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pH MODULATION OF TRANSIENT STATE KINETICS OF ENZYMES

I. SIMPLE THEORETICAL MODELS

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The effect of proton concentration on pre-steady-state kinetics has been investigated theoretically for enzyme reactions involving the breaking of one substrate into two products. Even for the simple double-intermediate mechanism the approach to the steady state may exhibit a rather complex kinetics, which is pH-dependent. This process may even exhibit damped oscillations. A change of pH may completely change this transient kinetics and even suppresses the oscillatory regime. A simple method is presented which allows estimation of the values of the rate and ionization constants. This procedure allows one to distinguish the simple double-intermediate mechanism from a more complex process where the 'fast' binding of the substrate induces a 'slow' conformation change of the enzyme.

1. Introduction

The experimental finding that some enzymes exhibit a burst kinetics before a steady state is reached has prompted theoretical studies on the transient phase of enzyme reactions [1]. These studies have been performed, in most cases, with the so-called double-intermediate mechanism, where it is assumed that the enzyme binds its substrate and releases two products following a compulsory order. Most hydrolases follow this simple reaction scheme.

Moreover, it has been outlined in a few cases [2,3] that, during the pre-steady-state phase, damped oscillations of any enzyme-substrate or enzyme-product intermediate may occur. Although this possibility has been considered, the precise conditions which allow these oscillations to arise have not been derived for any realistic model of enzyme reaction. Moreover, this interesting effect has not yet been observed.

Although numerous studies have been performed on pH effects of enzyme reactions [4], a possible pH modulation of transient phase behaviour does not seem to have been considered. Such a study, however, may be of biological interest when performed with enzymes located on cell envelopes, either cell walls or biomembranes, where proton concentration may change dramatically under different conditions [5].

The aims of this paper are three-fold:

To derive the pre-steady-state equations for various simple realistic models of enzyme reaction;

To determine the conditions which allow damped oscillations of a reaction intermediate during the transient phase;

To predict how pH changes of the reaction medium may modulate the pre-steady-state kinetics of the reaction process.

2. Theory

The first model that will be considered is shown in fig. 1A. The substrate A is bound to the enzyme

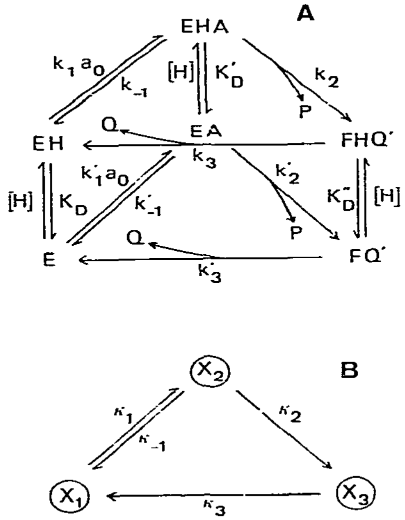

Fig. 1. Ionization of the enzyme in the double-intermediate mechanism. (A) The double-intermediate mechanism with ionization of free enzyme and enzyme-ligand complexes. (B) Node compression in the above mechanism. Ionization processes are supposed to be rapid as compared to the other reaction steps.

and two products P and Q are released following a compulsory sequence. The second step of product release corresponds to the hydrolysis of a covalent enzyme-ligand intermediate, FQ', for instance, an acyl-enzyme or a phosphoryl-enzyme complex. The rate constants k_3 and k'_3 are therefore apparent rate constants which contain in their expression water concentration. This scheme is quite realistic and has been found to apply to many hydrolytic reactions. Moreover, it is assumed that in the pH range investigated each enzyme form exists in two different ionization states. Ionization and protonation reactions are supposed to be fast processes as compared with the other reaction steps. Therefore, the scheme in fig. 1A may be represented by that shown in fig. 1B. The parameters (κ), which appear in this formal model, are apparent rate constants which can be written as

$$\begin{aligned} \kappa_1 &= k_1 f_1 + k'_1 f'_1 & \kappa_2 &= k_2 f_2 + k'_2 f'_2 \\ \kappa_{-1} &= k_{-1} f_2 + k'_{-1} f'_2 & \kappa_3 &= k_3 f_3 + k'_3 f'_3 \end{aligned} \tag{1}$$

