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Immobilized cycling enzyme active transport systems

pH feedback control

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Active transport can be induced by applying a pH gradient across a membrane containing a homogeneous mixture of two cycling enzymes. When the two reactions are inversely 'pH active', one producing protons and the other consuming them, a pH feedback control of the functional structure occurs and the active transport function of the membrane can be either stabilized or inhibited according to whether the endogenic pH modifications tend to enhance or reduce the exergonic pH gradient. When it is stabilized, the system looks like a thin active layer surrounded by two diffusive layers, leading to a fairly good model for biological transport systems. Under particular conditions, signals can be emitted.

1. Introduction

Active transport of ions or molecules is an important field of research in biophysics [1]. Mitchell's concept has stimulated new investigations in active transports related to pH gradients or proton pumps either in vivo [2,3] or in vitro [4] by using model systems such as liposomes. Most of the systems considered exhibit an asymmetrical spatial distribution of transport sites, essentially enzymatic sites, in the membrane and have been called 'permanent structures' [5]. Because very thin active biological membranes are always found in association with fairly thick diffusion or polarization layers, it is interesting to study thick membrane active transport models which can easily be analyzed using macroscopic laws (such as diffusion-reactions) and variables (concentration, pH, etc.).

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In addition, biotechnology is now developing rapidly and it is possible, by modifying genes of bacteria, to produce very valuable and complex biochemical compounds in industrial quantities. One of the most important problems remains the extraction of these molecules from the reactive mixture. A thick membrane active transport system using enzymes can thus be used as a specific extracting machine and so calls for very special attention.

In the past, thick membrane active transport models with a permanent structure have been proposed [5,6] and theoretically studied [7]. Using two enzymes of different pH dependence we showed, a few years ago, that the asymmetrical activation of homogeneously distributed enzymes by an external imposed pH gradient may induce asymmetrical 'functional structures' [8,9]. The evidence of such a structure was supported by an experimental glucose pump [10]. It should be noted that in such systems the role of the pH gradient in creating dissymetrical enzyme activity is entropic in the sense that there is neither an energetic nor a

stoichiometric link between proton flux and glucose pumping. In fact, the pH asymmetry replaces the structural asymmetry of the permanent structures. The reactions occurring inside the membrane must be driven by thermodynamics. In the glucose pump, for instance, the energy came from the dephosphorylation of ATP. Because of the high nonlinearity of the equations, the analytical solution [10] of a very simplified version of the model was completed by a numerical simulation of a more realistic system including typical shapes of substrate and pH dependences [11]; the time evolution of the system was also shown.

This paper presents in detail the regulations of active transport models by the feedback of protons: it shows how intramembrane reactions can themselves modify or stabilize the functional structure. When enzymes involved in the mechanism consume or produce protons in a medium which is not strongly buffered (such as hexokinase in the glucose pump considered), back actions of protons on the distribution of enzyme activities, i.e., on the functional structure, occur through the modification of the intramembrane pH profile. In monoenzymatic systems activation or inhibition results [12–16]; on the other hand, in mutlienzymatic systems other phenomena such as signals or pumps may appear when the two enzyme reactions are inversely pH active, i.e., one produces and the other consumes protons. According to whether or not the active proton flux is balanced by the inducing passive flux, the active transport function is either enhanced or reduced [17].

2. Theory

Two enzymes E_1 and E_2, catalyzing the cycling reactions

$$S_1 + XY \xrightarrow{E_1} S_2 + Y^- + H^+ \quad (1)$$

$$S_2 + H_2O \xrightarrow{E_2} S_1 + X^+ + OH^- \quad (2)$$

where S_1 is the pumped species, S_2 an intermediary product, XY a substrate; Y and X are reaction products. E_1 and E_2 are assumed to be purely of the Michaelis-Menten type. This means that, in solution, the rate of the enzyme reaction, V_i ($i = 1$ or 2), is a hyperbolic function of S_i when XY is taken in such high concentration that it can be considered to have no action on the kinetics ([XY] constant). This is written [11]:

$$V_i = V_{mi}\gamma_i\lambda_i \quad (3)$$

where:

$$\gamma_i = k_{ai}H/(k_{ai} + H)/(k_{bi} + H) \quad (4)$$

$$\lambda_i = S_i/(K_{mi} + S_i) \quad (5)$$

V_{mi}, γ_i, λ_i and K_{mi} being the maximum rate, pH dependence, substrate dependence and Michaelis constant of enzyme E_i in solution, respectively; k_{ji} are protonation constants relative to E_i. In the following, the products $V_m\gamma$ and $V_m\gamma\lambda$ will be called potential activity ($V(E)$) and effective activity ($V(\varepsilon)$), respectively.

