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MONITORING OF ARTIFICIAL ENZYME MEMBRANE SYSTEMS BY ELECTRIC CURRENTS

I. ANALYTICAL TREATMENT WITH DIRECT CURRENT AND BUFFERED CONDUCTIVITY

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Continuous electric fields (E) modify the transport flows and the intramembrane concentration profiles of protons or of ionic substrates or cofactors (inhibitors). These 'mediators' induce variations in enzyme activity, quantifiable by a generalized Damköhler group II ψ distinguishing electrotransport reactions from diffusion reactions. For three typical reaction schemas, using only one mediator, the steady-state equations have been established. Depending on boundary conditions, the direction of electric current (for asymmetrical systems) and the value of ψ, activations, inhibitions or activations followed by inactivations have been found. With buffered conductivity (supporting electrolyte), the limiting concentration profiles ($E \to \infty$) are uniformly equal to the boundary values; i.e., diffusion constraints are suppressed and the regime is controlled by the reaction. The calculations give the relative activity variations for partially suppressed transport controls.

1. Introduction

Enzyme technology has been considerably improved these last few years, particularly as far as immobilized enzymes are concerned. Meanwhile, new studies have provided a basis for the enzymic diffusion-reaction properties, especially concerning the phenomena of oscillations, hysteresis, chemical wave propagation and self-organization [1–3], as well as active transport models with functional or permanent structures [4–6].

Few works have taken into account the contribution of an electric field in these systems. Early results are essentially concerned with surface systems, i.e., systems for which the enzyme is fixed on an impermeable support; they allow the accumulation of a substrate, e.g., near the reactive surface [7–9]. The membrane systems (systems in which the enzyme is distributed homogeneously inside a matrix) have been considered only experimentally [10–13] and the results obtained are fragmentary. It was thought that a theoretical approach to the problem would be useful to determine the parameters, thus allowing better experimentation.

We are interested here in the modification of transport reactions by the action of an electric current on the transport of ionic species. The effects on the conformation of the enzyme protein or on the microenvironment of the active site are not taken into account; this is justified by the low values of imposed electric fields. We use profile analysis showing that the intramembrane diffusion-reaction concentration profiles of the chemical species are deformed by the action of electric currents when these species are charged; the enzyme activity may depend on the concentration of such species able to act in this case as 'mediators' between the activity and the field. To quantify these deformations we introduce the diffusion-electromigration-reaction parameter ψ (or transport-reaction parameter) [14], using the same approach that was used to define the enzyme diffusion-reaction parameter σ [4]. Finally, we intend to limit ourselves here to systems and conditions such that only one steady state exists.

2. Definition of the model

The structure of the model is symmetrical. The system is made up of a plane matrix of macroscopic thickness, chemically and electrically inert, but permeable to chemical species (fig. 1). The enzyme is distributed inside, homogeneously, and is immobilized. The medium is assumed to be isotropic and the enzyme protein not to contribute significantly to the local electric potential. The model is unidimensional (the transversal dimensions are much bigger than thickness l); moreover, the imposed electric current is perpendicular to the plane of the membrane. These two points enable us to consider the vectorial phenomena as being dependent only on the direction $X'X$, perpendicular to the plane of the membrane. Due to the presence of a high concentration of electrolyte which buffers the conductivity, the electric field E may be considered constant.

First, in order to simplify the treatment of the model, the presence of diffusion layers as well as the polarization phenomena on the boundaries are neglected; thus, we assume that the thickness of the membrane is large enough and the current intensity sufficiently small.

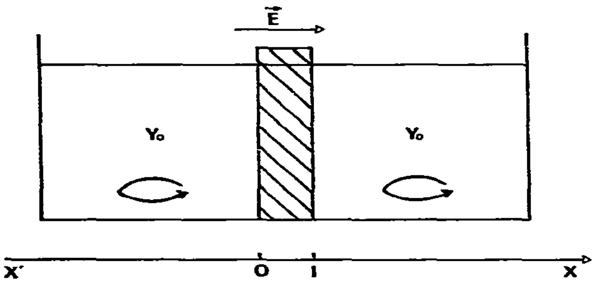

Fig. 1. Structure of the membrane model.

