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Autocatalytic cooperativity and self-regulation of ATPase pumps in membrane active transport

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Abstract. We investigate the effect of autocatalysis on the conformational changes of membrane pumps during active transport driven by ATP. The translocation process is described by means of an alternating access model. The usual kinetic scheme is extended by introducing autocatalytic steps and allowing for dynamic formation of enzyme complexes. The usual features of cooperative models are recovered, i.e., sigmoid shapes of flux versus concentration curves. We show also that two autocatalytic steps lead to a mechanism of inhibition by the substrate as experimentally observed for some ATPase pumps. In addition, when the formation of enzyme complexes is allowed, the model exhibits a multiple stationary states regime, which can be related to a self-regulation mechanism of the active transport in biological systems.

Key words: Active transport – ATPase pumps – Self-regulation mechanism – Multiple stationary states

Introduction

Active transport of small molecules and ions driven by membrane enzymes against the gradient of the electrochemical potential is a phenomenon that occurs in all living cells. Active transport is involved in the process of uptake of nutrients, excretion and energy storage of the cells. It is the main mechanism able to maintain, in far from equilibrium conditions, a specific singular chemical composition in the cell and in the cell compartments. The active transport mechanism is probably the most important key to understanding the self-control and self-regulation of autonomous living systems.

The ATPase pumps utilize ATP hydrolysis as an energy source and are responsible for many important metabolic functions of plants and animal cells. Various types of pumps may be classified according to the nature and the function of the membrane, nevertheless some general features are common for a broad class of active transport systems and may be described by general principles of pump functioning. An active transport pathway cannot be a permanently open channel in the membrane, as in passive transport. The translocation pathway must never be simultaneously accessible from both sides of the membrane because it must permit uphill movements of the transported molecule. The general model accepted to describe active transport is the alternating access model (Tanford 1983; Läuger 1984). In this model the protein has specific binding sites for the transported solute and at least two different conformational states. For a specific conformational state, the binding site is accessible from one side of the membrane, changing the conformation makes the site accessible from the other side. The transport occurs through specific kinetic steps involving the conformational changes of the protein and chemical binding of transported substrate and ATP at specific sites in the protein. Since there are many possible combinations, most of the variants of the alternating access model differ only in the ordering or number of these specific steps. As remarked by Tanford (1983) virtually all models for the active transport presented in the literature are variants of the alternating access model. Moreover, as pointed out by Läuger (1984), the most important thermodynamic and kinetic properties of the active transport are common to a broad class of models.

In this paper, instead of discussing any specific pump or a specific model for a given pump, we intend to investigate, from a general point of view, the cooperativity and self-regulation of ATPase pumps. Most of the active transport systems exhibit flux versus substrate concentration curves quite different from the usual Michaelis-Menten enzyme kinetics. Complex behaviour was reported, for example, in the proton-translocating ATPase of fungi (Brooker and Slayman 1983; Bowman 1983; Koland and Hammes 1986), in the Ca-ATPase both of the sarcoplasmic reticulum (Inesi and Hill 1983; Andersen 1989) and of the erythrocyte plasma membrane (Kosk-Kosicka

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et al. 1990) and in the Na^+/K^+ pumps (Rossi and Garrahan 1985; Repke 1986). Positive and negative cooperativity, variable stoichiometry, and inhibition by the substrate are experimentally observed in these systems. These behaviours are explained in different ways for different pumps, by, for example, assuming cooperativity between the ligand binding sites (Bowman 1983), or by taking into account the formation of cooperative oligomeric unities (Klingenberg 1981; Andersen 1989; Kosk-Kosicka et al. 1990), or even by considering the competition between different reaction pathways (Tanford et al. 1985). Although specific models for specific pumps are able to reproduce, more or less successfully, the particular behaviour of each kind of pump, it seems that cooperativity and regulation by substrate are common features of the ATPase pumps. The models presented in the literature usually involve only one pump in each kinetic step and cooperativity is related to the binding of transported substrate or ATP. The cooperative interactions between enzyme monomers are taken into account only by the equilibrium formation of dimers or oligomers, wich kinetically play the role of a single pump unit. We may ask what is the effect of dynamic interactions between pump units. The purpose of the present paper is to discuss this general aspect by introducing dynamic interactions between pumps units in the usual kinetic schemes, such as self-induced autocatalytic conformational changes and dynamic formation of protein complexes.

The central question in the proposed extension concerns the interactions between the transport ATPases. For sufficiently high pump concentrations, such as in the sarcoplasmic reticulum (30 000 Ca-ATPase/μm^2), the average distance between two proteins is of the order of their diameters (Andersen 1989), making it easy for them to interact with each other. In more dilute systems, such as the plasma membranes of fungi and plants where the concentration (Γ) is about 10 000 proton-ATPase/μm^2 (Serrano 1988), the interaction will only be possible owing to the fluidity of the membrane. As a rough estimate, for diffusion coefficients (D) of about 10^{-10} cm^2/s, the collision time (τ) for such concentrations is much less than 10^{-2} s ($\tau \ll 1/D\Gamma$). This leads to a collision rate higher than 10^2 s^{-1}, which is of the magnitude of the turnover rate of the pumps (Serrano 1988). This means that during the time of the complete reaction cycle, the number of estimated collisions are sufficiently large to justify the autocatalytic interaction during conformational changes.

