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AUTOCATALYTIC COOPERATIVITY AND SELF-REGULATION OF ATPase PUMPS IN MEMBRANE ACTIVE TRANSPORT

by

G. WEISSMÜLLER and P.M. BISCH

ABSTRACT

We investigate the effect of autocatalysis on the conformational changes of membrane pumps during the active transport driven by ATP. The translocation process is assumed to occur by means of an alternating access mechanism. 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 behavior was reported, for example, in the proton–translocating ATPase of fungi (Bowman, 1983), in the Ca–ATPase of the sarcoplasmic reticulum (Adersen, 1989) and in the Na^+/K^+ pumps (Repke, 1986). Positive and negative cooperativity, variable stoichiometry, and inhibition by the substrate are experimentally observed in these systems. These behaviors are explained in different ways for different pumps, by for example, assuming cooperativity between the ligand biding sites (Bowman, 1983), or by taken into account the formation of cooperative oligomeric unities (Klingenberg, 1981; Andersen, 1989), 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 behavior of each kind of pump, there is still lacking a general picture to be able to give a unified view and to explain the general trends common to all pumps.

Usually the models presented in the literature involve only one pump in each kinetic step and cooperativity is related to the binding of transported substrate or ATP. The cooperative interactions between enzime monomers are taken into account only by the equilibrium formation of dimers or oligomers, which 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, like the 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 like 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 then 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 due 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 concentration is much less than 10^{-2}s ($\tau << 1/D\Gamma$), which is of the magnitude of the turnover time of the pumps (Serrano, 1988). This means that the collision rate is larger than the turnover rate of the complete reaction cycle, justifying then the autocatalytic interaction during conformational changes.

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

In this article we intend to discuss the general kinetic properties resulting from autocatalytic cooperativity in membrane active transport. We are particularly interested in the existence of multiple stationary state regimes which should be related, as will be shown below, to self–regulation mechanism of the active transport.

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;

$$C + x_c \underset{K_c}{\rightleftharpoons} XC \qquad (1.a)$$

$$ATP + XC \underset{r}{\overset{p}{\rightleftharpoons}} XE + ADP \qquad (1.b)$$

$$XE \overset{K_e}{\rightleftharpoons} E + x_e \qquad (1.c)$$

$$E \underset{q}{\overset{k_{ec}}{\rightleftharpoons}} C + P_i \qquad (1.d)$$

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 subsequently release of P_i to begin the next cycle. This model is based on the following assumptions:

1) The phosphorilation and dephosphorilation 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 phosphorilation of the pumps occurs only by the ATP, consuming only one ATP molecule per cycle, direct phosphorilation by phosphate is neglected.

In solving the dynamic equations further simplifications are introduced;

4) The steps (1a) and (1c) are considered to be fast compared with steps (1b) and (1d), 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 $C_d^e C_p^e / C_t^e = K$, where K is the equilibrium constant of the ATP hydrolysis ($K \approx 10^5$ 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 K (Läuger, 1984);

$$\frac{C_d^e \; C_p^e}{C_t^e} = \frac{pK_c k_{ec}}{rK_e q} = K \qquad (2)$$

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

$$N_{xc} + N_{xe} + N_e + N_c = N \qquad (3)$$

$$N_c / N_{xc} = K_c / X_c \qquad (4)$$

$$N_e / N_{xe} = K_e / X_e \qquad (5)$$

$$\frac{d}{dt}[N_c + N_{xc}] = -qC_p N_c - pC_t N_{xc} + k_{ec} N_e + rC_d N_{xe} \qquad (6)$$

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

$$\phi = + pC_t N_{xc} - rC_d N_{xe} = + qC_d N_c - k_{ec} N_e \qquad (7)$$

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

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

where

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

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

and

$$D = pC_t + rC_d + (rC_d+qC_p)(K_c/X_c) + (p+k_{ec})(K_e/X_e) + (qC_p+k_{ec})(K_eK_c)/(X_eX_c) \tag{11}$$

Equation (9) defines the affinity $\mathcal{A}$ 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.