Now consider a porous or gel phase, such as a thick membrane with enzymes E_i distributed at random and immobilized in the gel of thickness e. By assuming the presence of a supporting electrolyte, electrical effects within the membrane can be neglected [18] and the local concentration C of each species is given by the classical diffusion-reaction law representing the mass balance [7,8]:

$$\frac{\partial C}{\partial t} = D_c\frac{\partial^2 C}{\partial x^2} + \sum_i V_{mi}\gamma_i\lambda_i \quad (6)$$

where t is the time, x the intramembrane space coordinate and D_c the diffusion coefficient of the species involved.

When a pH gradient is imposed through the membrane, the different enzyme functions are distributed inside the membrane in agreement with local pH through eq. 4 (fig. 1). Thus, as the reactive terms depend on several concentration levels they vary along the thickness of the membrane. Due to complexity and interdependence, only numerical computations can be used to solve eqs. 6. Since [XY] is assumed to be constant, only four species S_1, S_2, H^+ and OH^- need be taken into account in calculations. Using an explicit scheme [19], their concentrations are given by the

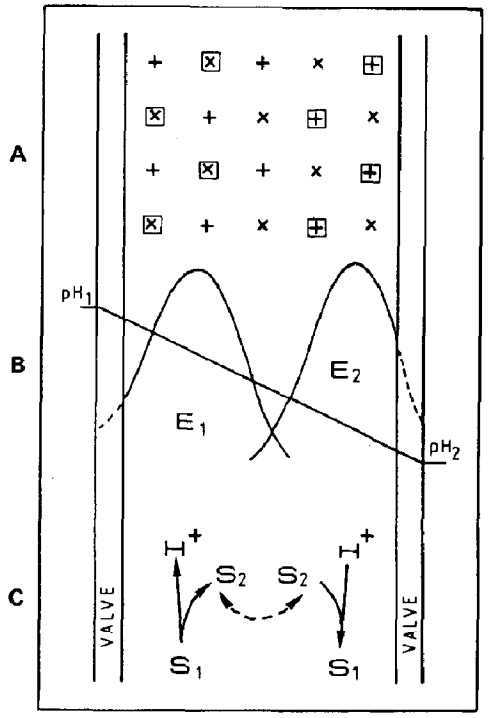

Fig. 1. Schematic diagram of an active transport membrane with asymmetrical functional structure. (A) Homogeneously distributed enzymes E_1 (×) and E_2 (+) and activity distribution by applying a pH gradient: activated enzyme (□); (B) activity profile of enzymes as a function of intramembrane pH; and (C) scheme of chemical reactions. The two valves are not permeable to S_2.

relation:

$$C_x^{t+\Delta t} = C_x^t + D_c\,\Delta t\frac{C_{x-\Delta x}^t + C_{x+\Delta x}^t - 2C_x^t}{\Delta x^2} + \Delta t\sum_i \nu_i V_i \qquad (7)$$

for S_1 or S_2, while H^+ and OH^- are included together in a symbolic species concentration H^*

$$H^* = [H^+] - [OH^-] \qquad (8)$$

thus taking into account the ionic equilibrium of water [12] (D being assumed to be the same for H^+ and OH^-). This gives:

$$H_x^{*t+\Delta t} = H_x^{*t} + D_h\,\Delta t\frac{H_{x-\Delta x}^{*t} + H_{x+\Delta x}^{*t} - 2H_x^{*t}}{\Delta x^2} + \Delta t\sum_i \nu_i V_i \qquad (9)$$

Computation of eqs. 7 and 9 gave the time evolution of the system.

3. Results

Depending on the relative positions of the enzyme pH dependences, γ_i, on the pH scale, two different models, I and A, could be distinguished where the functional structure, and thus the active transport function, are either inhibited or activated. In the first part, the inhibiting model I is presented which, in addition to its pumping function, can lead to a signal converter when associated with small compartments. In the second the activated model A is described which can lead to a fairly good model for biological membranes. In addition, an example of the time evolution of the system toward its steady state is presented.