It is well known in chemical kinetics that models involving autocatalysis can exhibit complex dynamic behaviour. In far from equilibrium conditions these systems are able to produce so called *dissipative structures*, such as limit cycles, spatial inhomogeneities, all or none transitions and periodic oscillations. These properties have been connected with many observed phenomena in the cells, such as chemical metabolic oscillations and self-regulation mechanisms (Nicolis and Prigogine 1977). Active transport is an example of a far from equilibrium process which would be expected to lead to dissipative structures under the appropriate conditions.

In this article we intend to discuss the general kinetic properties resulting from autocatalytic cooperativity in membrane active transport. The introduction of autocatalysis in the kinetic scheme is able to give a more compact description of the observed behaviour of the pumps. We expected that a more general view about the cooperativity of the pump mechanism would emerge from this approach. Further, autocatalytic non-linear steps are necessary to produce far from equilibrium transitions in the system, providing a model to discuss more complex phenomena. We are particularly interested in the existence of multiple stationary state regimes which should be related, as will be discussed below, to the self-control of substrate concentration levels in the cell compartments.

Alternating access model

As a basis for further analysis, we introduce a model which is thought to contain some of the essential features of real pumps. We adopt the simplest kinetic model for ATP-driven active transport, based on the alternating access mechanism as proposed by Läuger (1984) to describe the proton translocating ATPase of fungi. For the transport of one substract molecule per cycle, it is given by the following reaction steps;

$$C + x_c \underset{K_c}{\rightleftharpoons} XC \tag{1a}$$

$$\mathrm{ATP} + XC \underset{r}{\overset{p}{\rightleftharpoons}} XE + \mathrm{ADP} \tag{1b}$$

$$XE \overset{K_e}{\rightleftharpoons} E + x_e \tag{1c}$$

$$E \underset{q}{\overset{k_{ec}}{\rightleftharpoons}} C + P_i \tag{1d}$$

where C represents the dephosphorylated state of the pump protein, accessible only from the cytoplasmic side and E the phosphorylated state, accessible from the external side, which could be the intravesicular medium in the case of Ca-ATPases of sarcoplasmatic reticulum. XE and XC denote the corresponding conformational states with the binding site occupied by the transported substrate x. In a complete cycle, x is transported from the cytoplasm (x_c) to the external medium (x_e), via the transformation of ATP to ADP and a subsequent release of P_i to begin the next cycle. This model is based on the following assumptions:

1) The phosphorylation and dephosphorylation reactions ((1 b) and (1 d)) are assumed to be simultaneous with the conformational changes of the pump.
2) In states C and XC, the binding site is accessible only from the cytoplasm and in states E and XE only from the external side.
3) It is supposed that phosphorylation of the pumps occurs only by the ATP, consuming only one ATP molecule per cycle, direct phosphorylation by phosphate is neglected.

In solving the dynamic equation further simplifications are introduced:

4) The steps (1 a) and (1 c) are considered to be fast compared with steps (1 b) and (1 d), i.e., the ratio of concentrations in these steps are fixed by the equilibrium constants $K_c = N_c X_c / N_{xc}$ and $K_e = N_e X_e / N_{xe}$, where N_i is the number of pump molecules per unit area in the state i and X_c, X_e are the concentrations of the transported substrate.

5) The total number of membrane pumps per unit area (N) is kept fixed and the concentrations of ATP (C_t), ADP (C_d), P_i (C_p) and transported molecule (X_c, X_e) are considered as externally controlled parameters.

In thermodynamic equilibrium, one has $C_d^e C_p^e / C_t^e = \boldsymbol{K}$, where $\boldsymbol{K}$ is the equilibrium constant of the ATP hydrolysis ($\boldsymbol{K} \approx 10^5$ M). Equating the chemical potential on both sides of the membrane $X_c^e = X_e^e$, and considering also the chemical equilibrium at each step (1 a) to (1 d), it is easy to show the following relation between the kinetic constants and the equilibrium constant $\boldsymbol{K}$ (Läuger 1984):

$$\frac{C_d^e C_p^e}{C_t^e} = \frac{p K_c k_{ec}}{r K_e q} = \boldsymbol{K} . \tag{2}$$

Within the simplifications discussed above, we obtain from (1) the following set of equations,

$$N_{xc} + N_{xe} + N_e + N_c = N , \tag{3}$$

$$N_c / N_{xc} = K_c / X_c , \tag{4}$$

$$N_e / N_{xe} = K_e / X_e , \tag{5}$$

$$\frac{\mathrm{d}}{\mathrm{d}t} [N_c + N_{xc}] = -q C_p N_c - p C_t N_{xc} + k_{e\,c} N_e + r C_d N_{xe} . \tag{6}$$

The set of eqs. (3)–(6) must be solved to find the dynamic behaviour of the pumps. According to rate theory the flux of x from the cytoplasm to the external medium is given by:

$$\phi = +p C_t N_{xc} - r C_d N_{xe} = +q C_d N_c - k_{ec} N_e . \tag{7}$$

In the stationary state condition, i.e. $d[N_c + N_{xc}]/\mathrm{d}t = 0$, the flux is obtained from (2)–(7):

$$\phi / N = \frac{K_c \cdot q C_p \cdot r C_d}{X_c \cdot D} [\delta \cdot v - 1] \tag{8}$$

where

$$\delta \equiv \exp\{-\mathscr{A}/(RT)\} = (\boldsymbol{K} \cdot C_t)/(C_d \cdot C_p) , \tag{9}$$

$$v \equiv \exp\{+\Delta\mu/(RT)\} = X_c / X_e \tag{10}$$

and

$$D = p C_t + r C_d + (r C_d + q C_p)(K_c/X_c) + (p + k_{ec})(K_e/X_e) + (q C_p + k_{ec})(K_e K_c)/(X_e X_c) . \tag{11}$$

Equation (9) defines the affinity $\mathscr{A}$ of the direct ATP hydrolysis reaction, and $\Delta\mu$ is the difference of chemical potential of the transported substrate, between the cytoplasm and the external medium.