2.2. Formulation of the activity

2.2.1. Enzyme activity

The following notation was adopted for the enzyme reaction rate [14,15]:

$$v = V_m \gamma \lambda \delta \tag{1}$$

where V_m is the maximum rate and γ, λ and δ the mathematical expressions of the pH, substrate concentration and cofactor concentration dependences, respectively; with an inhibitor, δ is replaced by δ'. γ, λ, δ and δ' may vary from 0 to 1. The rational activity A is defined as:

$$A = v/V_m = \gamma\lambda\delta \tag{2}$$

The terms γ, λ, δ and δ' are usually [16] characterized by eqs. 3–6.

$$\gamma = \frac{K_a H}{(H+K_a)(H+K_b)} \text{ or } \gamma = \exp\left[-\beta(\text{pH}-\text{pH}')^2\right] \tag{3}$$

$$\lambda = \frac{S}{K_m + S} \text{ (Michaelian law)} \tag{4}$$

$$\delta = \frac{M}{K_c + M} \tag{5}$$

$$\delta' = \frac{K_i}{K_i + I} \tag{6}$$

where H, S, M and I are the concentrations of H^+, substrate, cofactor or inhibitor, respectively; K_a, K_b, K_c and K_i kinetic constants; K_m the Michaelis constant; pH′ the pH corresponding to the optimal pH of the enzyme and β a phenomenological coefficient characterizing the shape of the pH dependence.

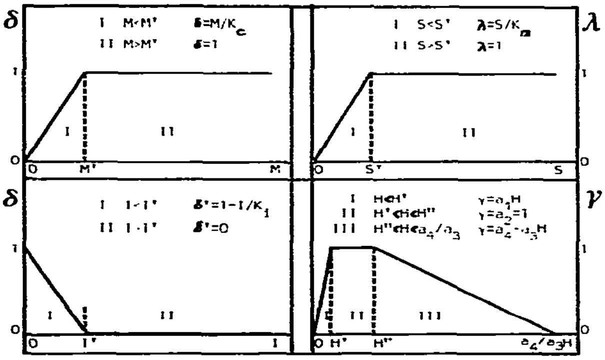

Fig. 2. Linearized forms of dependences of the enzyme activity: (top left) cofactor, (bottom left) inhibitor, (top right) substrate, (bottom right) pH.

Analytical solutions of the equations governing the piecewise system exist if γ, λ, δ and δ' are linearized piecewise (fig. 2).

To each domain of one of these functions (δ, δ', λ or γ) corresponds a specific solution.

All the results presented here were obtained with piecewise linearized functions and using only those domains where the activity varies (increases or decreases) as a function of the concentration of the mediator considered (a condition necessary to obtain activity modulations by electric fields).

It is convenient to introduce dimensionless variables to bring out the characteristic parameters:

$$x = X/l \tag{7}$$

where X is the dimensional abcissa, x the dimensionless one. K_m is used to rationalize the concentrations:

$$c_i = C_i/K_m \tag{8}$$

where C_i is the concentration of any charged or uncharged chemical species i.

2.2.2. *Rational membrane activity*

In order to characterize the enzyme activity of the membrane, we define the relative membrane activity $\bar{A}$ which integrates the local enzyme activity A throughout the thickness of the membrane, divided by the enzyme activity on the boundaries of the membrane (subscript o):

$$\bar{A} = \frac{\int_0^1 \gamma(x)\,\lambda(x)\,\delta(x)\,\mathrm{d}x}{\gamma_o \lambda_o \delta_o} \tag{9}$$

2.2.3. *Reaction schemas*

In order to render the analysis systematic, three typical reaction schemas (I–III) as simple as possible, characterized by one of the functions δ, λ or γ only, are considered:

$$\mathrm{S} \xrightarrow[\text{(I)}]{\mathrm{M}^+} \mathrm{P} \tag{I}$$

The cofactor (or the inhibitor) is the only variable affecting the activity. The monitoring of the activity results from the action of the electric field on δ (or δ'). λ and γ are assumed to be equal to 1 (zero order for the substrate and no production or consumption of H^+ by the enzyme reaction).