3.1. Self-inhibiting model I

3.1.1. Principle

When the optimal pH of the proton-producing enzyme E_1 (pH_1') is higher than that of the proton-consuming enzyme E_2, the pumping activity tends to decrease the intramembrane pH inside the layer where E_1 is active and to increase it inside the layer where E_2 is active (fig. 2). As a

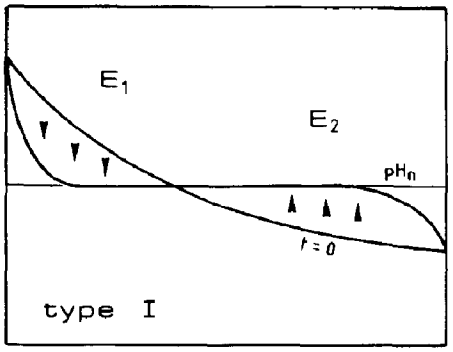

Fig. 2. Evolution tendency of the intramembrane pH in a type I active transport model: the pumping tends to decrease the intramembrane pH inside the layer where E_1 is active (high pH) and to increase it inside the layer where E_2 is active (low pH). Inhibition results.

consequence, in both layers the pH tends toward the neutral value pH_n where both reaction rates V_i are equal. No net activity remains in such a layer which degenerates into a pure diffusive layer. Of course, this evolution is slower in the border layers of the membrane which remain active longer. Fluxes of the pump, related to the asymmetrical functioning of the enzyme reactions, thus decrease with time, and the pump is progressively inhibited. The inhibition is enhanced by high substrate concentrations (fig. 3) since, in this case, the active border layers are thinner.

3.1.2. Fixed boundaries: the inhibited pump

With fixed boundaries, obtained either by monitoring pH in the compartments surrounding the membrane or by using large compartment volumes, the inducing pH gradient and thus the active transport function of the membrane do not completely disappear under the effect of enzyme activity. As the pH of the central layer approximates pH_n, the system tends toward a central pure diffusion layer surrounded by two thin active layers of E_1 and E_2 activities (fig. 4). While over-

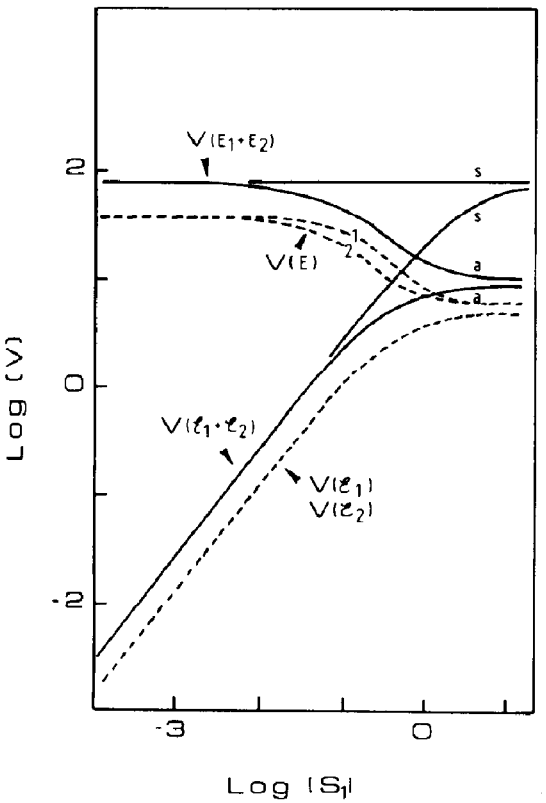

Fig. 3. Inhibition of the type I pump. High substrate concentrations enhance intramembrane pH modifications; inactivation of the pump results as shown by the decrease in potential activity $V(E)$. A deviation from the Michaelis-Menten type kinetics (curve s) occurs as shown by the effective activity curve $V(\varepsilon)$. Global membrane activities (———), and individual potential and effective activities (- - - - - -). Boundary pH values were 7 and 9.4 and the enzyme activity was given by $V_m e^2/K_m D = 75$.