The coefficients (f) are fractionation factors defined as

$$\begin{aligned} f_1 &= \frac{[\mathrm{H}]}{K_\mathrm{D}+[\mathrm{H}]} & f_2 &= \frac{[\mathrm{H}]}{K'_\mathrm{D}+[\mathrm{H}]} & f_3 &= \frac{[\mathrm{H}]}{K''_\mathrm{D}+[\mathrm{H}]} \\ f'_1 &= \frac{K_\mathrm{D}}{K_\mathrm{D}+[\mathrm{H}]} & f'_2 &= \frac{K'_\mathrm{D}}{K'_\mathrm{D}+[\mathrm{H}]} & f'_3 &= \frac{K''_\mathrm{D}}{K''_\mathrm{D}+[\mathrm{H}]} \end{aligned} \tag{2}$$

If e_0 and a_0 are the total concentrations of enzyme and substrate, setting

$$\begin{aligned} m &= [\mathrm{X}_2] = [\mathrm{EA}]+[\mathrm{EHA}] \\ n &= [\mathrm{X}_3] = [\mathrm{FQ'}]+[\mathrm{FHQ'}] \end{aligned} \tag{3}$$

one has

$$\begin{aligned} \frac{\mathrm{d}m}{\mathrm{d}t} &= \kappa_1 a_0 (e_0 - m - n) - (\kappa_{-1} + \kappa_2) m \\ \frac{\mathrm{d}n}{\mathrm{d}t} &= \kappa_2 m - \kappa_3 n \end{aligned} \tag{4}$$

These expressions are indeed valid if there is a large excess of substrate over enzyme concentration. This condition is usually fulfilled in most enzyme studies.

Integration of these linear differential equations yields (see appendix)

$$\begin{aligned} m &= \frac{\rho}{\xi} + \frac{\rho(\xi - \kappa_3 \lambda_2)}{\kappa_3 \xi (\lambda_2 - \lambda_1)} e^{-\lambda_1 t} + \frac{\rho(\kappa_3 \lambda_1 - \xi)}{\kappa_3 \xi (\lambda_2 - \lambda_1)} e^{-\lambda_2 t} \\ n &= \frac{\rho'}{\xi} - \frac{\rho' \lambda_2}{\xi (\lambda_2 - \lambda_1)} e^{-\lambda_1 t} + \frac{\rho' \lambda_1}{\xi (\lambda_2 - \lambda_1)} e^{-\lambda_2 t} \end{aligned} \tag{5}$$

where λ_1 and λ_2 are the Encke's roots of the characteristic equation associated with the differential system. ξ is the product of the two roots (see appendix), namely

$$\xi = \lambda_1 \lambda_2 = \kappa_1 a_0 (\kappa_2 + \kappa_3) + \kappa_3 (\kappa_{-1} + \kappa_2) \tag{6}$$

and the parameters ρ and ρ' have the following expressions

$$\begin{aligned} \rho &= \kappa_1 \kappa_3 e_0 a_0 \\ \rho' &= \kappa_1 \kappa_2 e_0 a_0 \end{aligned} \tag{7}$$

If p and q are the concentrations of products P and Q, one must have

$$\begin{aligned} \frac{\mathrm{d}p}{\mathrm{d}t} &= \kappa_2 m \\ \frac{\mathrm{d}q}{\mathrm{d}t} &= \kappa_3 n \end{aligned} \tag{8}$$

Eqs. 8 are readily integrated in the interval ($0,t$)

and one finds

$$\begin{aligned} p &= v_o t + \psi_1(1 - e^{-\lambda_1 t}) + \psi_2(1 - e^{-\lambda_2 t}) \\ q &= v_o t - \psi_1'(1 - e^{-\lambda_1 t}) + \psi_2'(1 - e^{-\lambda_2 t}) \end{aligned} \tag{9}$$

where v_o is the steady-state rate

$$v_o = \frac{\kappa_2 \rho}{\xi} = \frac{\kappa_3 \rho'}{\xi} \tag{10}$$

and the coefficients ψ and ψ' are given by the expressions

$$\begin{aligned} \psi_1 &= \frac{\kappa_2 \rho(\xi - \kappa_3 \lambda_2)}{\kappa_3 \lambda_1 \xi(\lambda_2 - \lambda_1)} \\ \psi_2 &= \frac{\kappa_2 \rho(\kappa_3 \lambda_1 - \xi)}{\kappa_3 \lambda_2 \xi(\lambda_2 - \lambda_1)} \\ \psi_1' &= \frac{\kappa_3 \lambda_2 \rho'}{\lambda_1 \xi(\lambda_2 - \lambda_1)} \\ \psi_2' &= \frac{\kappa_3 \lambda_1 \rho'}{\lambda_2 \xi(\lambda_2 - \lambda_1)} \end{aligned} \tag{11}$$

