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pH FEEDBACK CONTROL OF ENZYME MEMBRANES

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pH feedback on immobilized enzymes is theoretically examined with respect to substrate and pH levels, strength of acids produced by the reaction, buffering and asymmetry of the system. All the productions of proton by the different reactions are taken into account by using a 'symbolic species' H^*. The system of differential diffusion-reaction equations is then integrated using numerical methods. The local 'effective enzyme activity' modulated by an acidity factor enables us to predict and quantify evolutions of the systems: NonMichaelian behavior of an immobilized Michaelis-Menten-type enzyme is shown, even when pH back-actions are excluded; the analysis of intramembrane pH profiles shows that the shift of the optimal pH is a complex function of the substrate and pH levels, the intrinsic pH dependence of the enzyme, and the membrane characteristics. This study may easily be transposed to other types of effector such as divalent cations and used in examining self-regulations of multienzyme systems where pH-active reactions are involved.

1. Introduction

Immobilized enzyme kinetics has been extensively studied in the last 20 years; results and conclusions have been summarized in many reviews and books [1–6].

In recent years our group has mainly been interested in functional enzyme structures in which enzyme activity is monitored in space or time by different effectors, namely, enzyme cofactor, substrate or pH [7–9]. Most of the enzymes being pH dependent, the proton (H^+) may be considered as a universal effector. Externally imposed pH levels can control enzyme activity and its distribution in the matrix; on the other hand, 'pH-active' reactions in which protons are produced or consumed may lead to pH modifications giving rise to feedback control of the enzyme activity.

A number of different pH-activity effects have already been reported in the literature. The shift of the apparent (external) optimal pH, relative to the intrinsic one, caused by the accumulation of acids (or bases) produced in the matrix, was first reported [10–16]. We have shown in multienzyme systems that asymmetrical distribution of enzyme activities induced by imposed transmembrane pH gradients can lead to active transport pumping of substrate [7–9,17]; pH oscillations were also predicted in open homogeneous systems by using alternative pH-active reactions [18–20].

However, a careful examination of these results has shown that some significant parameters have been neglected or have only occasionally been taken into account. A more systematic analysis is still needed. Non-pH-active reactions and reactions producing strong acids in unbuffered systems are the boundary cases and are also the best known. However, the problem of production of weak acids or that of slightly buffered systems has not been studied in detail. Analytical equations of

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such feedback kinetics have been established and experimentally verified with pH-active enzyme reactions in solution [21]; the neceassary mathematical simplification came from the use of a symbolic species H^*, an function of the free proton concentration.

Our interest here is in extending the use of this species H^* to diffusion-reaction kinetics in heterogeneous systems. With this concentration H^*, the use of a new acidity factor enables us to quantify the back-action of protons in relation to substrate and pH levels, the strength of the involved acids or bases, buffereing and asymmetries.

For simplicitiy, we chose a reaction catalyzed by a Michaelis-Menten-type enzyme E [22]:

$$S + E \rightleftharpoons ES \rightarrow E + AH \tag{1}$$

which may correspond to many enzyme systems. The overall reaction rate v may be written as [21–24]:

$$v = V \frac{S}{K_m + S} = V\lambda \tag{2}$$

where S is the substrate concentration (no brackets will be used for concentrations), V the maximum reaction rate at a given pH, and K_m the Michaelis constant: in general, K_m is a function of pH but with many enzymes ionization constants of the free enzyme E and of the enzyme-substrate complex ES are not very different and variations of K_m with respect to pH are sufficiently small to be neglected. In this somewhat restricted situation (which will be assumed here), λ, a function only of the substrate concentration, is the substrate dependence of the enzyme E [17,19].

Due to protonation of amino acid residues located near the active site, V is not constant but depends on pH. The 'pH dependence' γ may be expressed by [20,21]:

$$\gamma = \frac{V}{V_m} = K_a H / (K_a + H)(K_b + H) \tag{3}$$

where K_a and K_b are the ionization constants of the protonatable groups and V_m the maximum rate at optimal pH, pH'.

In heterogeneous catalysis, for an uncharged substrate the local coupling between reaction and transport leads to the mass balance [7,26]:

$$\frac{dS}{dt} = \left(\frac{dS}{dt}\right)_{diffusion} - v \tag{4}$$

When the pH of the medium is constant, the kinetic laws are simpler; analytical steady-state expressions of substrate concentration profiles have been established [26] and time evolutions describing transient states have been calculated numerically [27].

When pH-active reactions are involved the laws are more complex and only a few particular aspects have been examined [5,10–13]: the intramembrane proton concentration depends on both the local reaction and transport rates and a curvilinear pH profile may appear.

In this systematic analysis, let us consider an acid AH which is ionized in water as shown in eq. 5:

$$AH \rightleftharpoons A^- + H^+ \tag{5}$$

Due to the ionic nature of the proton, the Nernst-Planck equation should be used [28]; but if high ionic strength is considered, the electric potential created by the spatial distribution of the H^+ in the membrane may be neglected [29]. Under these conditions, the problem may be reduced to a classical type of diffusion reaction. Nevertheless, in general, due to the complexity of equations, only numerical solutions are accessible.