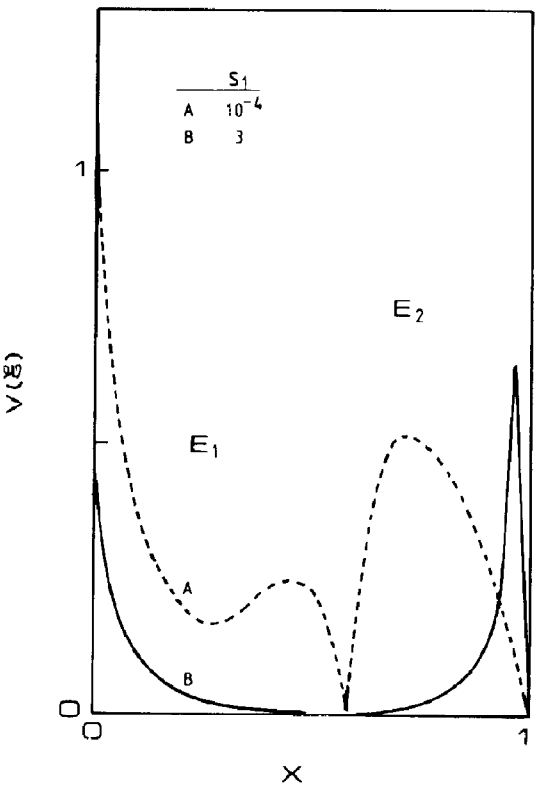

Fig. 4. The activity profile inside the membrane is highly dependent on the substrate concentration. At low substrate concentration (- - - - - -), enzyme activities are weak and intramembrane pH is almost unaffected; the effective activity profile is comparable to the potential activity profile (curve A). On the other hand, high substrate concentrations induce a great intramembrane pH modification; for model I, intramembrane pH becomes almost uniform and equal to pH_n, except close to the membrane solution interfaces. The medium layer is no longer active and looks like a pure diffusion layer (———). Conditions are the same as in fig. 3.

all activity decreases, the transport yield, defined by the ratio of the number of transported S_1 molecules over the number of reacting S_1 molecules, increases (fig. 5, curve i); the model is very efficient for substrate. This configuration is close to the previously studied 'square model' with a high n value [11]. Biologically, it may correspond to intra- and extracellular enzymes separated by the cell membrane, or even to membrane enzymes

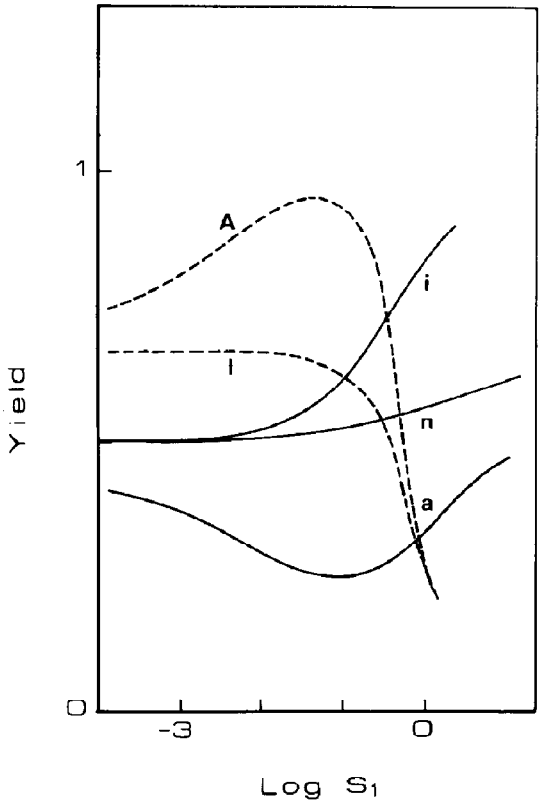

Fig. 5. The transport yield of the system, defined as being the ratio of the number of transported S_1 molecules over that of metabolized S_1 molecules, increases and stays between 0.5 and 1 with an active transport model of type I (curve i), although it remains under 0.5 for type A (curve a). Curve n represents the transport yield for a non-pH active model. Nevertheless, another type of efficiency can be defined as being the ratio of the number of transported S_1 molecules over that of enzyme molecules necessary for pumping. The influence of substrate concentration on this catalytic yield is in the opposite direction to the influence on the stoichiometric yield, i.e., model A (curve A) is now more efficient than model I (curve I). Curves A and I are normalized.

with an interfacial reactivity separated by the lipid bilayer.