The steady-state progress appearance of products P and Q is expressed by the equations

$$\begin{aligned} p_S &= \lim_{t \to \infty} p = v_o t + \psi_1 + \psi_2 \\ q_S &= \lim_{t \to \infty} q = v_o t - \psi_1' + \psi_2' \end{aligned} \tag{12}$$

After some algebra one can show that these expressions are equivalent to

$$p_S = \frac{\kappa_2 \rho}{\xi} t + \frac{\kappa_2 \rho}{\xi^2 \kappa_3}(\xi - \kappa_3 \sigma) \tag{13}$$

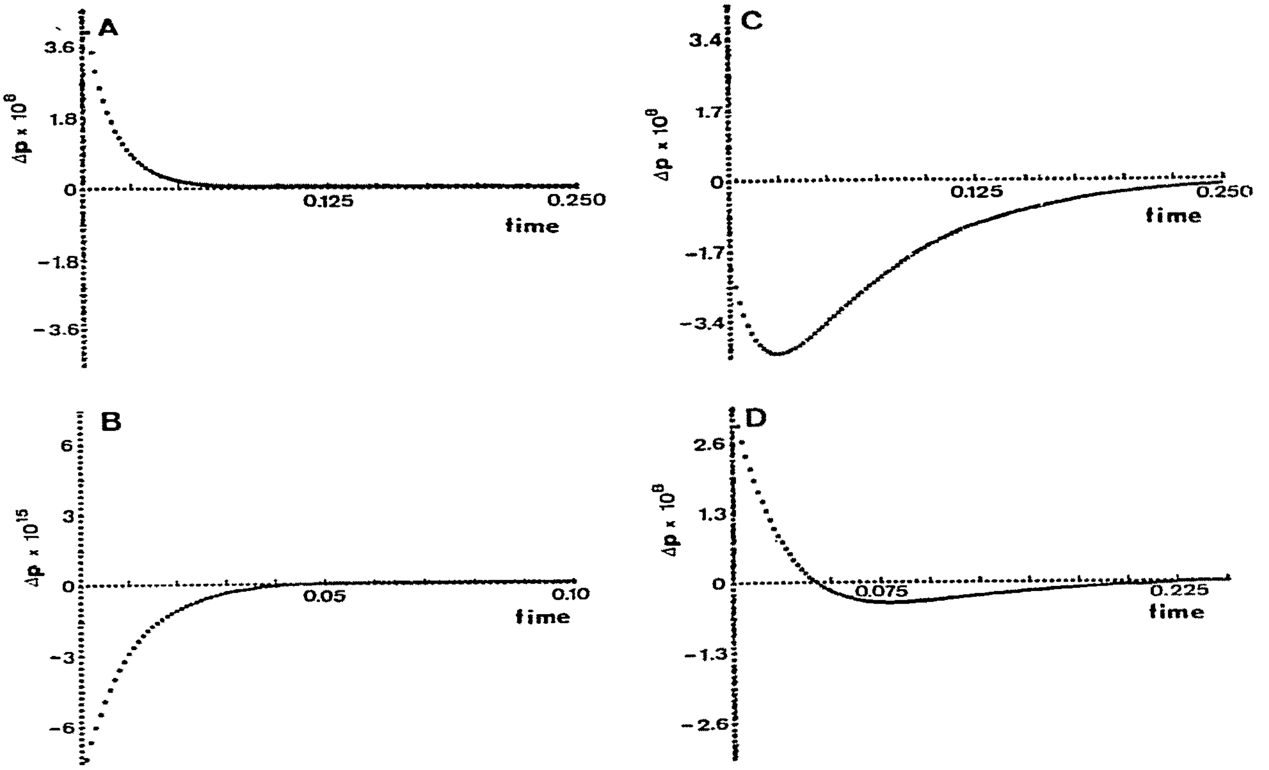

Fig. 2. Some possible different kinetic approaches to the steady state. The numerical values of the relevant parameters and ligand concentrations are: (A) $\kappa_3 = 9.76 \times 10^{-2}$, $\lambda_1 = 7.52 \times 10^{-2}$, $\lambda_2 = 8.75 \times 10^{-2}$, $\psi_1 = -7.26 \times 10^{-8}$, $\psi_2 = 2.41 \times 10^{-8}$, $[H] = 10^{-6}$, $a_o = 10^{-2}$; (B) $\kappa_3 = 0.1$, $\lambda_1 = 0.11$, $\lambda_2 = 20.09$, $\psi_1 = 8.11 \times 10^{-15}$, $\psi_2 = -4.46 \times 10^{-13}$, $[H] = 10^{-7}$, $a_o = 10^{-4}$; (C) $\kappa_3 = 1.19 \times 10^{-2}$, $\lambda_1 = 1.65 \times 10^{-2}$, $\lambda_2 = 6.05 \times 10^{-2}$, $\psi_1 = 8.71 \times 10^{-8}$, $\psi_2 = -6.82 \times 10^{-8}$, $[H] = 10^{-6}$, $a_o = 10^{-2}$; (D) $\kappa_3 = 1.66 \times 10^{-2}$, $\lambda_1 = 2.17 \times 10^{-2}$, $\lambda_2 = 2.95 \times 10^{-2}$, $\psi_1 = 8.95 \times 10^{-8}$, $\psi_2 = -1.23 \times 10^{-7}$, $[H] = 2.5 \times 10^{-7}$, $a_o = 10^{-3}$. The numerical values of rate constants have not been given for the sake of clarity.