Equation (8) gives the usual behaviour of active transport systems when electric effects are neglected. Even when X_e is larger than X_c ($v < 1$), a sufficiently large excess of ATP ($C_t \boldsymbol{K} \gg C_d C_p$, $\delta \gg 1$) could lead to the transport of the substrate from the cytoplasm to the external medium ($\phi > 0$). In this model, considering only a single transported molecule per reaction cycle, the flux curves have a Michaelis-like behaviour. In fact, by assuming constant concentrations C_p, C_d and X_e, (8) can be put in the following form:

$$\phi / N = \frac{a(\delta v - 1)}{b + (\delta v - 1)} , \tag{12}$$

where a and b are constants. Equation (12) reproduces a Michaelis-Menten process if we consider the concentration excess $\delta - 1$ (for $v = 1$) or $v - 1$ (for $\delta = 1$), as the concentration variables. This simple model then leads to the usual enzyme kinetics without any kind of cooperativity.

The reaction scheme of (1) represents the simplest model describing the basic features of an ATP-driven pump. If we consider that the concentrations of ATP, ADP, P_i and substrate are fixed externally controlled parameters, some extensions of this model are trivial and lead formally to the same results as given by (8)–(11). In this case the rate constants will be replaced by effective rate constants depending on the externally controlled concentrations. The following extensions could be considered:

i) The phosphorylation of the protein by the inorganic phosphate (P_i) or ATP hydrolysis and synthesis without transport of substratum, both reducing the thermodynamic efficiency of the pump (Läuger 1984).

ii) The existence of more than one binding site for the transported substrate and P_i or ATP binding, if this binding is made in only one step, leading to the usual cooperative behaviour (Bowman 1983).

iii) The co-transport of a second substrate, if its concentrations are also considered as externally controlled and if the exchange with the first substrate is made in a single step, as usually considered for the Na-K pumps (Repke 1986).

iv) The dependence on the transmembrane potential in the case of ionic transport. In this case the effective rate constants will depend on the transmembrane potential (Läuger 1984).

v) The ordering, where phosphorylation occurs, could also be changed by redefining the effective rate constants (Läuger 1984).

Although these extensions do not affect the general result (8)–(11) they could lead to a quite different flux curves and have been used by many authors to discuss the specific behaviour of some pumps.

Another class of generalisation of the reaction scheme (1) introduces new states of the pump, for example, by treating the phosphorylation-dephosphorylation steps and conformational transitions as separate processes (Läuger 1984) or by disregarding the equilibrium hypothesis of steps (1 a) and (1 c). Also, the competition between different reactions pathways may be considered (Tanford et al. 1985). These extensions give quite different solutions for the dynamic equations and also could be used to explain many properties of the pumps.

The alternating access models and their extensions have been used successfully to explain many of the prop-

erties of the pumps. However, the usual models consider only a single pump unit in each dynamic step. To our knowledge, the question of whether the dynamic interaction between pumps could reproduce the observed complex behaviour has never been addressed. In the following we investigate the effect of these dynamic interactions, starting with the simplest model containing the essential features of the active transport as described by (1).

Autocatalytic cooperative transport

In the chemical kinetics literature there are many reaction schemes that exhibit complex behaviour of dissipative structures (Pacaud et al. 1976; Nicolis and Prigogine 1977). However, in general they cannot be adapted to describe a reaction cycle for active transport such as the scheme of (1). We start in the simplest way by introducing one autocatalytic step in the reaction scheme:

$$C + x_c \underset{K_c}{\rightleftharpoons} XC \tag{13a}$$

$$\mathrm{ATP} + XC + XE \overset{p}{\underset{r}{\rightleftharpoons}} 2XE + \mathrm{ADP} \quad \text{(autocatalysis)} \tag{13b}$$

$$XE \overset{K_e}{\rightleftharpoons} E + x_e \tag{13c}$$

$$E \overset{k_{ec}}{\underset{q}{\rightleftharpoons}} C + P_i \tag{13d}$$

In the stationary state, with the same assumptions as before, one has to solve the set of equations formed by (3)–(5) and the following,

$$\frac{\mathrm{d}}{\mathrm{d}t}[N_c + N_{xc}] = -qC_pN_c - pC_tN_{xc}N_{xe} + k_{ec}N_e + rC_dN_{xe}^2 = 0. \tag{14}$$