3.1.3. Moving boundaries: signal converter

We obtain moving boundaries when the pH values in extramembrane compartments are slowly modified by the intramembrane reactions. With high enzyme activities, the pH of the whole system tends to become uniformly equal to pH_n for which no net enzyme activity remains, thus any active transport function at the steady state is destroyed. Nevertheless, due to the functioning of the active transport during the transition period, a signal (temporal flux of S_1) is emitted by the system (fig. 6) which depends on the compartment volumes and on the buffering power of the solutions. Only a new imposed external pH difference will induce another S_1 signal. This means that an H^+ signal has been converted into an S_1 signal.

3.1.4. Time evolution of the system

Although model I is characterized at the steady state by inhibition, the time evolution of the system is not monotonic, i.e., the membrane activity does not decrease continuously until the inhibited state is reached but shows a complex time evolution related to the double regulation of the system by substrates and pH. Fig. 7 shows the different phase of the time evolution: initially, only the E_1 reaction can run because product S_2 is not present. The pH thus decreases in the E_1 activity zone, decreasing the potential activity $V(E_1)$. Because of the diffusion, pH also decreases in the E_2 activity zone, inducing an increase in $V(E_2)$. In addition, S_1, being consumed by the reaction, decreases and induces a much greater drop in the effective activity $V(\varepsilon_1)$, although $V(\varepsilon_2)$ remains very low because not enough S_2 is produced. In the second phase, the increase in S_2 produced in the E_1 activity zone induces an increase in $V(\varepsilon_2)$, but by raising local pH, brings about a drop in $V(E_2)$; this drop, in turn, acts on $V(\varepsilon_2)$ until, eventually, equality between $V(\varepsilon_1)$ and $V(\varepsilon_2)$, which characterizes the steady state, is reached.

3.2. Self-activating model A

3.2.1. Principle

When pH_2' is higher than pH_1', the pumping

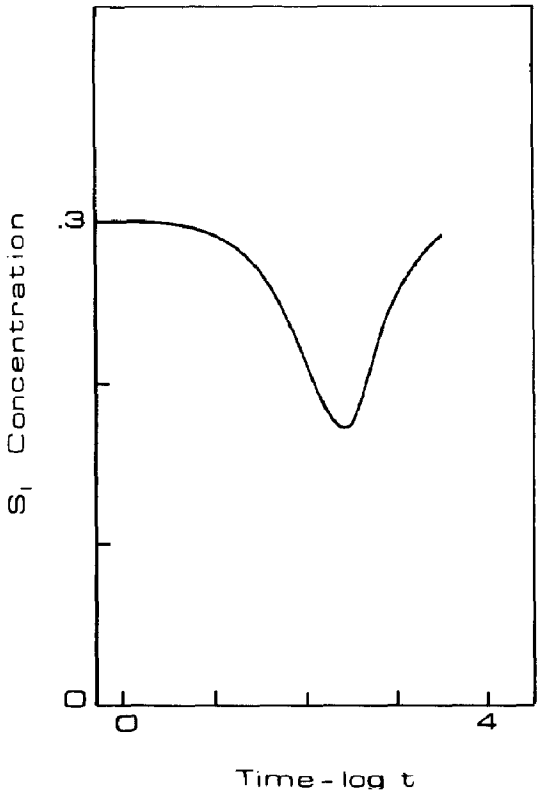

Fig. 6. Time evolution of the transported species concentration in the donor compartment for a type I model and a moving boundary system (boundary pH values not maintained at their initial levels). The active transport was efficient only during the first phase before the intramembrane pH modifications became too large. At the end, the intramembrane pH is uniform and no active transport function remains. A signal is emitted. $S/K_m = 0.3$; other initial conditions as in fig. 3.