$$q_S = \frac{\kappa_3 p'}{\xi} t - \frac{\kappa_3 p'}{\xi^2} \sigma$$

where σ is the sum of the two roots, namely

$$\sigma = \lambda_1 + \lambda_2 = \kappa_1 a_o + \kappa_2 + \kappa_{-1} + \kappa_3 \tag{14}$$

Therefore, the intercept of the steady-state progress curve of product P may be positive, zero or negative, at a fixed pH, depending on the respective values of ξ and $\kappa_3\sigma$. Conversely, the intercept of the progress curve for product Q is of necessity negative at any pH. One may show after some algebra that the condition

$$\xi > \kappa_3 \sigma \tag{15}$$

which generates a burst kinetics is equivalent to

$$\frac{k_1[\mathrm{H}] + k_1' K_\mathrm{D}}{K_\mathrm{D} + [\mathrm{H}]} \cdot \frac{k_2[\mathrm{H}] + k_2' K_\mathrm{D}'}{K_\mathrm{D}' + [\mathrm{H}]} a_o > \frac{(k_3[\mathrm{H}] + k_3' K_\mathrm{D}'')^2}{(K_\mathrm{D}'' + [\mathrm{H}])^2} \tag{16}$$

Changing the pH as well as the substrate concentration may change this inequality and therefore may alter the kinetics of the pre-steady-state phase.

The approach to the steady-state appearance of products P and Q may be expressed as

$$\Delta p = p - p_S = -\psi_1 e^{-\lambda_1 t} - \psi_2 e^{-\lambda_2 t} \tag{17a}$$

$$\Delta q = q - q_S = \psi_1' e^{-\lambda_1 t} - \psi_2' e^{-\lambda_2 t} \tag{17b}$$