2. Theory

2.1. The equations

For a membrane-bound-enzyme reaction, the disappearance of the substrate is expressed by the classical diffusion-reaction equation [4,8,26]:

$$\frac{\partial S}{\partial t} = D_s \frac{\partial^2 S}{\partial X^2} - v \tag{6}$$

X being the space coordinate. Since protons are involved in several reactions (R_i), such as water ionic equilibrium (R_w), acid-base equilibria of the acid produced, AH (R_{ah}) (see eq. 5), and of any non-reactive buffer, BH (R_{bh}), added, the com-

plete scheme may be written:

$$\begin{aligned} &S \rightarrow AH \quad \text{(enzyme reaction rate } v\text{)} \\ &H_2O \rightleftharpoons H^+ + OH^- \quad (\text{with } K_w = H \times OH) \\ &AH \rightleftharpoons H^+ + A^- \quad (\text{with } K_{ah} = H \times A/AH) \\ &BH \rightleftharpoons H^+ + B^- \quad (\text{with } K_{bh} = H \times B/BH) \end{aligned} \tag{7}$$

The global H^+ production rate is expressed by:

$$\left(\frac{\partial H}{\partial t}\right)_{\text{total}} = \left(\frac{\partial H}{\partial t}\right)_{\text{diffusion}} + \left(\frac{\partial H}{\partial t}\right)_{R_w} + \left(\frac{\partial H}{\partial t}\right)_{R_{ah}} + \left(\frac{\partial H}{\partial t}\right)_{R_{bh}} \tag{8}$$

By writing similar expressions for the other species:

$$\left(\frac{\partial OH}{\partial t}\right)_{\text{total}} = \left(\frac{\partial OH}{\partial t}\right)_{\text{diffusion}} + \left(\frac{\partial OH}{\partial t}\right)_{R_w} \tag{9}$$

$$\left(\frac{\partial AH}{\partial t}\right)_{\text{total}} = \left(\frac{\partial AH}{\partial t}\right)_{\text{diffusion}} + \left(\frac{\partial AH}{\partial t}\right)_{R_{ah}} + v \tag{10}$$

$$\left(\frac{\partial B}{\partial t}\right)_{\text{total}} = \left(\frac{\partial B}{\partial t}\right)_{\text{diffusion}} + \left(\frac{\partial B}{\partial t}\right)_{R_{bh}} \tag{11}$$

$$\left(\frac{\partial A}{\partial t}\right)_{\text{total}} = \left(\frac{\partial A}{\partial t}\right)_{\text{diffusion}} + \left(\frac{\partial A}{\partial t}\right)_{R_{ah}} \tag{12}$$

noting that:

$$\begin{aligned} &(\partial H/\partial t)\, R_w = (\partial OH/\partial t)\, R_w \\ &(\partial H/\partial t)\, R_{ah} = -(\partial AH/\partial t)\, R_{ah} = (\partial A/\partial t)\, R_{ah} \\ &(\partial H/\partial t)\, R_{bh} = (\partial B/\partial t)\, R_{bh} \end{aligned} \tag{13}$$

and assuming that all the diffusion coefficients are equal, eq. 8 becomes:

$$\left(\frac{\partial(H - OH + AH - B)}{\partial t}\right)_{\text{total}} = \left(\frac{\partial(H - OH + AH - B)}{\partial t}\right)_{\text{diffusion}} + v \tag{14}$$

where the symbolic species $H^* = H - OH + AH - B$ obeys the classical diffusion-reaction law:

$$\left(\frac{\partial H^*}{\partial t}\right)_{\text{total}} = \left(\frac{\partial H^*}{\partial t}\right)_{\text{diffusion}} + v \tag{15}$$

which may be expressed as a function of space and time by:

$$\frac{\partial H^*}{\partial t} = D_s \frac{\partial^2 H^*}{\partial x^2} + v \tag{15a}$$

As in homogeneous kinetics [21], H^* may be expressed as a function of the proton concentration:

$$H^* = H - K_w/H + H/(K_{ah} + H)\mathscr{A} - K_{bh}/(K_{bh} + H)\mathscr{B} \tag{16}$$

where $\mathscr{B}$ is the total buffer concentration ($\mathscr{B} = B + BH$) and $\mathscr{A}$ the amount of the species produced by the reaction; it is seen from eqs. 10 and 12 that the concentration of this species $\mathscr{A}$ also obeys the diffusion-reaction law:

$$\left(\frac{\partial \mathscr{A}}{\partial t}\right)_{\text{total}} = \left(\frac{\partial \mathscr{A}}{\partial t}\right)_{\text{diffusion}} + v \tag{17}$$

Remarks

(a) The assumption of equality of diffusion coefficients enabled us to obtain a general diffusion-reaction law for the symbolic species H^*. In a more careful examination of the system the inequality of these diffusion coefficients must be considered because, while the diffusion coefficients of B and AH may often be equal (weak acids protonated or unprotonated), the coefficient of H^+ is generally much greater (at least 4- or 5-times) than that of either AH or B. In this case, eqs. 14 and 15 are still valid but their expression as a function of space and time changes and eq. 15a can no longer be used. Analytically, it should be a problem; but numerically, it is sufficient to replace eq. 15a by eq. 15b:

$$\frac{\partial H^*}{\partial t} = D_s \frac{\partial^2 H^{**}}{\partial x^2} + v \tag{15b}$$

where:

$$H^{**} = \frac{D_h}{D_s} H - \frac{D_{oh}}{D_s} OH + \frac{D_{ah}}{D_s} AH - \frac{D_b}{D_s} B \tag{15c}$$

(b) In the limiting case where K_{ah} and K_{bh} are both high (no weak acids) and $D_h \simeq D_{oh}$, H^+ simplifies to H-OH [30].

2.2 Solving the equations

With respect to eqs. 3 and 4, the H^+ and S concentration profiles are interdependent and no analytical solution exists. Numerical methods are then necessary to obtain kinetic evolutions of the system. If an explicit scheme is used [27], the kinetics is given by expressing the local concentration at time $t + \Delta t$ as a function of concentrations at time t:

$$s_x^{t+\Delta t} = s_x^t + \Delta t \left[\frac{s_{x-\Delta x}^t + s_{x+\Delta x}^t - 2s_x^t}{\Delta x^2} - \sigma(\gamma\lambda)_x^t\right] \tag{18}$$

and

$$h_x^{*t+\Delta t} = h_x^{*t} + \Delta t\left[\frac{h^{*t}_{x-\Delta x} + h^{*t}_{x+\Delta x} - 2h_x^{*t}}{\Delta x^2} + \sigma(\gamma\lambda)_x^t\right] \quad (19)$$

where the dimensionless parameters are:

$$s = S/K_m; \quad h^* = H^*/K_m; \quad x = X/e$$
$$t' = tD_s/e^2 \quad \text{and} \quad \sigma = V_m e^2/K_m D_s \quad (20)$$

e being the membrane thickness, D_s the diffusion coefficient of S and σ the diffusion-reaction parameter [4].