$$\mathrm{S}^- \rightarrow \mathrm{P}^- \tag{II}$$

The ionic substrate is the only mediator of activity control and leads to the action of the electric field on λ. The parameters δ and γ are assumed to be equal to 1 (cofactor not necessary or in excess, and no production or consumption of H^+ by the enzyme reaction).

$$\mathrm{S} \rightarrow \mathrm{P}^- + \mathrm{H}^+ \tag{III}$$

H^+ being the only mediator affecting the activity, the control will involve the action of the electric field on γ. Parameters δ and λ are assumed to be equal to 1. This schema corresponds to H^+ production. H^+ consuming reactions will not be considered as they can be deduced from the results presented here.

The use of these schemas requires further assumptions:

The product P is assumed to have no influence on the enzyme activity,

For reaction schema I, the nonlinear cofactor (or inhibitor) profiles are evaluated with asymmetrical boundary conditions.

For reaction schema III, the linearization of the differential equation governing the system limits the treatment to low values of the pH ($pH < 6$) (OH^- concentration neglected).

3. Mathematical treatment

3.1. *Setting the equations*

Analytical solutions may be found in the steady states. They are determined by the expression of the mass balance for each species i (R, reaction; T, transport):

$$\left(\frac{\partial C_i}{\partial t}\right)_R + \left(\frac{\partial C_i}{\partial t}\right)_T = 0 \tag{10}$$

The transport term encompasses diffusion and electromigration. The reactive term is expressed by:

$$\left(\frac{\partial C_i}{\partial t}\right)_R = \nu_i v \tag{11}$$

where ν_i is a stoichiometric coefficient.

The transport term $(\partial C_i/\partial t)_T$ is obtained from the Nernst-Planck relation:

$$J_i = -D_i \frac{\partial C_i}{\partial X} + D_i \frac{z_i F}{RT} C_i E \tag{12}$$

Using Fick's second Law, we have:

$$\left(\frac{\partial C_i}{\partial t}\right)_T = -D_i\frac{\partial^2 C_i}{\partial X^2} - D_i\frac{z_i F}{RT}E\frac{\partial C_i}{\partial X} \tag{13}$$

assuming that the diffusion coefficients are independent of concentration.

Combining eqs. 10, 11 and 13 leads to the general steady-state equation:

$$D_i\frac{d^2C_i}{dX^2} - D_i z_i\frac{FE}{RT}\frac{dC_i}{dX} + \nu_i v = 0 \tag{14}$$

or in dimensionless form:

$$\frac{d^2c_i}{dx^2} - z_i\frac{FEl}{RT}\frac{dc_i}{dx} + \frac{V_m l^2}{K_m D_i}\nu_i\gamma\lambda\delta = 0 \tag{15}$$

By introducing the dimensionless diffusion-reaction parameter $\sigma = V_m l^2/K_m D_s$ and the electrical one $\zeta = FEl/RT$, eq. 14 becomes:

$$\frac{d^2c_i}{dx^2} - z_i\zeta\frac{dc_i}{dx} + D_s/D_i\nu_i\sigma\gamma\lambda\delta = 0 \tag{16}$$

This is the fundamental equation leading to the intramembrane profile of concentration of each chemical species. When the treatment of reaction schema I is considered, the only equation to be taken into account is the one concerning M^+ or I^+; e.g., for M^+:

$$\frac{d^2m}{dx^2} - z_M\zeta\frac{dm}{dx} = 0 \tag{17}$$

For reaction schema II the following equation is to be solved:

$$\frac{d^2s}{dx^2} - z_S\zeta\frac{ds}{dx} - \sigma\lambda = 0 \tag{18}$$

and finally, for reaction schema III:

$$\frac{d^2h}{dx^2} - \zeta\frac{dh}{dx} + D_S/D_H\sigma\gamma = 0 \tag{19}$$

3.2. Transport-reaction parameter ψ

A transport-reaction parameter was defined allowing a quantitative prediction of the evolution of the system. To define ψ, it was necessary to solve eq. 18 in order to express $s(x)$. The calculations show that the slope of the s profile near its entering face inside the membrane is proportional to the term ψ:

$$\psi = \frac{\sigma}{1 + |z_S\zeta|/2} \tag{20}$$

This expression defines the transport-reaction parameter enabling the evaluation of the distortion of the profiles when an electric field is applied, and guarantees the continuity between the diffusion-reaction and the transport-reaction fields as for $\zeta = 0$, $\psi = \sigma$ (table 1).

4. Results

Theoretical results concerning reaction schemas I–III include, on the one hand, the concentration profiles in cofactor (or inhibitor) and substrate, and the pH profiles, and on the other, the variations of the membrane activity $\bar{A}$ as a function of E (or ζ).

Table 1

Different types of control of the system depending on diffusion-reaction, electric and diffusion-electromigration-reaction parameters

Parameter			Type of control
Diffusion-reaction	Electric	Transport-reaction	
$\sigma \ll 1$		$\psi \ll 1$	No constraint of diffusion control by the reaction
$\sigma \gg 1$	$\zeta = 0$ (or $\zeta \ll 1$)	$\psi \gg 1$	Diffusion reaction
	$\zeta \simeq 1$	ψ	Diffusion-electromigration reaction
	$\zeta \gg \sigma$	$\psi \ll 1$	Electromigration control by the reaction

4.1. Cofactor (δ) mediated electric regulations

4.1.1. Theoretical results

Solving eq. 17 gives the profiles of m (or i):

$$m=\frac{(m_2-m_1)\exp(z_{\mathrm{M}}\zeta x)+m_1\exp(z_{\mathrm{M}}\zeta)-m_2}{\exp(z_{\mathrm{M}}\zeta)-1} \tag{21}$$

where m_1 and m_2 are the values of m on the boundaries $x=0$ and $x=1$, respectively.

These profiles are represented in fig. 3; the linear diffusion profile of m is distorted by the action of the electric field giving a concavity depending on the direction of the electric field:

$$\zeta>0 \quad \frac{\mathrm{d}^2m}{\mathrm{d}x^2}<0$$

$$\zeta<0 \quad \frac{\mathrm{d}^2m}{\mathrm{d}x^2}>0$$

Applying a positive electric field causes a filling of the membrane with cofactor, while a negative electric field gives the opposite result.

With a cofactor the expression of the membrane activity as a function of ζ is the following:

$$\bar{A}=\frac{(m_2-m_1)(\exp(z_{\mathrm{M}}\zeta)-1)+(m_1\exp(z_{\mathrm{M}}\zeta)-m_2)\,z_{\mathrm{M}}\zeta}{\frac{m_1+m_2}{2}z_{\mathrm{M}}\zeta(\exp(z_{\mathrm{M}}\zeta)-1)} \tag{22}$$

The variations in $\bar{A}$ predicted by eq. 22 are illustrated in fig. 4a.

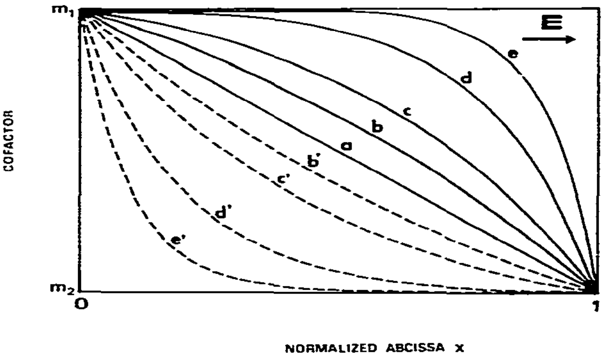

Fig. 3. Intramembrane profiles of cofactor concentration for different values of ζ:

	a	b	c	d	e	b'	c'	d'	e'
ζ:	0	1	2	5	10	−1	−2	−5	−10

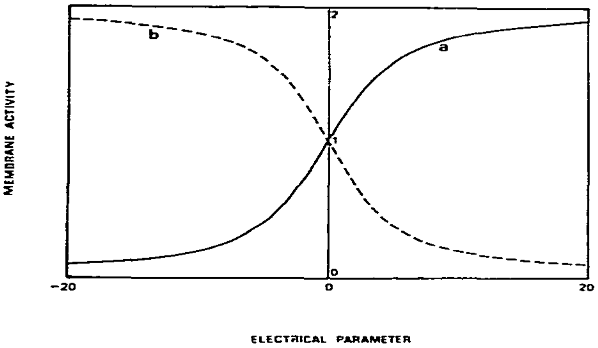

Fig. 4. $\bar{A}(\zeta)$ curves analytically obtained for reaction schema I. (a) Case of an activator; (b) case of an inhibitor.

With an inhibitor, the expression of the membrane activity becomes:

$$\bar{A}=\frac{1-\dfrac{K_{\mathrm{m}}}{K_{\mathrm{I}}}\dfrac{(i_2-i_1)(\exp(z_{\mathrm{I}}\zeta)-1)+(i_1\exp(z_{\mathrm{I}}\zeta)-i_2)\,z_{\mathrm{I}}\zeta}{z_{\mathrm{I}}\zeta(\exp(z_{\mathrm{I}}\zeta)-1)}}{1-\dfrac{K_{\mathrm{m}}}{K_{\mathrm{I}}}(i_1+i_2)/2} \tag{23}$$

These variations are reported in fig. 4b.

These results show that it is possible to regulate the enzyme activity of a membrane by the action of an electric field on a cofactor or an inhibitor; and, more especially, by choosing the appropriate direction of the electric field, the system can be activated either by accumulating the cofactor or by excluding the inhibitor.

4.1.2. Experimental illustration

The concentration profile of a cofactor ion inside the membrane was obtained with and without an applied electric field. The ion was labelled with ^{14}C; membranes were made up of agarose gels (3.5% weight) of 5 mm thickness.

When the steady state was reached, the membrane was cut parallel to its faces with a deep-freezing microtome, and the radioactivity of each slice was measured. The concentration profile of cofactor was obtained from the results (fig. 5).

The diffusion profile is approximately linear while the diffusion-electromigration profile deviates a great deal from this line. It may be cor-

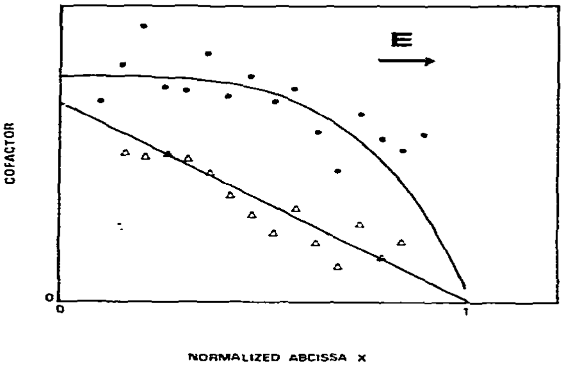

Fig. 5. Ionic profiles experimentally obtained (reaction schema I): (△) Diffusion; (●) diffusion-electromigration.

rectly fitted by a second-degree polynomial in agreement with a development in a series of the expression (eq. 21) of the theoretical profile of m.

4.2. *Substrate (λ) mediated electric regulation*

The substrate concentration profiles are obtained as solutions of eq. 18 with symmetric boundary conditions, $s(0) = s(1) = s_o$:

$$s = s_o \frac{(1-\exp(r_2))\exp(r_1 x) + (\exp(r_1)-1)\exp(r_2 x)}{\exp(r_1)-\exp(r_2)} \quad (24)$$

with $r_1, r_2 = \frac{-z_S\zeta \pm \sqrt{z_S^2\zeta^2 + 4\sigma}}{2}$

The evolution of s profiles with E (or ζ) is presented in fig. 6. Fig. 6 illustrates the role of parameter ψ which quantifies the profile distortions: different values of σ and ζ lead to profiles very alike when they correspond to the same value of ψ.