Eq. (8) gives the usual behavior 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 ($C_tK >> C_dC_p$, $\delta >> 1$) could leads 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 behavior. In fact by assuming constant concentrations C_p, C_d and X_e, Eq.(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. Eq. (12) reproduces a Michaelis–Menten process if we consider the concentration excess δ–1 (for v=1) or v–1 (for δ=1), as the concentration variables. This simple model leads then to the usual enzyme kinetics without any kind of cooperativity.

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

i) The phosphorylation of the protein by the inorganic phosphate (P_i) or the 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 subtrate and P_i or ATP binding, if this binding is made in only one step, leading to the usual cooperative behavior (Bowman, 1983).

iii) The cotransport of a second substrate, if its concentrations are also considered as external controlled and if the exchange with the first substrate is made in a single step, as usually consider 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 transmenbrane potencial (Läuger, 1984).

v) The ordering, where phosphorilation 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 behavior of the pumps 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 phosphorilation-dephosphorilation 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 reaction 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 model and their extensions have been used successfully to explain many of the properties of the pumps. However, the usual models consider only one single pump unit in each dynamic step. To our knowledge, the question whether the dynamic interaction between pumps could reproduce the observed complex behaviour has never been adressed. 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 Equation (1).

AUTOCATALYTIC COOPERATIVE TRANSPORT

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

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

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

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

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

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

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

Using Eq.(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,

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

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

$$\gamma = (pC_tN/k_{ec})/[(K_e/X_e+1)(K_c/X_c+1)]$$
$$= \delta(rC_dN/k_{ec})(K_e/K_c)(qC_p/k_{ec})/[(K_e/X_e+1)(K_c/X_c+1)] \tag{15.c}$$

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

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

The flux curves for this model are given in Figures 1 and 2 for typical values of the parameters. Fig. 1 shows the normalized flux of one pump molecule ϕ/N as a function of $\delta = (KC_t)/(C_dC_p)$ 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 behavior 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 for both ATP and substrate varying 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 (13.d), instead of in step (13.b), a similar result is found. It is also important to note that α and γ in Eq.(15) depend explicitly on the total number of pumps (N), which means that, contrary to the usual cooperative models, the normalized rate of turnover depends on the concentration of pump molecules.

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

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

$$ATP + XC + XE \underset{r}{\overset{p}{\rightleftharpoons}} 2XE + ADP \quad \text{(auto–catalysis)} \qquad (16.b)$$

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

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

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

$$\frac{d}{dt}[N_c+N_{xc}] = -k_{ce}C_pN_c^2 - pC_tN_{xc}N_{xe} + k_{ec}N_eN_c + rC_dN_{xe}^2 = 0 \qquad (17)$$

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

$$\phi = +pC_tN_{xc}N_{xe} - rC_dN_{xe}^2 = k_{ec}N_eN_c - k_{ce}C_pN_c^2 \qquad (18)$$

Replacing the results of Eqs. (3)–(5) and (17) into Eq.(18) one finds;

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

where,

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

and

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

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

$$\gamma_1 = (pC_t/k_{ec})/[(K_e/X_e+1)\cdot(K_c/X_c+1)] = \delta(K_e/K_c)(rC_d/k_{ec})(qC_p/k_{ec})/[(K_e/X_e+1)\cdot(K_c/X_c+1)] \qquad (20.d)$$

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

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

The properties of this analytical solution are shown in Figs. 3 and 4. It is easily to see that besides the sigmoid curves, the increase of concentrations of both the ATP and the transported substrate leads to an inhibition of the flux rate. 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 the steps (16.a) and (16.b), 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 (20.d) since the conformational change E → 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 → C in step (13.d) does not depend on the C concentrations. In fact, for the

reaction scheme (13), involving only one autocatalitic 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 and 2.

The autocatalysis in the conformational changes of pumps molecules introduced in this section is able to explain, at least qualitatively, the cooperative behavior 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 considering much more complexe reaction pathways (Tanford, 1985; Tanford *et al.* 1985; Repke, 1986; Pedemonte, 1988). Moreover the normalized flux in this model has an explicit dependence on the total concentration of pump units (Eqs. (15), (19) and (20)). 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.