Initial concentration profiles and boundary conditions being known, evolutions of profiles with respect to time can be calculated step by step from eqs. 18 and 19.

The enzyme activity of the membrane is characterized by two expressions:

(a) the global potential enzyme activity $V(E)^t$:

$$V(E)^t = \int_0^1 \sigma\gamma_x^t \mathrm{d}x = \int_0^1 v(E)_x^t \mathrm{d}x \quad (21)$$

with its local equivalent, $v(E)_x^t$, being related to the pH profile inside the membrane and giving information about the distribution of enzyme activities, especially in multienzyme systems:

(b) the global effective enzyme activity:

$$V(\mathcal{E})^t = \int_0^1 \sigma\gamma_x^t\lambda_x \mathrm{d}x = \int_0^1 v(\mathcal{E})_x^t \mathrm{d}x \quad (22)$$

takes into account both the pH and substrate concentration levels, and corresponds to the real enzyme activity of the membrane (the local equivalent is $v(\mathcal{E})_x^t$).

In the steady state, this effective enzyme activity is proportional to the sum of the boundary fluxes:

$$V(\mathcal{E})^{t=\infty} = k\left(J_{x=1}^{t=\infty} - J_{x=0}^{t=\infty}\right)$$
$$= -kD_s\left[\left(\frac{\Delta S}{\Delta x}\right)_{x=1} - \left(\frac{\Delta S}{\Delta x}\right)_{x=0}\right] \quad (23)$$

All the calculations were made on the IBM 168-370 computer of the CIRCE at Orsay, and the program language was Fortran IV.

3. Results

Results are given by classifying systems according to whether or not they are affected by proton back-actions. Symmetry of the system is also taken into account. Moreover, the systems considered have only one steady state, independent of the initial state; since shorter computer times are required starting with an initially full instead of a void membrane, we decided to consider membranes initially full of substrate.

3.1. Enzyme systems without proton back-action

Such systems corresponding to reactions that do not produce (or consume) protons or to strongly

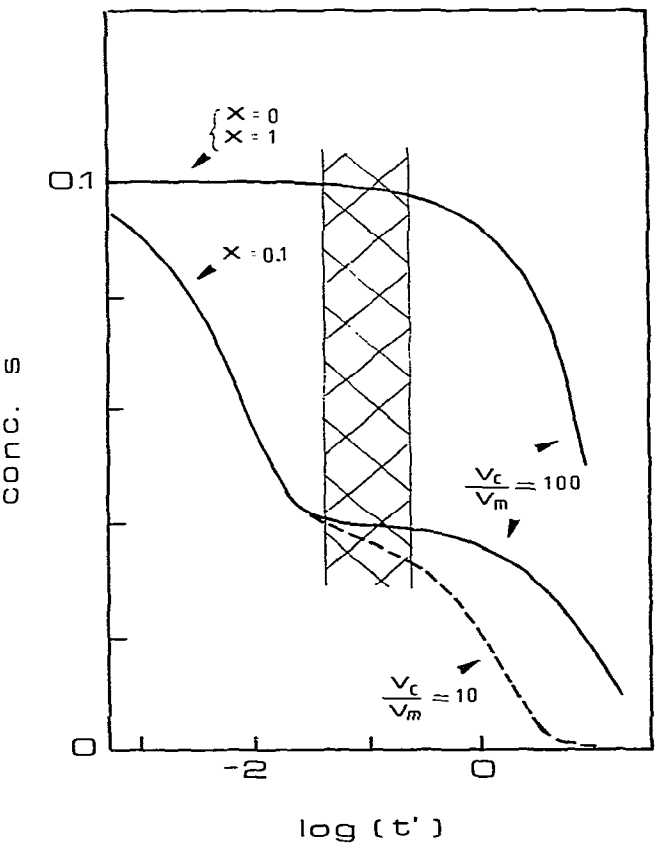

Fig. 1. Kinetics of the dimensionless substrate concentration s in different planes $x = 0$, $x = 0.1$ and $x = 1$ of the membrane. Zone A corresponds to the existence of successive quasi-steady states. This zone exists only when the volume ratio v_c/v_m is sufficiently large ($v_c/v_m > 100$).

buffered membrane and solutions may serve as *reference systems* to which the deviation due to proton back-actions can be compared.

3.1.1. Symmetrical systems

The local potential activity $v(E)'_x$ is independent of both the space (symmetry) and time (no back-action) coordinates. If the substrate concentration is high (zero-order reaction), this is also true for the local effective activity $v(\mathcal{E})'_x$. If not, $v(\mathcal{E})'_x$ becomes space and time dependent. Such systems have been extensively studied [1,26,27] and only two remarks should be made in analogy with two-enzyme systems [20]:

(a) First, even with moving boundaries, successive quasi-steady states exist only as long as the ratio of compartment and membrane volumes v_c/v_m remains large enough (fig. 1).

(b) Even in this simplest system, the coupling of diffusion and Michaelis-type reaction does not lead to a global Michaelis-type kinetics [31]; the deviation increases with the increasing σ value (fig. 2).

