibitor concentration may enhance the regulatory function of enzymic reactions that follow Michaelis–Menten kinetics in free solution.

This work was supported by a grant from the N.I.H., U.S. Public Health Service, no. GM 21084–01

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