MULTIPLE STATIONARY STATES

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

$$C + x_c \underset{K_c}{\rightleftharpoons} XC \qquad (21.a)$$

$$ATP + XC + XE \underset{r}{\overset{p}{\rightleftharpoons}} 2XE + ADP \quad \text{(auto–catalysis)} \qquad (21.b)$$

$$XE \overset{K_e}{\rightleftharpoons} E + x_e \qquad (21.c)$$

$$E + Y \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} W \quad \text{(enzime complex)} \qquad (21.d)$$

$$W \underset{q}{\overset{k_3}{\rightleftharpoons}} Y + C + Pi \qquad (21.e)$$

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 make then an additional assumption that, like 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 (21.a) and (21.c) 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 equations (22) to (25) and the following equations;

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

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

where we have introduced the definitions; $k_1=pC_t$, $k_{-1}=rC_d$ and $k_{-3}=qC_p$.

In the stationary state condition, replacing Eqs. (22) – (25) into Eqs. (26) and (27) we obtain;

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

$$(k_2+1)EC + k_2E^2 + C^2 + [(n-q)k_2+k_3]E + (n-q-k_3)C + k_3 n = 0 \tag{29}$$

where we have introduce the dimensionless variables;

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

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

and the following reduced set of dimensionless parameters;

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

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

$$k_1 = (k_1/k_{-3})/[K'_c(1+K'_e)]$$

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

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

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

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

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

$$K = \frac{pK_c k_2 k_{ec}}{rK_e k_{-2} q} = \frac{C_d^e\ C_p^e}{C_t^e} \tag{32}$$

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

By combining Eqs.(28) and (29) we obtain following forth order equation;

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

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

$$a_4 = (k_{-1}+k_1k_2-k_2^2+k_2)(k_{-1}+k_1) \tag{33.b}$$

$$a_3 = (n-q)[\ k_{-1}(k_1+k_2) + k_1k_2(k_1-k_2+2)\ ] +$$

$$(k_1+k_{-1})[\ (k_2-1) + k_3(k_2-k_1)\ ] \tag{33.c}$$

$$a_2 = k_3(qk_2^2+nk_1^2+k_1+k_{-1}) + (n+q)(k_{-1}-k_1k_2k_3) +$$

$$k_1k_2(q-n)(1+n-q) + q(k_{-2}-1)(k_1-k_2) + 2nk_1 \tag{33.d}$$

$$a_1 = k_3(qk_2-nk_1) + nq(k_1-k_2) + q(1-k_2) + q^2k_2 - n^2k_1 \tag{33.e}$$

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

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

$$\phi/N = (\ k_{-2}/n\ )\ \{\ k_1CE - k_{-1}E^2\ \} \tag{34}$$

Then, the flux is obtained by solving equation (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.5 as a function of ATP concentration, and in Fig.6 by varying the substrate concentration gradient. In both cases, there is a region of multiple stationary states presenting three solutions ($\Phi1<\Phi2<\Phi3$) for each value of δ or X_e/X_c. We have performed a linear stability analysis of the investigated stationary solutions, in particular those corresponding to Figs. 5, 6 and 7. We have shown that in all cases the lower ($\Phi1$) and the upper ($\Phi3$) branches are always stable and that the intermediate solution $\Phi2$ 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 mechanism of the pump. Starting from small cytoplasm concentrations ($X_c/X_e<10^{-3}$ in Fig.6), a large increase of concentration ($X_c/X_e>10^{-3}$ in Fig.6) 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 rapidly diminish the cytoplasm concentration and then to restore the initial situation of smaller concentrations. This provides the pump with a mechanism to stabilize the cytoplasm 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 thermodynamics properties of translocation of molecules against its own concentration gradients. The flux versus concentration curves show a Michaelis–Menten like behavior as the normal enzyme kinetics.

Introducing a single autocatalytic step on the reaction scheme, the flux versus substrate concentration curves exhibit a sigmoid shape typical of cooperative behavior.