Using (7) we find the flux of x as:

$$\phi/N = k_{ec}\left[\frac{\lambda+\beta}{2\cdot(\alpha+\gamma)}\left[[(\beta+\lambda-\gamma)^2 + 4(\alpha+\gamma)\lambda]^{1/2} - (\beta+\lambda-\gamma)\right] - \lambda\right], \tag{15}$$

where

$$\alpha = (rC_dN/k_{ec})/(K_e/X_e+1)\,, \tag{15a}$$

$$\beta = (K_e/X_e)/(K_e/X_e+1)\,, \tag{15b}$$

$$\gamma = (p - C_tN/k_{ec})/[(K_e/X_e+1)(K_c/X_c+1)] \tag{15c}$$

$$= \delta(rC_dN/k_{ec})(K_e/K_c)(qC_p/k_{ec})/[(K_e/X_e+1)(K_c/X_c+1)],$$

$$\lambda = (qC_p)\cdot(K_c/X_c)/[k_{ec}(K_c/X_c+1)]\,. \tag{15d}$$

In (15c) we have used (2) to relate (pC_tN/k_{ec}) to $\delta = (\boldsymbol{K}C_t)/(C_dC_p)$. It is important to note that the equilibrium at step (13b) gives the same relation between the concentrations as step (1b) in the previous scheme, leading to the same relation between kinetic and equilibrium constants (2).

The flux curves for this model are given in Figs. 1, 2 for typical values of the parameters. Figure 1 shows the normalized flux of one pump molecule ϕ/N as a function of $\delta = (\boldsymbol{K}C_t)/(C_dC_p)$ for fixed values of C_d, C_p. For the set of parameters indicated in Fig. 1 it clearly shows the sigmoid shape of the flux curve as a function of the ATP concentrations. In Fig. 2 the same behaviour is shown by varying the concentration X_c. These results demonstrate that an autocatalytic step in the reaction scheme leads to a cooperative effect in the dynamics of the active transport. Moreover, the cooperativity appears simultaneously on varying both ATP and substrate concentrations, since now it is related to the interaction between the pumps and not between specific binding sites at the same pump unit. It is easy to show that if the autocatalysis is introduced in step (13d), instead of in step (13b), a similar result is found. It is also important to note that α and γ in (15) depend explicitly on the total number of pumps (N), which means that, in contrast to the usual cooperative models, the normalized rate of turnover depends on the concentration of pump molecules.

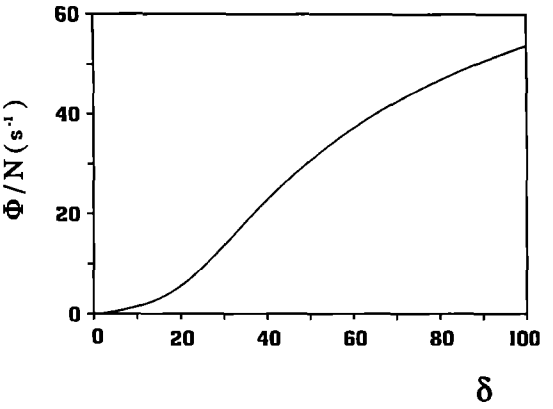

Fig. 1. Cooperative effect of a single autocatalytic step (13) on the normalized outgoing flux of the transported molecule (ϕ/N) as a function of ATP concentrations ($\delta = \boldsymbol{K}C_t/C_pC_d$). The number of pump unities and the ADP, P_i and substrate concentrations are fixed through the relations; $rC_dN/k_{ec} = 350$, $k_{ce}C_p/k_{ec} = 0.1$, $K_e/X_e = 15$, $X_c/X_e = 1$, within $K_e/K_c = 0.66$ and $k_{ec} = 100\ \mathrm{s}^{-1}$

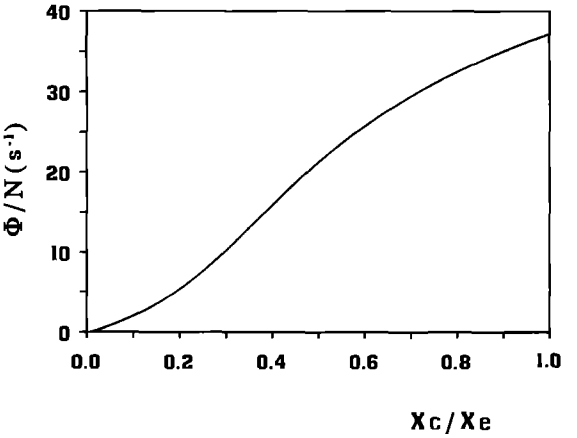

Fig. 2. Cooperative effect of a single autocatalytic step (13) on the normalized outgoing flux (ϕ/N) as a function of substrate concentration in the cytoplasm. The number of pump unities and the concentrations of ATP, ADP, P_i and x_e, are fixed through the relations: $rC_dN/k_{ec} = 200$, $k_{ce}C_p/k_{ec} = 0.05$, $\delta = 100$, $K_e/X_e = 15$, with $K_e/K_c = 0.01$ and $k_{ec} = 100\ \mathrm{s}^{-1}$

Let us now introduce two autocatalytic steps in the kinetic scheme:

$$C + x_c \underset{K_c}{\rightleftharpoons} XC \qquad (16a)$$

$$\mathrm{ATP} + XC + XE \underset{r}{\overset{p}{\rightleftharpoons}} 2XE + \mathrm{ADP} \quad \text{(autocatalysis)} \qquad (16b)$$

$$XE \underset{}{\overset{K_e}{\rightleftharpoons}} E + x_e \qquad (16c)$$

$$E + C \underset{q}{\overset{k_{ec}}{\rightleftharpoons}} 2C + P_i \ \text{(autocatalysis)} \qquad (16d)$$