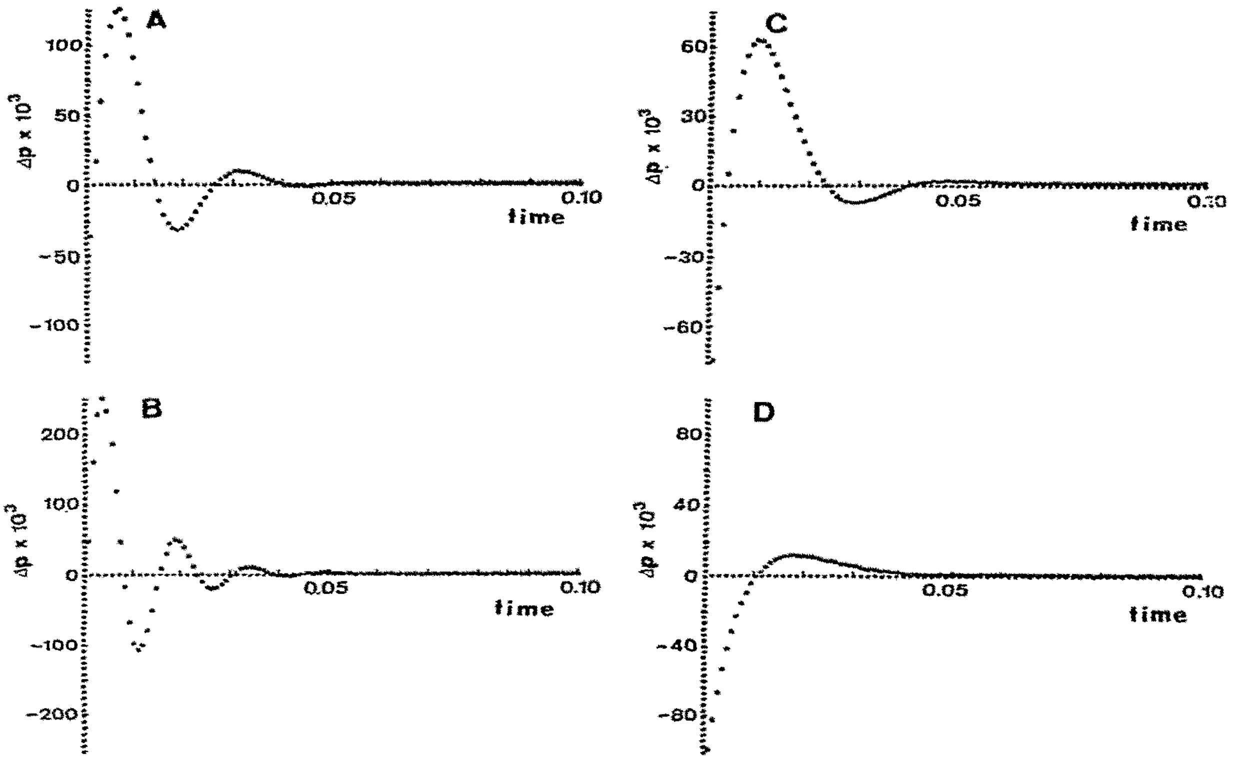

Fig. 3. Damped oscillations in the approach to the steady state. (A and B) Effect of substrate concentration on the frequency of damped oscillations. In both panels A and B the proton concentration is $[\mathrm{H}] = 10^{-8}$. Substrate concentration is $a_o = 2 \times 10^{-2}$ for panel A and $a_o = 5 \times 10^{-2}$ for panel B. (C and D) Effect of proton concentration on the oscillatory regime during the pre-steady state phase. In both panels C and D the substrate concentration is $a_o = 10^{-2}$. Proton concentration is $[\mathrm{H}] = 10^{-8}$ (panel C) and $[\mathrm{H}] = 10^{-7}$ (panel D). For any of the above simulations the numerical values of rate and ionization constants are: $k_1 = 90$, $k_{-1} = 90$, $k_1' = 50$, $k_{-1}' = 50$, $k_2 = 50$, $k_{-2}' = 4 \times 10^4$, $k_3 = 10^2$, $k_3' = 10^2$, $K_\mathrm{D} = 10^{-10}$, $K_\mathrm{D}' = 10^{-7}$, $K_\mathrm{D}'' = 10^{-8}$.

These expressions are analogous to relaxation spectra, where λ_1 and λ_2 are reciprocals of relaxation times and the pre-exponential terms are relaxation amplitudes [6–8].

One has indeed

$$\lambda_2 > \lambda_1 \tag{18}$$

and depending on the value of κ_3 with respect to those of λ_2 and λ_1, the two amplitudes in eq. 17a may have different signs. If

$$\kappa_3 > \lambda_2 > \lambda_1 \tag{19}$$

the first amplitude, $-\psi_1$, will be positive and the second, $-\psi_2$, negative. If

$$\lambda_2 > \kappa_3 > \lambda_1 \tag{20}$$

both amplitudes, $-\psi_1$ and $-\psi_2$, will be positive and if

$$\lambda_2 > \lambda_1 > \kappa_3 \tag{21}$$

the first amplitude, $-\psi_1$ will be negative and the second, $-\psi_2$, positive. Alternately, the first pre-exponential term, ψ'_1, of eq. 17b is always positive and the second, $-\psi'_2$, always negative. Some possible curve shapes are given in fig. 2.