However, since the catalytic cooperativity involves the interaction between two pumps units the sigmoid behavior is found simultaneously for varying substrate and ATP concentrations, even for the one to one stoichiometry considered. Furthermore, two auto–catalytic steps lead to an inhibition by substrate. These findings are in contrast with previous models which suppose cooperativity and/or competition between binding sites at the same transport unit to explain the complex behavior of the pumps (Bowman, 1983). Another way to reproduce sigmoid behavior of the flux and inhibition by substrate, is to introduce more complex reaction schemes involving competitive reaction pathways. (Tanford *et al.*1985; Pedemonte,1988). However in this case it should 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 both ATP and substrate varying concentrations, leading to a *hysteresis* and a finite jump between two quite different pumping regimes (see Figs. 5 and 6). The difference between these two regimes is explicitly demonstrated as a function of the pump concentration in Fig. 7. This behavior was not found in the usual models presented in the literature. 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 present approach deals with a relatively simple kinetic scheme and a small number of parameters. The main difference from the previous models is that the flux behavior depends on the pump concentration. The flux per pump unity 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 functioning depends only on the pump unit and, as a consequence, Φ/N is independent of concentration. This property eventually could be checked from experiments able to control the membrane pump concentrations. To our knowledge there is at least one experiment where the change of the flux regime could be attributed to change of the pump interactions. It was shown that detergent–solubilized monomers of Ca–ATPase isolated from sarcoplasmic reticulum exhibit quite different

behavior when the rate of ATP hydrolysis is compared with the membrane bound pumps (Andersen, 1989). For the model involving the formation of an enzyme complex, there are two regimes of the pump functioning as clearly shown in Fig.7, like in the Andersen experiments. Although this experiment can not be considered as a definitive proof of the model, since the behaviour of the momomer could be attributed to the different enviroment provide by the detergent, the proposed model gives an alternative interpretation of the experimental results. Unfortunatly, to our knowledge, in the literature there is no systematic experimental investigation of the pump concentration dependence of the fluxes in native or reconstructed lipid membranes.

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

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FIGURE CAPTIONS

FIG.1– Cooperative effect of a single autocatalytic step (Eq.13) on the normalized outgoing flux of the transported molecule (ϕ/N) as a function of ATP concentrations ($\delta = KC_t/C_pC_d$). The number of pump unities and the ADP, Pi 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 $k_{ec}=100\ s^{-1}$.

FIG.2– Cooperative effect of a single autocatalytic step (Eq.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, Pi 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 $k_{ec}=100\ s^{-1}$.

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

FIG.4– Self–inhibition of the normalized outgoing flux (ϕ/N) of the transported substrate due to the effect of combining two auto–catalytic steps (Eq.17). The number of pump unities, the concentrations of ATP, ADP, Pi and x_e, are fixed through the relations; $Nk_{ec}=2000s^{-1}$, $\delta=100$, $rC_d/k_{ec}=0.2$, $k_{ce}C_p/k_{ec}=0.002$, $K_e/X_e=0.1$, with $K_e/K_c=0.02$.

FIG.5– Multiple steady states for the outgoing flux due to the formation of a regulatory complex combined with an autocatalytic step (see Eq.21). The normalized flux (ϕ/N) is plotted as a function of ATP concentration ($\delta = K[C_t/C_pC_d]$).The broken line represents unstable states, the arrows indicate the jump between stable states. The total number of co–enzymes and pump unities, and the concentrations of ADP, Pi and substrate, are fixed through the relations; $k_{-3}=qC_pN/k_{-2}=4000$, $Q/N=0.9$, $k_{-1}/k_{-3}=rC_d/qC_p=20$, $k_2/k_{-3}= k_2/qCp= 80$, $k_3/k_{-2}=100$, $K_e/X_e=1$, $X_c/X_e=1$, with $K_e/K_c=0.02$ and $k_2=1s^{-1}$.

FIG.6– Multiple steady states for the outgoing flux due to the formation of a regulatory complex combined with an autocatalytic step (Eq.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, Pi and x_e, are fixed through the relations; $k_{-3}=qC_pN/k_{-2}=4000$, $Q/N=0.9$, $k_{-1}/k_{-3}= rC_d/qC_p=20$, $k_2/k_{-3}=k_2/qCp=80$, $k_3/k_{-2}=100$, $\delta=1.23\times10^5$, $K_e/X_e=0.8$, with $K_e/K_c=6.15$ and $k_2=1s^{-1}$.