3.1.2. Asymmetrical systems

When asymmetry is imposed on the membrane by using a transmembrane pH difference, the potential activity $v(E)'_x$ and the effective activity $v(\mathcal{E})'_x$ become functions of the space coordinate. When a nonzero order of reaction is considered, $v(\mathcal{E})'_x$ also becomes a function of time. If σ is large enough, i.e., the reaction turnover V_m/K_m is larger

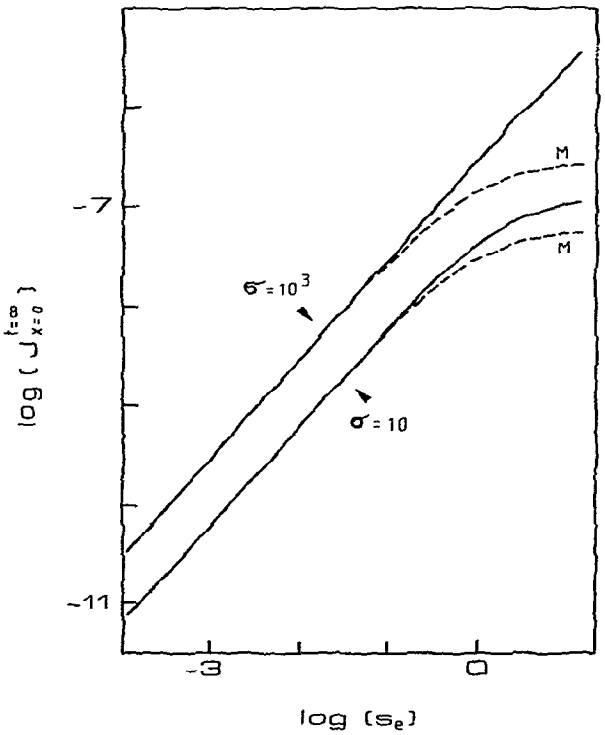

Fig. 2. NonMichaelian behavior of a membrane containing a Michaelis-Menten-type enzyme: The product fluxes of the enzyme membrane (———) and of a fictive Michaelis-type system (- - - - -) are compared. The deviation increases with the parameter σ.

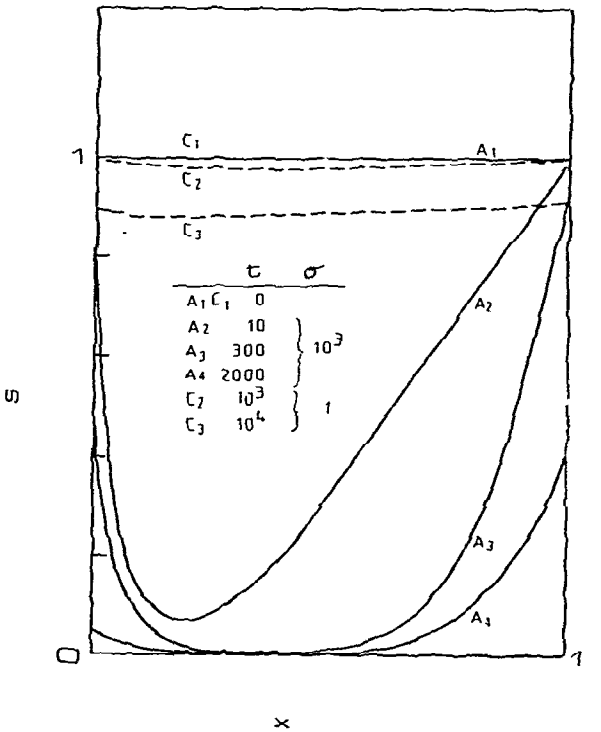

Fig. 3. The composition asymmetry imposed by a transmembrane pH gradient is transmitted to the system only if $V(\mathcal{E})\xi > 1$ (curves A). When $V(\mathcal{E})\xi < 1$, the system is governed by diffusion and remains symmetrical (curves C).

than the transport rate D_s/e^2, this asymmetry affects the concentration profiles. The rate is controlled by diffusion and the shape of concentration profiles, imposed by the reaction, is asymmetrical (fig. 3).

The main interest of such asymmetrical systems resides in functional structures: starting with a homogeneous enzyme composition, asymmetrical distribution of enzyme activity may be induced by a transmembrane pH difference [7–9].

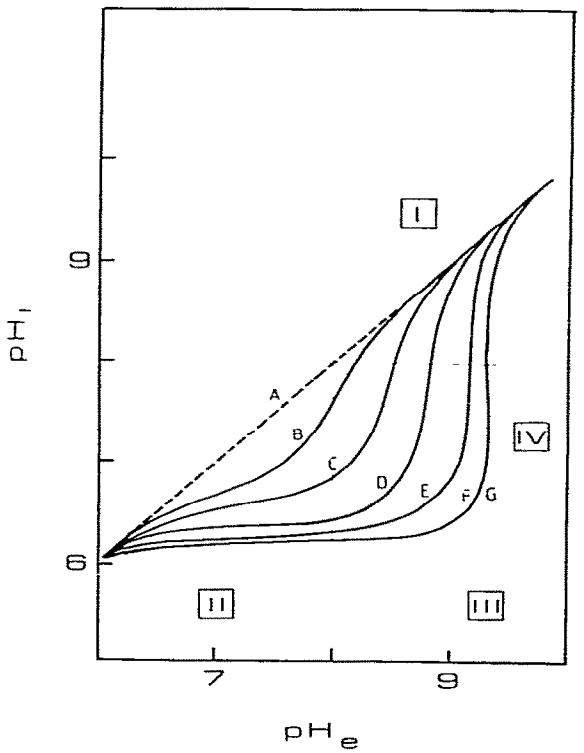

Fig. 4. Mid-plane pH, plotted against external pH_e for different substrate concentrations. In zone I, γ has a maximum and in zone IV, γ has two symmetrical maxima, but in the two zones pH_i is lower than pH_e and remains near the pH' value (pH' = 8): so, the system is activated. In zone II, pH_i deviates from pH' and the system is inhibited. In zone III, detailed calculations are needed. Results are given for $\sigma = 10$ and the dimensionless substrate concentration values are: 0 (A), 0.03 (B), 0.1 (C), 0.3 (D), 1 (E), 3 (F) and 30 (G).