Another important question is to know whether the time constants λ_1 and λ_2 may be complex. This is so when

$$4\xi > \sigma^2 \tag{22}$$

This situation may indeed occur. Then the expressions of the two roots assume the forms

$$\begin{aligned} \lambda_1 &= \tfrac{1}{2}\left(\sigma - i\sqrt{4\xi - \sigma^2}\right) \\ \lambda_2 &= \tfrac{1}{2}\left(\sigma + i\sqrt{4\xi - \sigma^2}\right) \end{aligned} \tag{23}$$

Setting

$$\omega = \sqrt{4\xi - \sigma^2} \tag{24}$$

the equations that describe the approach to the steady state assume the form

$$\Delta p = \exp\left(-\frac{\sigma}{2}t\right)\left(-\psi_1 e^{i\omega t} - \psi_2 e^{-i\omega t}\right) \tag{25}$$

$$\Delta q = \exp\left(-\frac{\sigma}{2}t\right)\left(\psi'_1 e^{i\omega t} - \psi'_2 e^{-i\omega t}\right) \tag{26}$$

From eq. 14 σ is a linear function of substrate concentration. Therefore, increasing that concentration results in an increased damping and frequency of oscillations during the transient phase. This is illustrated by computer simulation in fig. 3.

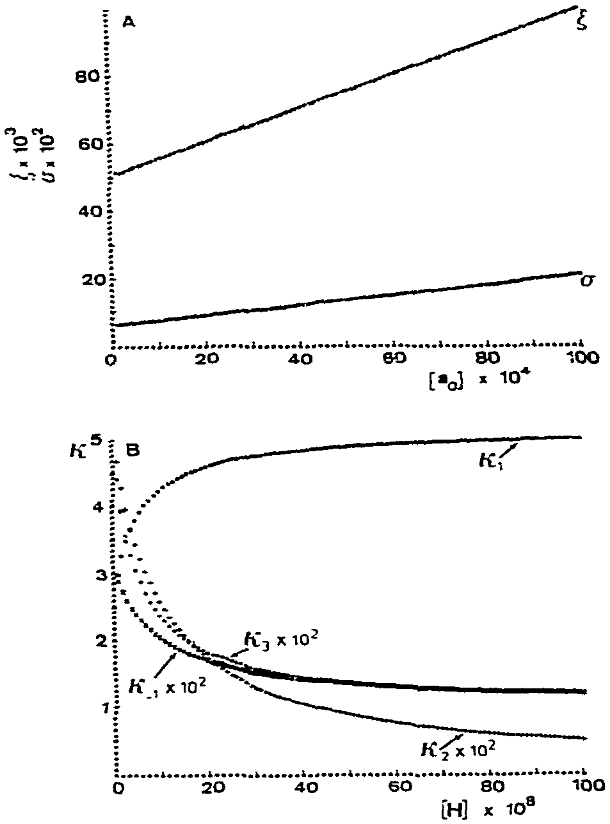

Fig. 4. Determination of rate and ionization constants of model of fig. 1. (A) Plot of ξ and σ as a function of substrate concentration. (B) Plot of κ as a function of proton concentration. The numerical values of rate and ionization constants are: $k_1 = 5$, $k_{-1} = 10^{-2}$, $k'_1 = 2.5$, $k'_{-1} = 3 \times 10^{-2}$, $k_2 = 10^{-4}$, $k'_2 = 5 \times 10^{-2}$, $k_3 = 10^{-2}$, $k'_3 = 5 \times 10^{-2}$, $K_D = 5.5 \times 10^{-8}$, $K'_D = 10^{-7}$, $K''_D = 5 \times 10^{-8}$.

Since ξ and σ depend on pH, changing the pH may create or suppress oscillations (fig. 3). Both ξ and σ are linear functions of substrate concentration a_0 at constant pH. Plotting ξ and σ as a function of a_0 allows one to determine the four κ values (fig. 4). Plotting these values as a function of proton concentration allows the estimation of

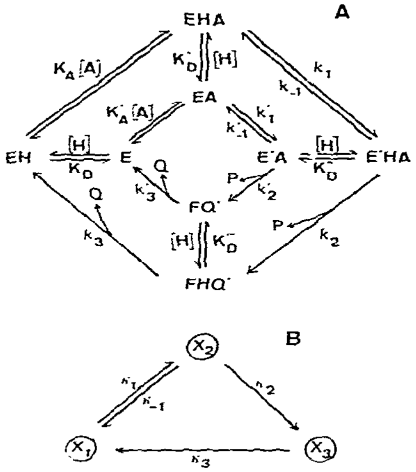

Fig. 5. Double-intermediate mechanism with a slow conformation change of the enzyme.

the real rate constants as well as of the ionization constants K_D, K'_D and K''_D. Some possible variations of the κ values as a function of [H] are shown in fig. 5B.