An increasing value of ψ leads to an increase in the slope of the s profile; at the same time, an increasing asymmetry is observed. The accumulation of S produces the activation of the system as shown through the variations of the membrane activity with E (or ζ):

$$\bar{A} = \frac{(\exp(r_1)-1)(\exp(r_2-1))}{\exp(r_1)-\exp(r_2)}\left(\frac{1}{r_2}-\frac{1}{r_1}\right) \quad (25)$$

where r_1 and r_2 are defined in the same way as in eq. 24. The variations in activity are shown in fig. 7 for different values of σ; for high electric fields, the different $\bar{A}(\zeta)$ curves tend toward the same limit, viz., 1. This is explained by the fact that the electric field tends to cancel the limitations by diffusion and to increase intramembrane S concentration to the level of the concentrations on the boundaries of the system.

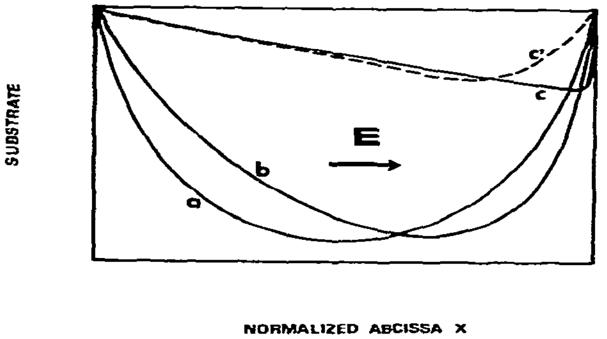

Fig. 6. Substrate concentration intramembrane profiles, obtained analytically, for different values of ζ, for reaction schema II:

	ζ	ψ	σ
a:	0	40	50
b:	1	33.3	50
c:	118.5	0.83	50
c':	10	0.83	5

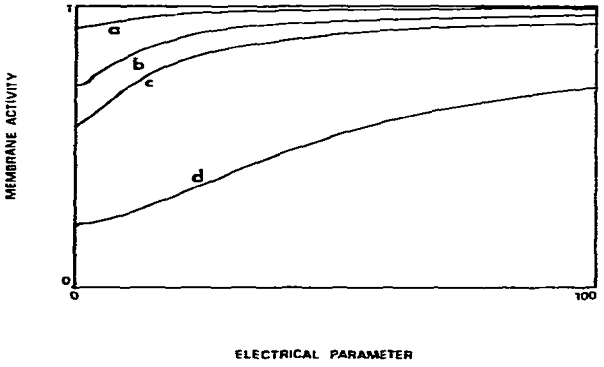

Fig. 7. $\bar{A}(\zeta)$ curves analytically obtained for reaction schema II for different values of σ. $\sigma = 1$ (a), 5 (b), 10 (c), 75 (d).

The possibility of activating the enzyme system by the action of an electric field on the substrate dependence λ is shown here.

4.3. pH (γ) mediated electric regulations

These are more complex owing to the fact that the pH dependence γ exhibits a maximum. An analytical solution, i.e., the expression of the concentration profile of H$^+$, can be obtained in each domain of the piecewise linearized shape of γ. The different types of solutions are given in table 2.

The solutions of domain I of the pH dependence are illustrated in fig. 8 which shows the slackening of the h profiles for increasing values of E.

The expressions of the membrane activity calculated for the three domains of the linearized shape of the pH dependence are summarized in table 3. The variations of $\bar{A}$ are illustrated with domains I and III for different values of σ (fig. 9): domain I decreases and domain III increases in $\bar{A}$