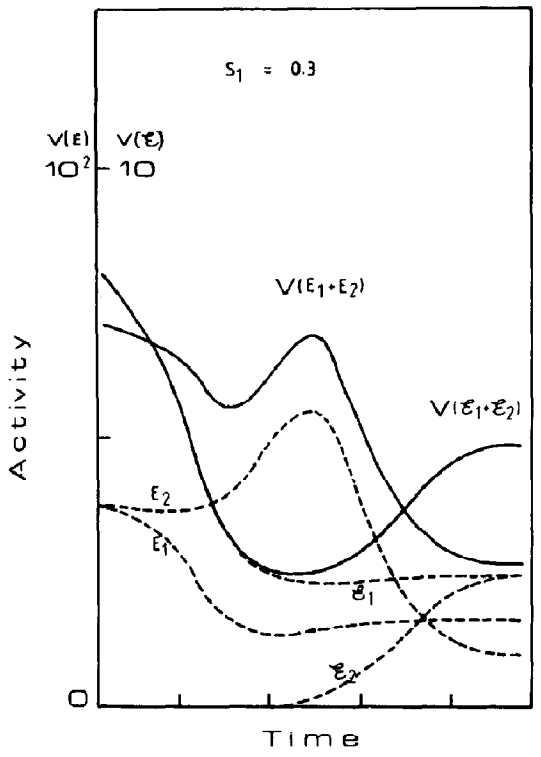

Fig. 7. Time evolution of the enzyme activities of the active transport membrane for model I at fixed boundaries. (- - - - -) Individual effective activities ($V(\varepsilon_1)$ and $V(\varepsilon_2)$) and the individual potential activities ($V(E_1)$ and $V(E_2)$) of enzymes; (———) evolution of the global potential membrane activity, $V(E)$, and of the global effective membrane activity, $V(\varepsilon)$, which is proportional to transport fluxes. Conditions as in fig. 3 and $S/K_m = 0.3$.

tends to increase the local pH in the E_2 activity layer where it is already high, and to decrease it in the E_1 activity layer where it is initially low (fig. 8). Thus, unlike model I, model A tends to stabilize two distinct intramembrane pH values, one for each layer and thus to localize the largest pH gradient in the central part of the membrane. We can again distinguish two cases according to whether the pH at the membrane limits is fixed or free to change.

3.2.2. *Fixed boundaries: model for thin membrane*

At fixed boundaries, when the limiting pH val-

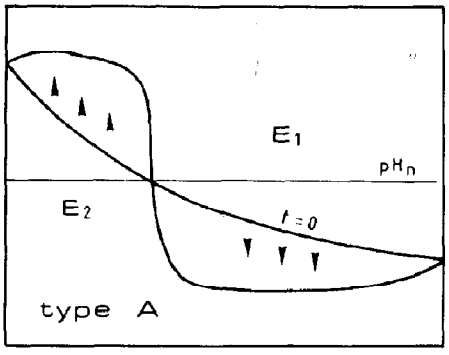

Fig. 8. Evolution tendency of the intramembrane pH in a type A active transport model: the pumping tends to increase local pH in the E_2 activity layer where it is already high, and to decrease it in the E_1 activity layer where it is initially low. Activation results.

ues are located between pH_n and pH_i', the system is strongly activated. The activation is enhanced at high substrate concentrations because, under such conditions, the pH in each E_i activity layer is close to pH_i'. The deviation from Michaelis-type behavior thus increases and active fluxes may even show a sigmoidal curve as a function of substrate concentration (fig. 9). This is related to the autocatalytic effect of H^+ since at a given external pH, the intramembrane pH is a function of the S concentration [20]. On the other hand, when boundary pH values are chosen far from the optimal values pH_i', the whole pH variation is limited to the central zone of the membrane which then looks like an asymmetrical active layer surrounded by two purely diffusive layers. Biologically, this system is a fairly good model of a thin two-enzyme active transport membrane surrounded by its two diffusion or polarization layers (fig. 10) [17,21]. This configuration is close to that of biological membranes for which transport activity is probably always limited by the thickness of the polarization layers. So, even though this model is studied through macroscopic techniques, laws and concepts, it is of great conceptual importance as it can give significant information about the behav-