Another model, which will now be considered, is shown in fig. 5. In this model it is assumed that the binding of the substrate is a 'fast' process followed by a 'slow' conformation change. Thus, the model may be depicted as shown in fig. 5B, which is very similar to that of fig. 1B. The expression of the κ parameters is still that given by eq. 1, but now the fractionation factors assume the form

$$\begin{aligned}
f_1 &= \frac{K_D K'_A [\mathrm{H}] a_0}{K_D K'_A a_0 (K'_D + [\mathrm{H}]) + K'_D (K_D + [\mathrm{H}])} \\
f'_1 &= \frac{K_D K'_D K'_A a_0}{K_D K'_A a_0 (K'_D + [\mathrm{H}]) + K'_D (K_D + [\mathrm{H}])} \\
f_2 &= \frac{[\mathrm{H}]}{K''_D + [\mathrm{H}]} \qquad f'_2 = \frac{K''_D}{K''_D + [\mathrm{H}]} \\
f_3 &= \frac{[\mathrm{H}]}{K'''_D + [\mathrm{H}]} \qquad f'_3 = \frac{K'''_D}{K'''_D + [\mathrm{H}]}
\end{aligned} \tag{26}$$

The analytical treatment of the model depicted in fig. 5B is similar to that of the model in fig. 1B, but the expressions of σ and ξ are not linear functions of a_0. The equation of ξ and σ is now that of a hyperbola as illustrated in fig. 6 by computer simulation. In practice, a plot of ξ or σ as a function of a_0 may allow one to discriminate between the models of figs. 1 and 6.

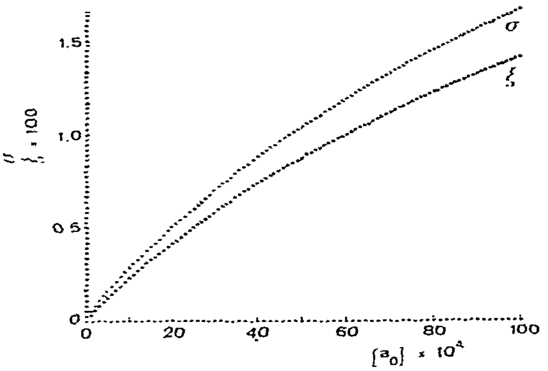

Fig. 6. Plot of ξ and σ as a function of substrate concentration for model of fig. 5 (slow conformation change of the enzyme). The numerical values of rate and ionization constants are: $k_1 = 5$, $k_{-1} = 10^{-2}$, $k'_1 = 2.5$, $k'_{-1} = 3 \times 10^{-2}$, $k_2 = 10^{-4}$, $k'_2 = 5 \times 10^{-2}$, $k_3 = 10^{-4}$, $k'_3 = 5 \times 10^{-4}$, $K_D = 5.5 \times 10^{-8}$, $K'_D = 10^{-7}$, $K''_D = 5 \times 10^{-8}$, $K''' = 10^{-8}$.

3. Discussion

It is well known that many hydrolytic enzyme reactions follow the so-called 'double-intermediate' mechanism [1]. During this process, a covalent intermediate, such as an acyl-enzyme or a phosphoryl-enzyme is formed. The first product to be released may exhibit either a burst or a lag kinetics in the pre-steady-state phase. The results presented in this paper show that, if the enzyme exists in at least two different ionization states, a change of pH may completely change the pre-steady-state behaviour.

Even with the simple, realistic, double-intermediate mechanism, the reaction intermediates may exhibit damped oscillations during the approach of the steady state. The appearance, or the suppression, of the oscillatory regime may be induced by a pH change. The possibility of the existence of oscillations, during the approach of the steady state, had already been considered with complex models [2] and these oscillations had even been simulated with a rather unrealistic model [3].