In the steady state condition the set of equations is now formed by (3)–(5) and the following equation:

$$\frac{\mathrm{d}}{\mathrm{d}t}[N_c + N_{xc}] = -k_{ce} C_p N_c^2 - p C_t N_{xc} N_{xe} + k_{ec} N_e N_c + r C_d N_{xe}^2 = 0 . \qquad (17)$$

The flux from the cytoplasm to the extra-cellular medium is now given by:

$$\phi = +p C_t N_{xc} N_{xe} - r C_d N_{xe}^2 = k_{ec} N_e N_c - k_{ce} C_p N_c^2 . \qquad (18)$$

Replacing the results of (3)–(5) and (17) in (18) one finds:

$$\phi/N = N k_{ec} [\gamma_1 B - (\alpha_1 + \gamma_1) B^2] \qquad (19)$$

where

$$B = \{[(\beta_1 - \gamma_1 + 2\lambda_1)^2 + 4(\alpha_1 - \beta_1 + \gamma_1 - \lambda_1)\lambda_1]^{1/2} - (\beta - \gamma_1 + 2\lambda_1)\}/\{2(\alpha_1 - \beta_1 + \gamma_1 - \lambda_1)\} \qquad (20a)$$

and

$$\alpha_1 = (r C_d/k_{ec})/[(K_e/X_e + 1)^2] , \qquad (20b)$$

$$\beta_1 = (K_e/X_e)(K_c/X_c)/\{(K_e/X_e + 1)(K_c/X_c + 1)\} , \qquad (20c)$$

$$\gamma_1 = (p C_t/k_{ec})/[(K_e/X_e + 1) \cdot (K_c/X_c + 1)] \qquad (20d)$$

$$= \delta (K_e/K_c)(r C_d/k_{ec})(q C_p/k_{ec})/[(K_e/X_e + 1) \cdot (K_c/C_c + 1)],$$

$$\lambda_1 = (q C_p/k_{ec})[(K_c/X_c)/(K_c/X_c + 1)]^2 . \qquad (20e)$$

As before we have used (2) to display the dependence of δ in (20d).

The properties of this analytical solution is exemplified in Fig. 3. It is easily to see that besides the sigmoid curves, the increase of ATP concentrations leads to an inhibition of the flux rate. Similar behaviour was found on varying the transported substrate concentration (data not shown). This could be understood by the fact that an increase of ATP or x_c concentrations leads to an increase of XE and E, through steps (16a) and (16b), and consequently a decrease of the concentration of C, since the total number of pump molecules is kept fixed. The inhibition of the flux is due to the autocatalytic step (20d), since the conformational change $E \rightarrow C$, necessary to restart the process, is proportional to the decreasing concentration of C, as the concentrations of x_c or ATP are increasing. This kind of inhibition was not found in the reaction scheme (13), since the rate $E \rightarrow C$ in step (13d) does not depend on the C concentrations. In fact, for the reaction scheme (13), involving only one autocatalytic step, the effect of keeping the total number of pumps fixed was only to determine a limit flux as the concentrations are increased, as shown in Figs. 1, 2.

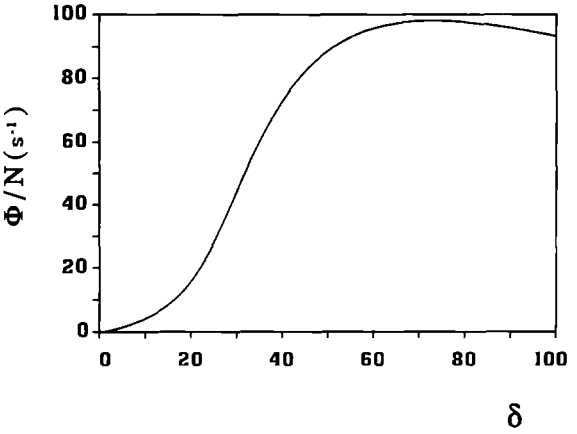

Fig. 3. The effect of combining two autocatalytic steps (17) on the normalized outgoing flux (ϕ/N) as a function of ATP concentration ($\delta = K[C_t/C_p C_d]$). The number of pump unities and the ADP, P_i and substrate concentrations, are fixed through the relations: $Nk_{ec} = 4500\ \mathrm{s}^{-1}$, $r C_d/k_{ec} = 0.2$, $k_{ce} C_p/k_{ec} = 0.002$, $K_e/X_e = 0.1$, $X_c/X_e = 1$, with $K_e/K_c = 0.02$

Positive cooperativity in the transport of substrate has being observed for most of the ATPase pumps, leading to the sigmoid flux curves such as in Fig. 2 (see for example Inesi and Hill 1983; Rossi and Garrahan 1989a; Kosck-Kosicka et al. 1990). Inhibition by the transported substrate has also been reported (Rossi and Garrahan 1989b). Further, the Ca-ATPase of the sarcoplasmic reticulum (Andersen 1989) and the Na^+/K^+ pumps (Rossi and Garrahan 1985) show a biphasic response to the concentration of ATP, ensuring cooperative behaviour, at least in a certain range of concentrations. For the proton translocating ATPase of fungi plasma membranes the sigmoid shape of the flux curve as a function of MgATP concentration was clearly demonstrated (Brooker and Slayman 1983; Bowman 1983; Koland and Hammes 1986). Inhibition by ATP and Mg was also found by the same authors.