3.2. Enzyme systems with strong acid back-actions

If the pH in the system is neither strongly buffered nor strongly acid or alkaline, both the $v(E)^t_x$ and $v(\mathcal{E})^t_x$ activities become space and time dependent. From this feedback action, curvilinear pH profiles result and activations or inhibitions may appear; the maximum activity of the system may correspond to a pH different from the intrinsic optimum pH and an apparent pH dependence different from the intrinsic one may result.

3.2.1. pH profile

Even if the pH at the boundaries is monitored and constant, the H^+ concentration profile shows a curvature and γ varies as a function of the space coordinate. Modifications of the local pH depend,

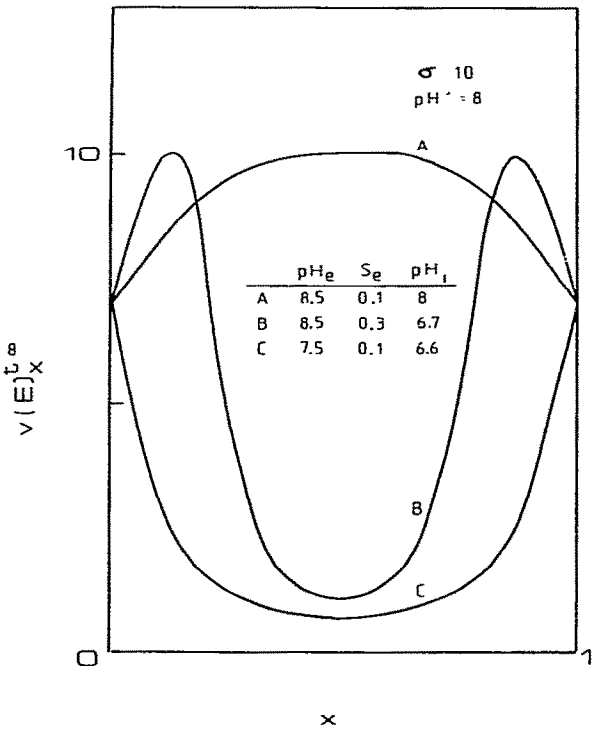

Fig. 5. Potential activity profiles corresponding to three groups shown in fig. 4: activation corresponding to zone I (A); activation corresponding to zone III (B) and inhibition corresponding to zone II (C).

first of all, on the ratio of reaction and diffusion rates of protons, i.e., $v(\mathscr{E})'_x$, but also on the local pH value, which can be taken into account in a phenomenological acidity factor ξ:

$$\xi = 10^{-|pH-7|}$$

which gives an account of the concentration of the ions in water, H^+ and OH^-, and is maximum when the sum of their concentrations is minimum, i.e., when a given amount of proton has a maximum effect on the modification of the pH value.

When $|v(\mathscr{E})\xi|'_x \ll 1$, the pH profile, scarcely affected by the reaction, is similar to that without back-action. When $|v(\mathscr{E})\xi|'_x > 1$, the local pH is modified (fig. 4), but due to the logarithmic pH scale, the pH profile is flatter than the substrate concentration profile; so, the largest pH variations are mainly located near the boundaries of the membrane. Relations between the external pH, pH_e, and the mid-plane pH, pH_i, are shown in Fig. 4; numbered zones define the activation of the system.

3.2.2. Membrane activity

Such pH profiles correspond to typical activity profiles. The relative values of the optimum pH of the enzyme, pH', and pH_e may define three types of back actions: one for $pH' > pH_e$ and two more for $pH' \leqslant pH_e$ (fig. 5).

When $pH' > pH_e$, the steady-state potential activity profile $\sigma\gamma(x)$ always has a minimum. As $\lambda(x)$ always decreases from boundaries to the mid-plane of the membrane, the effective activity profile $[v(\mathscr{E})](x)$ also shows a depression. The greater the σ value, the deeper the depression. A a consequence, the enzyme membrane is more or less inhibited (fig. 5C).

When $pH' \leqslant pH_e$, pH_i overshoots or does not reach pH' according to whether the initial $V(\mathscr{E})$ value is high or low (zones I and III–IV in fig. 4). In the latter case (zone I), $\gamma(x)$ has a single maximum and $[v(\mathscr{E})](x)$ resulting from the combination of $\gamma(x)$ and $\lambda(x)$ can present either a maximum or a minimum: At high substrate concentration, $\lambda(x)$ remains constant and the $\gamma(x)$ profile prevails over the $\lambda(x)$ profile; so, the membrane is activated (fig. 5A). When the substrate concentration decreases, the $\lambda(x)$ profile becomes very depressed and prevails of the $\gamma(x)$ profile and the membrane is inhibited. However, when pH_i overshoots pH' (zones III and IV), the $pH(x)$ profile intersects pH' in two different and symmetrical planes of the membrane and the $\gamma(x)$ profile has two maxima (fig. 5B). At high initial $v(\mathscr{E})$ value, the two maxima are located near the boundaries and pH_i is much smaller than pH' (zone III); the central layer of the membrane may, in this way, degenerate into a pure diffusion layer. Only detailed examination can decide whether activation or inhibition of the membrane results.

3.2.3. Potential pH dependence of the membrane

First, the following points may be mentioned:

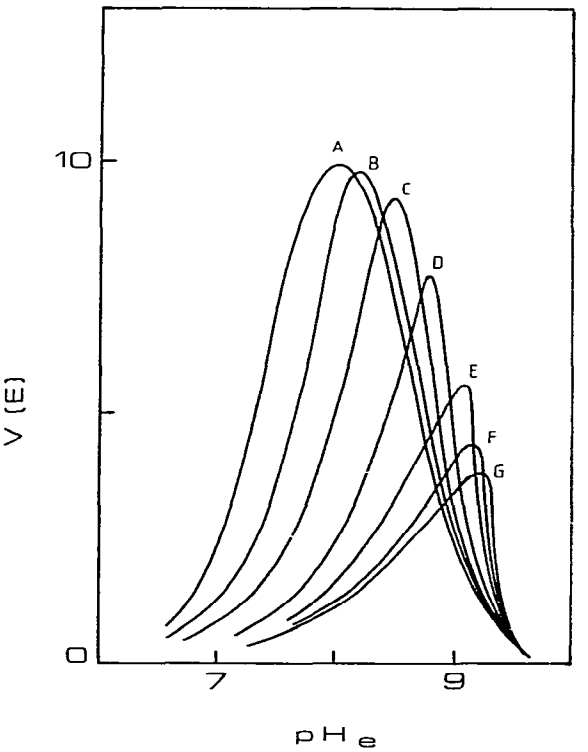

Fig. 6. Steady-state potential pH dependence $V(E) = f(pH_e)$ as a function of the substrate concentration s (same values as in fig. 4). When enzyme activity increases (via λ value) both the deviation between pH' and $pH'(E)$, and the curvature of the pH profile increase, while the maximum activity decreases.