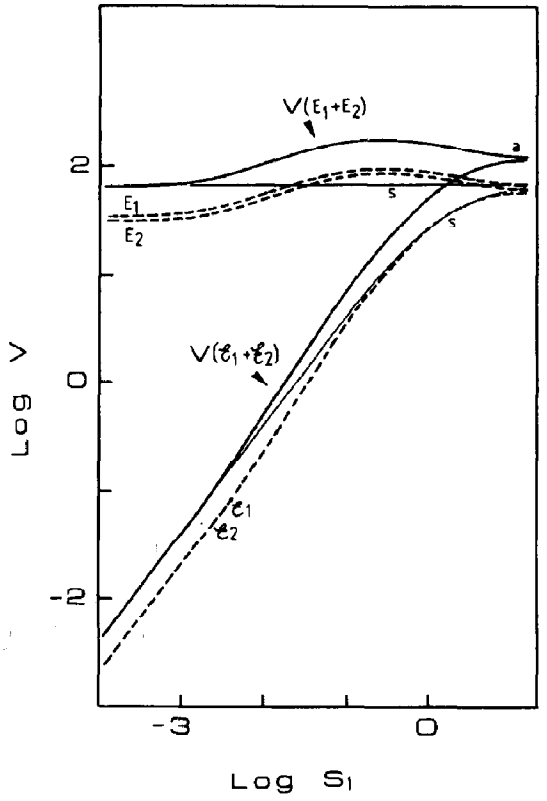

Fig. 9. Activation of the type A pump. High substrate concentrations enhance intramembrane pH modifications; activation of the pump results as shown by the increase in both potential activity $V(E)$ and effective activity $V(\varepsilon)$. A sigmoidal shape of $V(\varepsilon)$ (which is proportional to active fluxes) as a function of the substrate concentration is numerically predicted. It shows that an apparent allosteric behavior can be obtained for an active transport mechanism in which only two pure Michaelis-Menten type enzymes are involved. Boundary pH values were 6 and 8 respectively and $V_m e^2/K_m D = 75$.

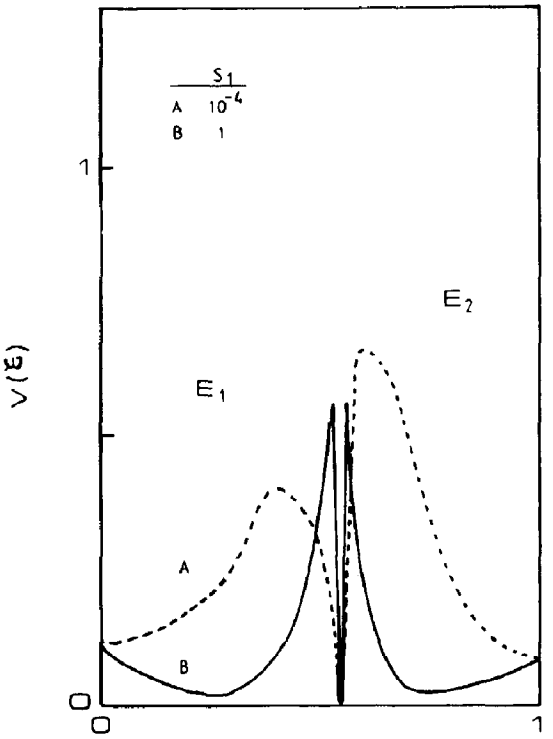

Fig. 10. For model A, the intramembrane pH modifications induced by high substrate concentrations (———) lead to the accumulation of effective activities in a thin central layer of the membrane. The model is a fairly good 'thin membrane model'. At low substrate concentrations (- - - - -), the activity profile is similar to that calculated for model I (see fig. 3). Conditions as in fig. 9.

ior of very thin membranes. In particular, model A was shown to be less efficient than model I, since the stoichiometric yield always remains lower than 0.5 (fig. 5, curve a). This is essentially due to the proximity of the two active zones and to the low level of diffusion constraints between these zones. On the other hand, as the model is activated, it does not need high enzyme concentration immobilized in the membrane and so it constitutes another type of efficiency: efficiency with respect to enzyme (fig. 5, curves A and I).

3.2.3. Moving boundaries: signal amplification

As above, the H^+ pump flows in the opposite direction to the passive H^+ flux and there is a self-activating process. At moving boundaries, the steady-state pH difference between the two membrane boundaries is a function of the enzyme activity immobilized in the membrane. For high enzyme activity, the stabilization of the oscillatory pH profile tends to enhance the initial pH difference between boundaries. Whatever the initial pH limits, the evolution of this model tends toward the 'thin membrane model' described above, i.e., an asymmetrical active transport membrane surrounded by two purely diffusive layers.