The possible existence, with a simple realistic model, of damped oscillations is, probably, an interesting result. Up to now, however, no real enzyme has been shown to display such a behaviour. This matter is considered in the accompanying paper [9].

Recent results have shown that the local pH within the outer envelope of isolated plant cells may vary markedly in response to a change of the external milieu [5]. Some hydrolytic enzymes of this cell wall exhibit, under steady-state conditions, a surprising pH dependence [10]. One may tentatively speculate that this pH modulation might play an important role in the hydrolysis and transport of solutes within the cell. It is therefore of interest to determine experimentally whether these pH changes may quantitatively modify the pre-steady-state kinetics of a plant cell wall enzyme, as suggested by the present theoretical study. This is the aim of the companion paper [9].

Appendix

A1. Integration of eqs. 4

The initial conditions of eqs. 4 of the main text are

$$\begin{aligned} m(o) &= 0 \\ n(o) &= 0 \end{aligned} \tag{A1}$$

Solving the linear differential eqs. 4 of the main text is readily done by solving the linear system

$$\begin{bmatrix} s(s+\kappa_1 a_o+\kappa_{-1}+\kappa_2) & s\kappa_1 a_o \\ \kappa_2 & -(s+\kappa_3) \end{bmatrix} \begin{bmatrix} \mathscr{L}\{m\} \\ \mathscr{L}\{n\} \end{bmatrix} = \begin{bmatrix} \kappa_1 e_o a_o \\ 0 \end{bmatrix} \tag{A2}$$

where $\mathscr{L}\{m\}$ and $\mathscr{L}\{n\}$ are the Laplace transforms of variables m and n, and s the usual operator.

Setting

$$\begin{aligned} \sigma &= \kappa_1 a_o+\kappa_2+\kappa_{-1}+\kappa_3 \\ \xi &= \kappa_1 a_o(\kappa_2+\kappa_3)+\kappa_3(\kappa_{-1}+\kappa_2) \\ \rho &= \kappa_1\kappa_3 e_o a_o \\ \rho' &= \kappa_1\kappa_2 e_o a_o \end{aligned} \tag{A3}$$

One finds from the system, eq. A2,

$$\mathscr{L}\{m\} = \frac{\rho+\kappa_1 e_o a_o s}{s(s^2+\sigma s+\xi)}$$

$$\mathscr{L}\{n\} = \frac{\rho'}{s(s^2+\sigma s+\xi)} \tag{A4}$$

If λ_1 and λ_2 are the Encke's roots of the quadratic equation which appears in the denominator of eqs. A4 above, these expressions may be decomposed in simple fractions as

$$\mathscr{L}\{m\} = \frac{\rho}{\xi}\cdot\frac{1}{s}+\frac{\rho(\xi-\kappa_3\lambda_2)}{\kappa_3\xi(\lambda_2-\lambda_1)}\cdot\frac{1}{s+\lambda_1} + \frac{\rho(\kappa_3\lambda_1-\xi)}{\kappa_3\xi(\lambda_2-\lambda_1)}\cdot\frac{1}{s+\lambda_2} \tag{A5}$$

and

$$\mathscr{L}\{n\} = \frac{\rho'}{\xi}\cdot\frac{1}{s}+\frac{\rho'\lambda_2}{\xi(\lambda_2-\lambda_1)}\cdot\frac{1}{s+\lambda_1}+\frac{\rho'\lambda_1}{\xi(\lambda_2-\lambda_1)}\cdot\frac{1}{s+\lambda_2} \tag{A6}$$

Therefore

$$m = \mathscr{L}^{-1}\{m\} = \frac{\rho}{\xi}+\frac{\rho(\xi-\kappa_3\lambda_2)}{\kappa_3\xi(\lambda_2-\lambda_1)}e^{-\lambda_1 t} + \frac{\rho(\kappa_3\lambda_1-\xi)}{\kappa_3\xi(\lambda_2-\lambda_1)}e^{-\lambda_2 t} \tag{A7}$$

and

$$n = \mathscr{L}^{-1}\{n\} = \frac{\rho'}{\xi}-\frac{\rho'\lambda_2}{\xi(\lambda_2-\lambda_1)}e^{-\lambda_1 t}+\frac{\rho'\lambda_1}{\xi(\lambda_2-\lambda_1)}e^{-\lambda_2 t} \tag{A8}$$

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