(a) Due to proton production, when $pH_e = pH'$, pH_i is always lower than pH′ and the global activity is lower than that obtained with a pH value equal to pH′. On the other hand, if, for a given $pH_e > pH'$, pH_i is comparable to pH′, the membrane activity is almost maximum and so, the potential optimum pH, $pH'(E)$ is shifted toward higher values. (b) Even under optimal conditions, $pH(x)$ is never uniformally equal to pH′ and the membrane activity is always lower than the activity at pH′ of the same quantity of enzyme in solution, i.e. $\int_0^1 \gamma(x)dx < 1$.

Now, the potential pH dependence of the membrane, i.e. the potential membrane activity $V(E)$ as a function of pH_e, can be drawn. The shift of the optimal pH, $pH'(E)$, linked to modifications of the intramembrane pH, increases with σ and λ (section 3.2.1). For a given enzyme membrane (σ constant) and an increasing λ value, the shift of $pH'(E)$ increases and the maximum potential activity ($V(E)$ at $pH = pH'(E)$) decreases (fig. 6). As a consequence, for a given pH_e value, $V(E)$ has a maximum as a function of S_e.

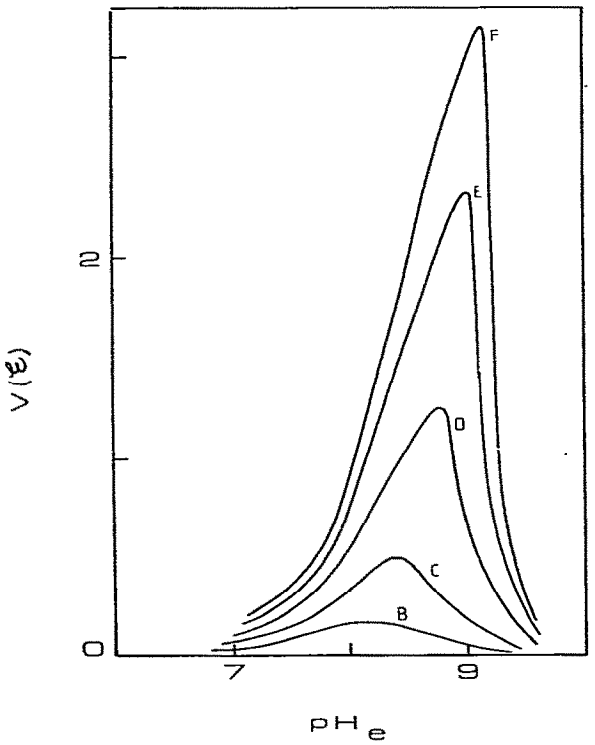

Fig. 7. Steady-state effective pH dependence $V(\mathscr{E}) = f(pH_e)$ as a function of the substrate concentration s (same values as in fig. 4). The effective and potential pH-dependences are not homothetic (see fig. 6) and the optimal $pH'(\mathscr{E})$ is different from $pH'(E)$. The real pH dependence of the membrane is a function of the substrate level, the membrane parameter σ and, of course, the intrinsic γ function.

3.2.4. *Effective pH dependence of the membrane*

The real membrane activity is characterized by

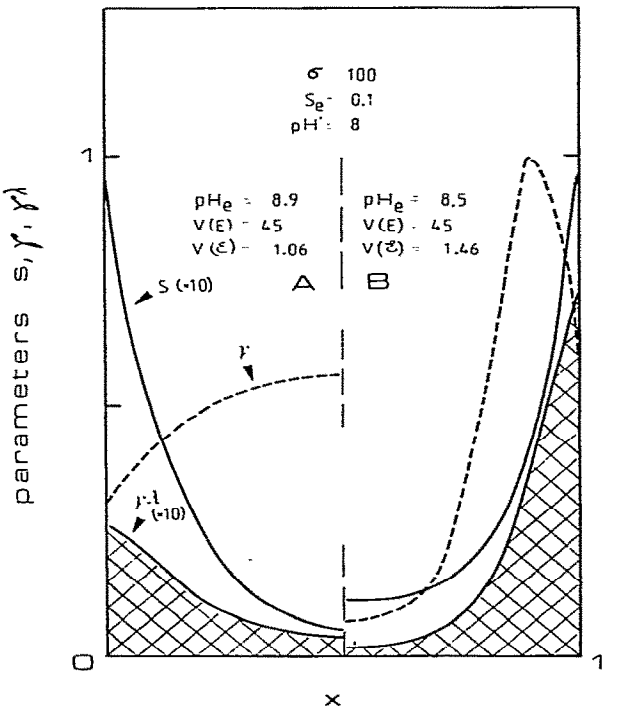

Fig. 8. The real membrane activity is a function of the λ and γ profiles. Due to the difference between $pH'(\mathscr{E})$ and $pH'(E)$, to one $V(E)$ value may correspond two different $V(\mathscr{E})$ values. One is characterized by a two-maximum γ function (B) and the second by a single-maximum γ function (A) depending on whether or not $pH(x)$ crosses pH′; the highest $V(\mathscr{E})$ value is obtained when the maxima of $\lambda(x)$ and $\gamma(x)$ correspond to each other, i.e., $\gamma(x)$ has two maxima located near the membrane boundaries (case B; zone IV in fig. 4). This is clearly shown by the global effective activity represented by the cross-hatched area.