The time evolution of the system is similar to that described for model I and we shall not go into more detail about it.

4. Conclusion

When two cycling enzymes are inserted in an artificial membrane, a pH gradient imposed through the membrane can induce an active transport of one of the enzyme substrates, S_1. When reactions are inversely pH active, a cotransport (symport and antiport) H^+/S_1 occurs. The stoichiometry of the coupling is equal to that of the reactions involved. Depending on the relative positions of the two enzyme optimal pH values on the pH scale, this transport system makes protons move either in the thermodynamic direction or against their electrochemical potential gradient. Two different types of behavior are exhibited: (i) When the active transport and the passive diffusion of protons have the same direction, the pump is rapidly inhibited and signals can be emitted. When pH is not maintained at boundaries, only signals are emitted. (ii) When the transports are in opposite directions, the active H^+ flux maintains the inducing pH difference and the S and proton pumps are activated and self-stabilized. Under particular conditions, the system looks like a thin active layer surrounded by two purely diffusive layers; it is a fairly good model for biological transport systems which can be studied using macroscopic laws and variables.

The yield of the active transport model is close to 0.5 transported molecule per reacting molecule; it increases at high substrate concentrations for the inhibited model , although it remains under 0.5 for the activated version of the model which for the same amount of S_1 molecules transported consumes more energy (XY species), but uses much fewer enzyme molecules.

In addition at moving boundaries, i.e., when boundary pH values are not maintained at their initial levels, the system can also be used as either a signal converter for the inhibited model I or a signal amplifier for the activated model A.

When the proton pump is self-stabilized, it is also possible to find conditions under which it functions without any asymmetry of structure or composition, only one pulse of asymmetry being needed. This aspect will be examined in detail in a later paper.

References

1 B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts and J.D. Watson, Molecular biology of the cell (Garland, New York, 1983) p. 286.
2 J.C. Skou and J.G. Norby, Na^+-K^+ATPase: structure and kinetics (Academic Press, New York, 1979).
3 J.C. Vincent, C. Kumar, A. Naqui and B. Chance, Communication, Biophysical Society Meeting, San Diego, USA, February 1983. (Abstract: Biophys. J. 41 (1983) 321a.)
4 E. Racker, A new look at mechanisms in bioenergetics (Academic Press, New York, 1976) p. 127.
5 E. Selegny, in: Polyelectrolytes, eds., E. Selegny, M. Mandel and U.P. Strauss (Reidel, Dordrecht, 1974) p. 419.
6 D. Thomas and S.R. Caplan, in: Membrane separation processes, ed. P. Meares (Elsevier, Amsterdam, 1976) p. 351.
7 D. Thomas, Dr. Sci. Thesis, Rouen (1971).
8 J.C. Vincent, Dr. Sci. Thesis, Rouen (1980).

9 E. Selegny and J.C. Vincent, Biophys. Chem. 12 (1980) 93.
10 E. Selegny and J.C. Vincent, Biophys. Chem. 12 (1980) 107.
11 J.C. Vincent, E. Selegny and M. Metayer, Biophys. Chem. 14 (1981) 159.
12 J.C. Vincent, J.M. Valleton and E. Selegny, Biophys. Chem. 18 (1983) 369.
13 D. Vallin and C. Tran Minh, Biochim. Biophys. Acta 571 (1979) 321.
14 A. Friboulet and D. Thomas, Biophys. Chem. 16 (1982) 153.
15 J.M. Engasser and C. Horvath, Biochim. Biophys. Acta 358 (1974) 178.
16 J. Ricard, G. Noat, M. Crasnier and D. Job, Biochem. J. 195 (1981) 357.
17 J.C. Vincent, J.M. Valleton and E. Selegny, in: Physical chemistry of transmembrane ion motions, ed. G. Spach (Elsevier, Amsterdam, 1983) p. 123.
18 J.M. Valleton, Dr. Sci. Thesis, Rouen (1984).
19 J.P. Kernevez, Dr. Sci. Thesis, Paris VI (1972).
20 J.C. Vincent and M. Thellier, Biophys. J. 41 (1983) 23.
21 J.C. Vincent, Spectra Suppl. 13 (1985) 27.