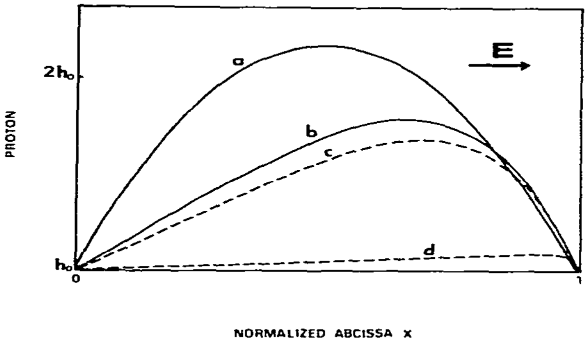

Fig. 8. H$^+$ concentration profiles, analytically obtained for reaction schema III, with the first domain of the pH dependence, for different values of ζ:

	a	b	c	d
ζ:	0	4	5	50
ψ:	5	1.7	1.4	0.2

(———) Sinusoidal form; (------) exponential form.

Table 2

Mathematical expressions of intramembrane H$^+$ concentration profiles, for reaction schema III depending on the domain of the pH dependence.

Domain I

Low values of E, $4\sigma\frac{D_S}{D_H}a_1' - \zeta^2 > 0$

$$h = h_o \exp(a'x)\cos(bx) + \frac{\exp(-a') - \cos(b)}{\sin(b)}$$

$$a' = \frac{\zeta}{2},\ b = \frac{1}{2}\sqrt{4\sigma\frac{D_S}{D_H}a_1' - \zeta^2},\ a_1' = K_m a_1$$

High values of E, $4\sigma\frac{D_S}{D_H}a_1' - \zeta^2 < 0$

$$h = h_o\frac{[1-\exp(r_2)]\exp(r_1 x) + [\exp(r_1) - 1]\exp(r_2 x)}{\exp(r_1) - \exp(r_2)}$$

$$r_1, r_2 = \frac{\zeta \pm \sqrt{\zeta^2 - 4\sigma D_S/D_H a_1'}}{2}$$

Domain II

$$\mathrm{h} = \frac{\sigma D_S a_2}{D_H \zeta[\zeta - \exp(\zeta)]}[\{\exp(\zeta x) - 1\} + h_o + x\{\zeta - \exp(\zeta)\}]$$

Domain III

$$h = (h_o - \frac{a_4}{a_3})\frac{[1-\exp(r_2)]\exp(r_1 x) + [\exp(r_1) - 1]\exp(r_2 x)}{\exp(r_1) - \exp(r_2)}$$

$$a_3' = K_m a_3,\quad r_1, r_2 = \frac{\zeta \pm \sqrt{\zeta^2 + 4\sigma D_S/D_H a_3'}}{2}$$

Table 3

Mathematical expressions of membrane activity as a function of ζ for reaction schema III depending on the domain of the pH dependence

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Domain I

Low values of E

$$\bar{A}=\frac{b[\exp(2a')+1]-2\exp(a')\cos(b)}{(a'^2+b^2)\exp(a')\sin(b)}$$

for definition of a' and b see table 2

High values of E

$$\bar{A}=\frac{[\exp(r_2)-1][\exp(r_1)-1]}{\exp(r_2)-\exp(r_1)}(\frac{1}{r_1}-\frac{1}{r_2})$$

for definition of r_1 and r_2 see table 2

Domain II

$$\bar{A}=1$$

Domain III

$$\bar{A}=\frac{[\exp(r_2)-1][\exp(r_1)-1]}{\exp(r_2)-\exp(r_1)}(\frac{1}{r_1}-\frac{1}{r_2})$$

for definition of r_1 and r_2 see table 2

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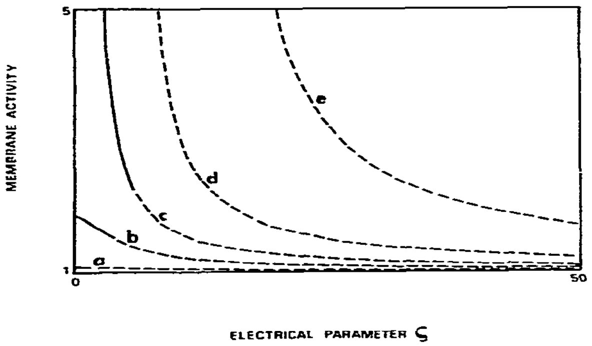
with increasing values of E. It is evident that a third type of variation exists that cannot be shown analytically and that corresponds to the passing of the intramembrane h concentration through the h' value. In this case the $\bar{A}(\zeta)$ curve has a maximum. A numerical treatment has confirmed the existence of this shape [14].