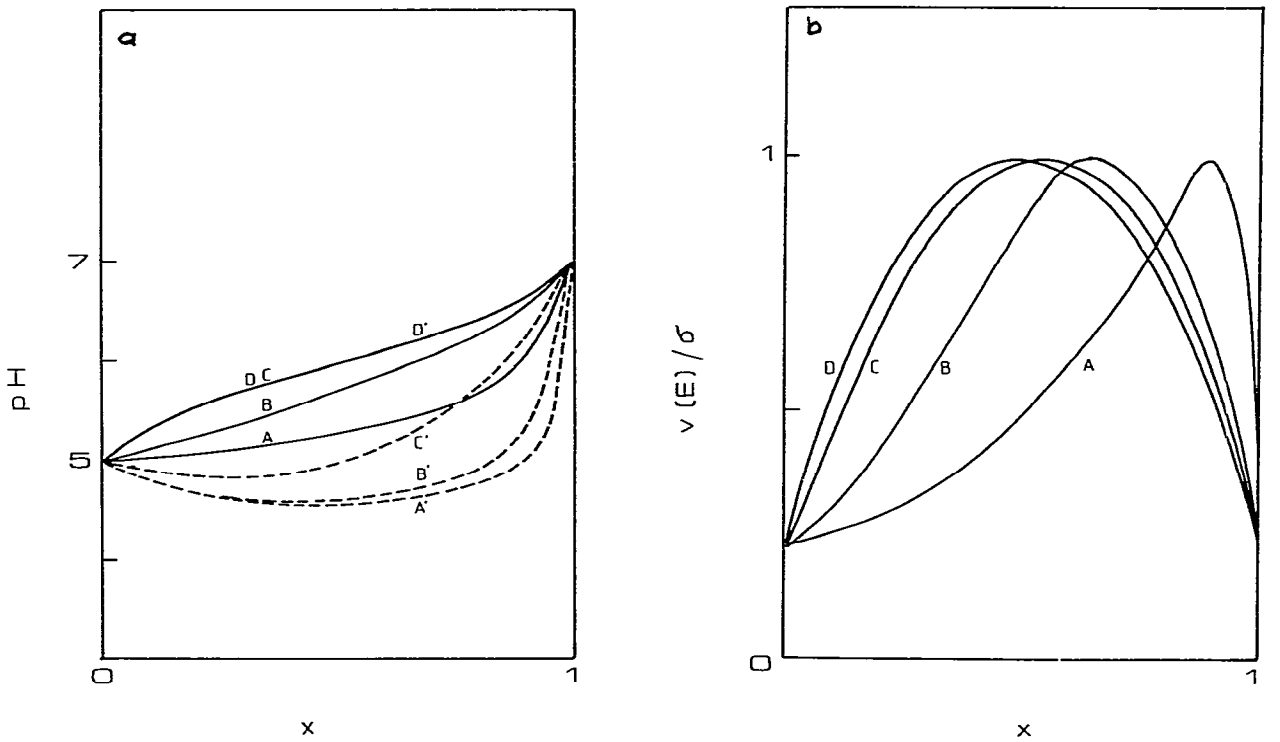

Fig. 9. Effect of a nonreactive pH buffer BH on enzyme membrane activity: (a) The pH profile follows the titration curve of the buffer BH; if $pK_{bh} = 1/2\ (pH_{e^1} + pH_{e^2})$, the linearity of pH profiles increases with the $\mathscr{B}$ concentration. Conditions are: zero-order reaction, $\sigma = 10$, $pH' = 6$ and $\mathscr{B} = 10^{-3}$ (A), 0.1 (B), 1 (C) and 100 (D). The figure shows the initial pH profiles (———) and the steady-state pH profiles (- - - - -) in every case. (b) A symmetrical activity profile $\gamma(x)$ is obtained when $pK_{bh} = pH'$ (same conditions as in panel a).

$V(\mathscr{E})$. In solution, the potential and effective pH dependences are homothetic and both are centered on the intrinsic optimal pH; pH'. This is different in membrane systems (figs. 6 and 7) where the potential optimal pH, $pH'(E)$, and the effective one, $pH'(\mathscr{E})$, can be distinguished. Nevertheless, $pH'(\mathscr{E})$ is always situated between pH' and $pH'(E)$.

Moreover, when $pH_e > pH'$, $V(E)$ is maximum for a pH_i value close to pH'; two equal $V(E)$ values may thus be obtained, corresponding to $pH_i < pH'$ for the first and to $pH_i > pH'$ for the second. However, they correspond to two totally different $V(\mathscr{E})$ values (even at the same S_e value), the highest one being obtained when the maxima of $\gamma(x)$ and $\lambda(x)$ correspond to each other, i.e., when $pH_i < pH'$ (fig. 8).

3.3. Enzyme systems with weak acid back-actions

Now, we will consider conditions when weak acids are involved: either a nonreactive pH buffer, BH, is added to the medium or the acid produced, AH, is weak.