The autocatalysis in the conformational changes of pump molecules introduced in this section is able to explain, at least qualitatively, the cooperative behaviour and the inhibition by substrate experimentally observed. These results are obtained from a very simple reaction pathway and even considering only a single binding site per pump, in contrast with previous models which considered much more complex reaction pathways (Tanford et al. 1985; Repke 1986; Pedemonte 1988; Rossi and Garrahan 1989a, b). Moreover, the normalized flux in this model has an explicit dependence on the total concentration of pump units (15) and (19). This property could be checked from experiments to decide whether the cooperativity comes from the interaction between binding sites in the same pump unit, or from the dynamic self-induced trans-conformational changes of interacting pump molecules.

We note that the model presented could be extended and adapted to describe a specific pump, by including the available data about that pump. As discussed in the previous section, some extensions are trivial, in the sense that they do not change the formal result obtained from the dynamic equations, as for example, by introducing different stoichiometries if the concentrations of the substrates are kept constant. In this case, even more complex behaviour is expected, such as biphasic flux curves. However, this task is beyond the purpose of the present paper which aims to give only a first general picture about autocatalysis in active transport systems.

Multiple stationary states

The Edelstein model for enzymatic reactions (Edelstein 1970) could be adapted to the active transport in the following way:

$$C + x_c \underset{K_c}{\rightleftharpoons} XC \tag{21a}$$

$$\mathrm{ATP} + XC + XE \overset{p}{\underset{r}{\rightleftharpoons}} 2XE + \mathrm{ADP} \quad \text{(autocatalysis)} \tag{21b}$$

$$XE \overset{K_e}{\rightleftharpoons} E + x_e \tag{21c}$$

$$E + Y \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} W \text{ (enzyme complex)} \tag{21d}$$

$$W \overset{k_3}{\underset{q}{\rightleftharpoons}} Y + C + P_i . \tag{21e}$$

In this scheme, a complex W is formed from the active enzyme E and a co-enzyme Y. The species Y could be a membrane protein or another kind of molecule present in the membrane or in the membrane environment. We then make an additional assumption that, as for the enzyme pumps, the total number Q of the co-enzymes in the system is kept fixed, leading to the following conditions:

$$N_{xc} + N_{xe} + N_e + N_c N_w = N , \tag{22}$$

$$N_y + N_w = Q . \tag{23}$$

As before, we consider (21a) and (21c) as equilibrium steps:

$$N_c/N_{xc} = K_c/X_c = K'_c , \tag{24}$$

$$N_e/N_{xe} = K_e/X_e = K'_e . \tag{25}$$

The dynamic evolution of the system is now given by the set of (22) to (25) and the following:

$$\frac{\mathrm{d}}{\mathrm{d}t}[N_{xe} + N_e] = k_1 N_{xc} N_{xe} - k_{-1} N_{xe}^2 - k_2 N_e N_y + k_{-2} N_w , \tag{26}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[N_y] = -k_2 N_e N_y + k_{-2} N_w + k_3 N_w + k_{-3} N_c N_y , \tag{27}$$

where we have introduced the definitions: $k_1 = p C_t$, $k_{-1} = r C_d$ and $k_{-3} = q C_p$.

In the stationary state condition, substituting (22)–(25) in (26) and (27) we obtain:

$$(k_1 - k_2) EC - (k_1 + k_2) E^2 + [k_2(n-q) - 1] E - C + n = 0 , \tag{28}$$

$$(k_2 + 1) EC + k_2 E^2 + C^2 + [(n-q) k_2 + k_3] E + (n - q - k_3) C + k_3 n = 0 \tag{29}$$

where we have introduced the dimensionless variables:

$$E = k_{-3} N_{xe} \{[(1 + K'_e) K'_c]/[(1 + K'_c) k_{-2}]\} , \tag{30a}$$

$$C = N_{xc} \{K'_c k_{-3}/k_{-2}\} \tag{30b}$$

and the following reduced set of dimensionless parameters:

$$q = k_{-3} Q \{K'_c/[k_{-2}(1 + K'_c)]\} , \tag{31a}$$

$$n = k_{-3} N \{K'_c/[k_{-2}(1 + K'_c)]\} , \tag{31b}$$

$$k_1 = (k_1/k_{-3})/[K'_c(1 + K'_e)] = \delta (K_c/K_e)/[(k_2/k_{-3})(k_3/k_{-2}) K'_c(1 + K'_e)] , \tag{31c}$$

$$k_{-1} = (k_{-1}/k_{-3})(1 + K'_c)/[K'_c(1 + K'_e)^2] , \tag{31d}$$

$$k_2 = (k_2/k_{-3})[K'_e(K'_c + 1)]/[K'_c(K'_e + 1)] , \tag{31e}$$

$$k_3 = (k_3/k_{-2}) + 1 . \tag{31f}$$

Owing to the additional step (21d), we note that instead of (2) the relation between kinetic and equilibrium constants is now given by:

$$\boldsymbol{K} = \frac{p K_c k_2 k_{ec}}{r K_e k_{-2} q} = \frac{C_d^e C_p^e}{C_t^e} . \tag{32}$$

This relation has been used in deriving (31c).