Thus, more varied possibilities of regulation are predictable when an electric field acts on the γ term: an increase in activity, a decrease in activity or an activation followed by a deactivation may be obtained depending on parameters pH_0, pH' and σ.



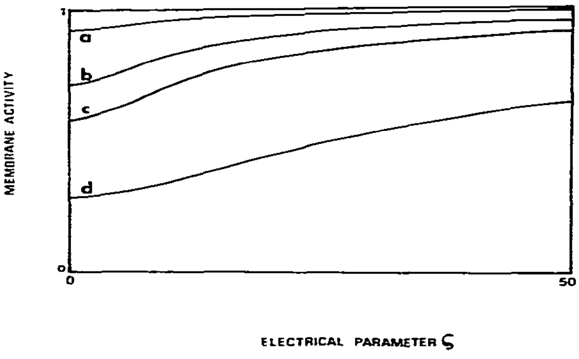


Fig. 9. $\bar{A}(\zeta)$ curves for reaction schema III, for different values of σ.

(a) (Upper) Domain I of the pH dependence.

	a	*b*	*c*	*d*	*e*
σ:	1	5	10	20	50

(————) Sinusoidal form; (------) exponential form.

(b) (Lower) Domain III of the pH dependence

	a	*b*	*c*	*d*
σ:	1	5	10	50.

5. Conclusion

It was shown that the action of an electric field led to the asymmetrical deformation of concentration profiles of cofactor M^+, inhibitor I^+, substrate S^- or H^+. For high electric fields the concentration profile of an ionic species tends toward the value of its concentration at its entering interface of the membrane.

This allowed us to bring out possibilities of regulating the membrane activity $\bar{A}$ through the terms $\delta(M)$, $\delta'(I)$, $\lambda(S)$ and $\gamma(pH)$. For high values of the electric field the enzyme activity $\bar{A}$ tends toward 1. These results are summarized in table 4. The factors of regulation include: the typical reaction schemas used, the conditions on the boundaries, the intensity of the electric field (and its direction for asymmetrical systems) and the value of parameters σ and ψ.

Where the electrical regulations by action on γ are concerned, the results established here analytically for low pH values ($pH < 6$) were numerically extended to higher pH values [14]. It is also possible to extrapolate the results concerning the production of H^+ to consumption of H^+. The results reported here concern a medium of constant conductivity; we established that a weak level of conductivity, when liable to modulation by the enzyme reaction in presence of an electric field, led to other types of phenomena [14]. The combination of parameters δ, λ and γ by more sophisticated reaction schemas led to more complex transport-reaction phenomena [14].

Finally, multiple steady states have not been referred to here; they are the subject of another paper containing theoretical analysis and experiments [17].

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Table 4

Typical variations of membrane activity as a function of ζ, with a high electrolyte concentration, for independent regulations by action of the electric field on δ, λ and γ terms

Mediator	Reaction schema	Shape of the dependence in mediator	Boundary conditions		E (ζ)	$\bar{A}$
M^+	$S \xrightarrow{M^+} P$		Asymmetrical		↗	↗
	$\lambda = 1$					
	$\gamma = 1$				↘	↘
I^+	$S \xrightarrow{I^+} P$		Asymmetrical		↗	↗
	$\lambda = 1$					
	$\gamma = 1$				↘	↗
S^-	$S^- \rightarrow P^-$		Symmetrical		↗	↗
	$\delta = 1$					
	$\gamma = 1$					
H^+	$S \rightarrow P^- + H^+$		Symmetrical	$pH_o > pH'$	↗	↗ ↘
	$\lambda = 1$			$\sigma \gg 1$		
	$\delta = 1$			$pH_o > pH'$	↗	↘
				$\sigma < 1$		
				$pH_o < pH'$	↗	↗

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