3.3.1. pH-buffered solutions

In a symmetrical configuration, the kinetic behavior of buffered systems in which a strong acid H^+ is produced is intermediary between strong acid production in an unbuffered medium (pH_e

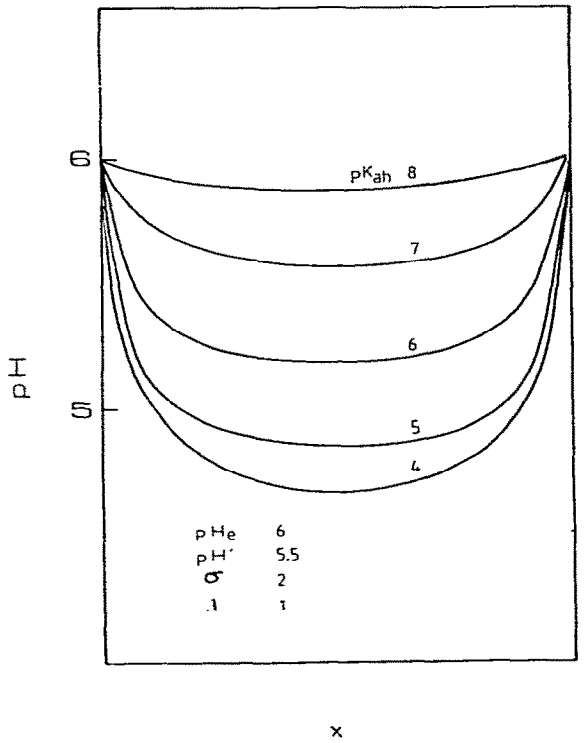

Fig. 10. Effect of the weak acid AH produced by the reaction: pH profiles as a function of pK_{ah} values are shown. The plateau type of the pH profile appears when the intramembrane pH is close to pK_{ah}.

very different from pK_{bh} or $\mathscr{B} \cong 0$) (see section 3.2) and a system without any back-action ($pH_e \simeq pH_{bh}$ and high $\mathscr{B}$ value) (section 3.1). That means that the time evolution of the intramembrane pH is slow and the difference $pH_i - pH_e$ remains small.

The most interesting case is given by an asymmetrical system where the buffering acid can modify the shape of the initial pH profile. Calculations show that the pH profile follows the titration curve of the weak acid BH (eq. 14); for instance, if $pK_{bh} = (pH_1 + pH_2)/2 = pH'$, high buffer concentrations tend to linearize the initial pH profile and to render the activity profile symmetrical (fig. 9), increasing the thickness of the active layer of the membrane.

3.3.2. Weak acid AH produced by the reaction

The behavior of the system depends on the pH value, namely, if $pH_e > pK_{ah} + 2$, AH acts as a strong acid, but if $pH_e < pK_{ah} - 2$, AH has no pH effect and so pH profiles remain flat. In the intermediary pH_e range, the reaction increases the buffering power of the solution with time and pH_i decreases without being able to go below the $pK_{ah} - 2$ value. A typical pH profile may be observed (fig. 10). Such profiles have been obtained with the hydrolysis of urea by using urease [32].

4. Conclusions

We have shown that back-actions by proton production in immobilized enzyme systems can be quantified by the potential enzyme activity $V(E)$ which may characterize functional structures and by the effective enzyme activity $V(\mathscr{E})$ which is directly responsible for substrate and product fluxes. When the initial $V(\mathscr{E})$ value, corrected by the acidity factor ξ, is smaller than unity, pH back-actions have no effect on the behavior of the system; the existence of quasi-steady states and nonMichaelian behavior of a Michaelis-Menten-type immobilized enzyme were shown, in agreement with studies on two-enzyme models [17,31]. When $V(\mathscr{E}) \gg 1$, pH profiles are modified by the reaction and activations or inhibitions result (table 1). Contrary to enzymes in solution, the potential and effective pH dependences are not homothetic and the optimum pH is shifted toward higher values with proton-producing reactions. The apparent optimal pH depends on the intrinsic pH dependence, substrate levels and membrane characteristics grouped in the diffusion-reaction parameter σ.

Weak acid effects were also included in the treatment thanks to a symbolic species H^*, which is a function of the free proton concentration only; naturally weak acids are found to behave in the same way as strong acids but with lesser effects.

All the results may easily be transposed to base-producing reactions by interchanging H^+ and OH^-. This treatment may also be transposed to other types of effector such as bivalent cations (Mg^{2+} for hexokinase, Ca^{2+}...) which may react

Table 1

Back-actions of an acid-producing enzyme reaction on the activity of the membrane, with respect to a nonpH-active reaction

Initial values of			Profiles of		Results (see numbered zones in fig. 4)
$\int_0^1 \xi V(\xi)\mathrm{d}x$	pH_e	s_e	$\lambda(x)$	$\gamma(x)$	
<1	$\forall\, pH_e$	$\forall\, s_e$	1 minimum	none	no modification
>1	$pH_e \leq pH^*$	$\forall\, s_e$	1 minimum	1 minimum	inhibition (zone II)
		>1	0 minimum	2 maxima + 1 minimum	inhibition (zone III)
	$pH_e > pH^*$	<1	1 minimum	2 maxima + 1 minimum	activation (zone IV)
		$\ll 1$	1 minimum	1 maximum	activation (zone I)

with any complexing agents added in the medium.

Relations between imposed boundary asymmetries and functional asymmetries are also examined. It is clear that this paper is mostly concerned with pH asymmetries, i.e., γ gradients; but results would be similar if other asymmetries which concern the reaction term $\sigma\gamma\lambda$ were considered: Asymmetry in substrate concentration (λ gradient) or in cofactor concentration or asymmetry in enzyme distribution (σ gradient), as described in ref. 33, may also produce asymmetrical fluxes of substrate and product. Nevertheless, pH effects are more complex and more interesting because the reaction itself may increase or decrease local pH. The corresponding results are of fundamental importance in predicting self-modifications of pH profiles in multienzyme systems and will be very useful in showing how an active transport of proton can be self-stabilized [9] and become a dissipative structure due to autocatalytic mechanisms.

This study also constitutes a basic work for diffusion-electromigration-reaction studies in which we are engaged [28,29].

The H^* species may be inserted in the Nernst-Planck equation in order to examine modifications of proton profiles and of activity profiles in an enzyme membrane placed in an electric field.

This study has also a few other implications: It shows that optimum pH has no meaning in heterogeneous enzyme catalysis if all the conditions are not defined (substrate concentration, membrane parameter σ, γ function). This may also be very useful in interpreting results obtained with specific enzyme electrodes based on pH measurements.

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