By combining (28) and (29) we obtain following fourth-order equation:

$$a_4 E^4 + a_3 E^3 + a_2 E^2 + a_1 E + a_0 = 0 \tag{33a}$$

where the coefficients $\{a_i\}$ are given by:

$$a_4 = (k_{-1} + k_1 k_2 - k_2^2 + k_2)(k_{-1} + k_1) , \tag{33b}$$

$$a_3 = (n-q)[k_{-1}(k_1 + k_2) + k_1 k_2 (k_1 - k_2 + 2)] + (k_1 + k_{-1})[(k_2 - 1) + k_3 (k_2 - k_1)] , \tag{33c}$$

$$a_2 = k_3 (q k_2^2 + n k_1^2 + k_1 + k_{-1}) + (n+q)(k_{-1} - k_1 k_2 k_3) + k_1 k_2 (q-n)(1 + n - q) + q(k_{-2} - 1)(k_1 - k_2) + 2n k_1 , \tag{33d}$$

$$a_1 = k_3 (q k_2 - n k_1) + n q (k_1 - k_2) + q(1 - k_2) + q^2 k_2 - n^2 k_1 , \tag{33e}$$

$$a_0 = -nq . \tag{33f}$$

The flux from the cytoplasm to the external phase in terms of the dimensionless concentrations is now given by:

$$\phi/N = (k_{-2}/n)\{k_1 CE - k_{-1} E^2\} . \tag{34}$$

Then, the flux is obtained by solving (33) and replacing the values in the expressions (28) and (34). Each solution should be tested to be physically acceptable, i.e., to correspond to real positive concentrations. For a particular set of parameters the result is shown in Fig. 4 as a function of the substrate concentration gradient. A similar curve is

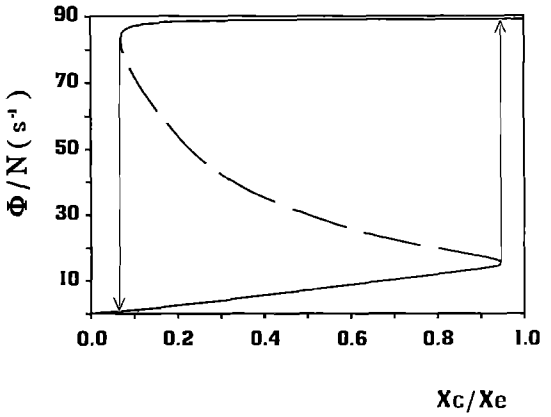

Fig. 4. Multiple steady states for the outgoing flux due to the formation of a regulatory complex combined with an autocatalytic step (21). The normalized flux (ϕ/N) is plotted as a function of the cytoplasm concentration of the transported substrate. The broken line represents unstable states, the arrows indicate the jump between stable states. The total number of pumps unities and co-enzymes, the concentrations of ATP, ADP, P_i and x_e, are fixed through the relations: $k_{-3} = qC_pN/k_{-2} = 4000$, $Q/N = 0.9$, $k_{-1}/k_{-3} = rC_d/qC_p = 20$, $k_2/k_{-3} = k_2/qC_p = 80$, $k_3/k_{-2} = 100$, $\delta = 1.23 \times 10^5$, $K_e/X_e = 0.8$, with $K_e/K_c = 6.15$ and $k_2 = 1\ s^{-1}$

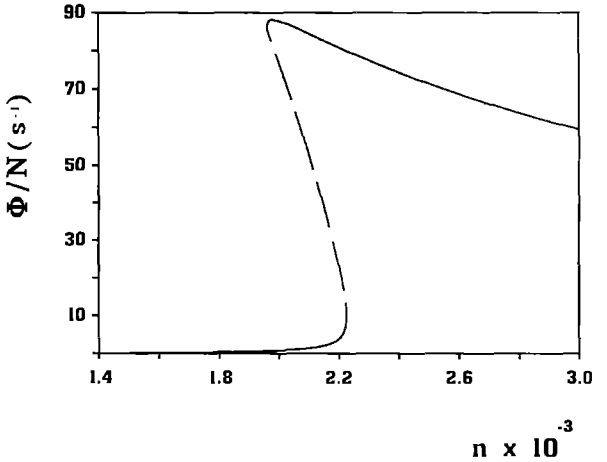

Fig. 5. Two pumping regimes for the outgoing flux of substrate show as a function of pump concentration ($n = [qC_pN/k_{-2}]/[K_c/(1+K_c)]$) resulting from the combined effect of the presence of a co-enzyme and autocatalytic step (see (21)). The broken line represents unstable states. The total number of co-enzymes, the concentrations of ATP, ADP, P_i and substrate are fixed through the relations: $k_{-3} = qC_pQ/k_{-2} = 3600$, $k_{-1} = rC_d/qC_p = 20$, $k_2/k_{-3} = k_2/qC_p = 80$, $k_3/k_{-2} = 100$, $\delta = 2 \times 10^4$, $K_e/X_e = 1$, $X_c/X_e = 1$, with $K_e/K_c = 1$ and $k_{-2} = 1\ s^{-1}$

found by varying the ATP concentrations (data not shown). In both cases, there is a region of multiple stationary states presenting three solutions ($\Phi 1 < \Phi 2 < \Phi 3$) for each value of X_e/X_c or δ. We have performed a linear stability analysis of the investigated stationary solutions, in particular those corresponding to Figs. 4, 5. We have shown that in all cases the lower ($\Phi 1$) and the upper ($\Phi 3$) branches are always stable and that the intermediate solution $\Phi 2$ is always unstable (Weissmüller G. 1989, Ms.C. Thesis, CBPF, Rio de Janeiro). This means that a continuous increase of δ leads to a finite jump from the lower to the upper branch. On the other hand, starting on the upper branch a decrease of δ leads to a jump from the upper to the lower branch.

This kind of *hysteresis* can be associated with the regulation mechamism of the pump. Starting from small cytoplasmic concentrations ($X_c/X_e < 10^{-3}$ in Fig. 4), a large increase of concentration ($X_c/X_e < 10^{-3}$ in Fig. 4) could lead to an increase of the flux of the order of ten compared with the initial situation. The increase of the outgoing flux tends to diminish rapidly the cytoplasmic concentration and then to restore the initial situation of smaller concentrations. This provides the pump with a mechanism to stabilize the cytoplasmic concentrations around a fixed value, even when submitted to a sudden large increase of substrate concentrations.

General discussion

We have started with the simplest scheme for the alternating access model to describe the active transport driven by ATP enzymes. This model reproduces the essential kinetic and thermodynamic properties of translation of molecules against their own concentration gradients. The flux versus concentration curves show a Michaelis-Menten like behaviour as for normal enzyme kinetics.

Introducing a single autocatalytic step in the reaction scheme makes the flux versus substrate concentration curves exhibit a sigmoid shape typical of cooperative behaviour. However, since the catalytic cooperative involves the interaction between two pump units the sigmoid behaviour is found simultaneously for varying substrate and ATP concentrations, even for the one to one stoichiometry considered. Furthermore, two autocatalytic steps lead to an inhibition by substrate. Although a direct comparison between the model and the available experimental data requires further investigation, it should be stressed that the general features of the model are recovered in the experiments as discussed in the previous sections. Indeed, for example, in the experiments with membrane-bound Ca-ATPase of the sarcoplasmic reticulum, besides the sigmoid curves found as a function of Ca concentration (Inesi and Hill 1983), a secondary cooperative rise in the millimolar concentration range of ATP was observed (Andersen 1989). The above findings are in contrast with previous models which propose cooperativity and/or competition between binding sites at the same transport unit to explain the complex behaviour of the pumps (Bowman 1983; Repke 1986; Andersen 1989). Another way to reproduce sigmoid behaviour of the flux and inhibition by substrate is to introduce more complex reaction schemes involving competitive reaction pathways (Tanford et al. 1985; Pedemonte 1988; Brzezinski et al. 1988). However, in this case it would be necessary to introduce many unknown kinetic constants which could be difficult to obtain from experimental data.

When a regulatory complex is introduced in the reaction scheme, we find a domain of multiple steady states for varying both ATP and substrate concentrations, leading to a *hysteresis* and a finite jump between two quite different pumping regimes (see Fig. 4). The difference between these two regimes is explicitly demonstrated as a

function of the pump concentration in Fig. 5. This behaviour was not found in the usual models presented in the literature. Further, the model suggests a new mechanism of regulation of the pumps. To our knowledge, this is the first time that a model for active transport predicts a self-regulation mechanism for the transport of substrate. The *hysteresis* cycle and the regulation mechanism could be tested in experiments able to maintain both ATP concentrations and transported substrate gradients in far from equilibrium conditions.

The present approach deals with relatively simple kinetic schemes and a small number of parameters. The main difference from the previous models is that the flux behaviour depends on the pump concentration. The flux per pump unit Φ/N is a function of the density of pumps in the autocatalytic models presented here, in contrast with the previous models where the pump function depends only on the pump unit and, as a concequence, Φ/N is independent of concentration. This property could be eventually be checked from experiments where one is able to control the membrane pump concentrations. There is one example where the change of the flux regime could be attributed to a change of the pump interactions in experiments with purified Ca-ATPase from erythrocyte membranes. This is an in vitro system of mixed phospholipid/detergent micelles, which should mimic the in vivo conditions (Kosk-Kosicka et al. 1990). It was shown that ATP hydrolysis is a function of the enzyme concentration when the detergent concentration was kept fixed and the molar ratio of phospholipid to enzyme was constant (Kosk-Kosicka and Bzedega 1988). Based on fluorescence energy transfer experiments the authors attributed the change of pumping regime to a Ca-dependent self-association of the enzyme (Kosk-Kosicka et al. 1989). Although the activation observed at high concentrations could be due to the formation of stable oligomers, probably dimers, as suggested by the authors, the kinetic effects can also be explained in the context of the present model. First, it should be remarked that autocatalysis is a highly cooperative dynamic process and a strong self-associative mechanism, involving a dynamic formation of metastable dimers. Further, as should be deduced from the present findings, the autocatalysis produces sigmoid cooperative flux curves, and when combined with the formation of as regulatory complex, produces sharp transitions between different pumping regimes, as in the experiments. Unfortunately, in the literature there is still little information on the pump concentration dependence of the flux in native or reconstructed lipid membranes.

The kinetic cooperative steps introduced in the model are able to reproduce the complex behaviour observed in the function of some biological pumps, and suggest a new mechanism of self-control of the pumps. This theoretical procedure has the advantage of explaining, at least qualitatively, the essential behaviour of the ATP pumps within a still restricted number of parameters. The idea of enzymatic autocatalytic and regulatory kinetics introduces a new point of view of pump function and should be extended to analyze more complex and more realistic situations, to be compared with specific experimental data on pump function in biological systems.

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