FIG.7– 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 an autocatalytic step (see Eq.21). The broken line represents unstable states. The total number of co–enzymes, the concentrations of ATP, ADP, Pi and substrate are fixed through the relations; $k_{-3}=qC_pQ/k_{-2}=3600$, $k_{-1}=rC_d/qC_p=20$, $k_2/k_{-3}=k_2/qCp=80$, $k_3/k_{-2}=100$, $\delta=2\times10^4$, $K_e/X_e=1$, $X_c/X_e=1$, with $K_e/K_c=1$ and $k_{-2}=1s^{-1}$.

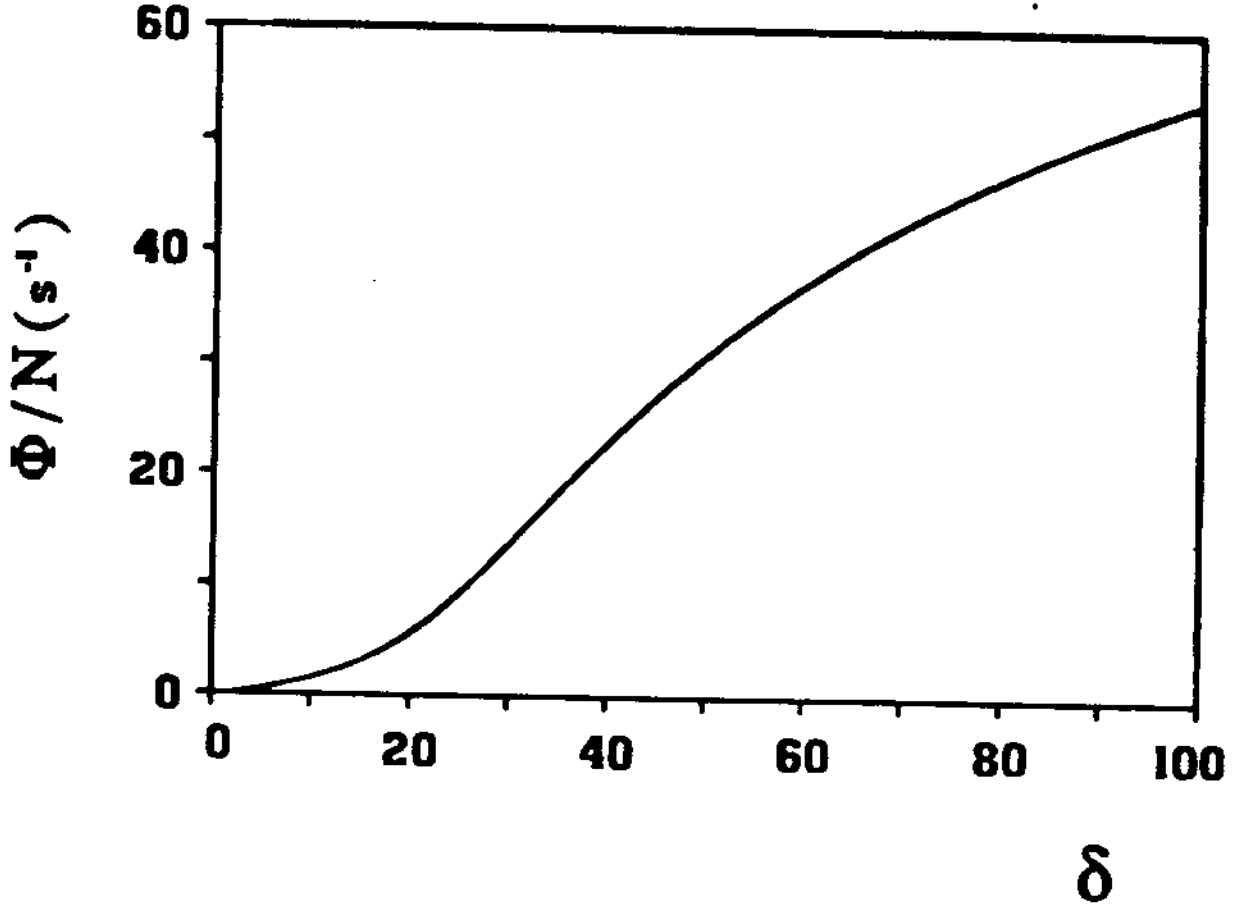

Fig.1

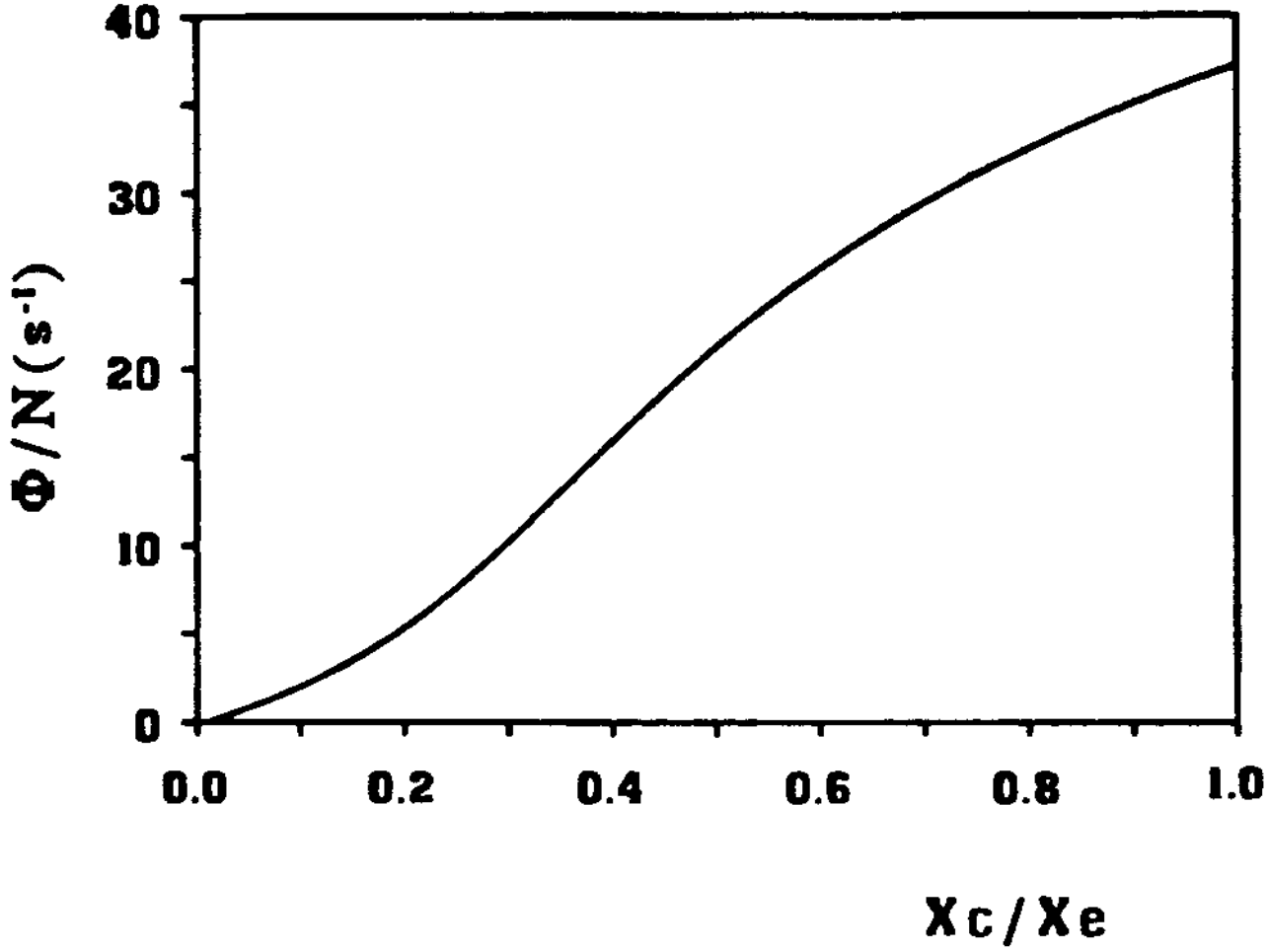

Fig.2

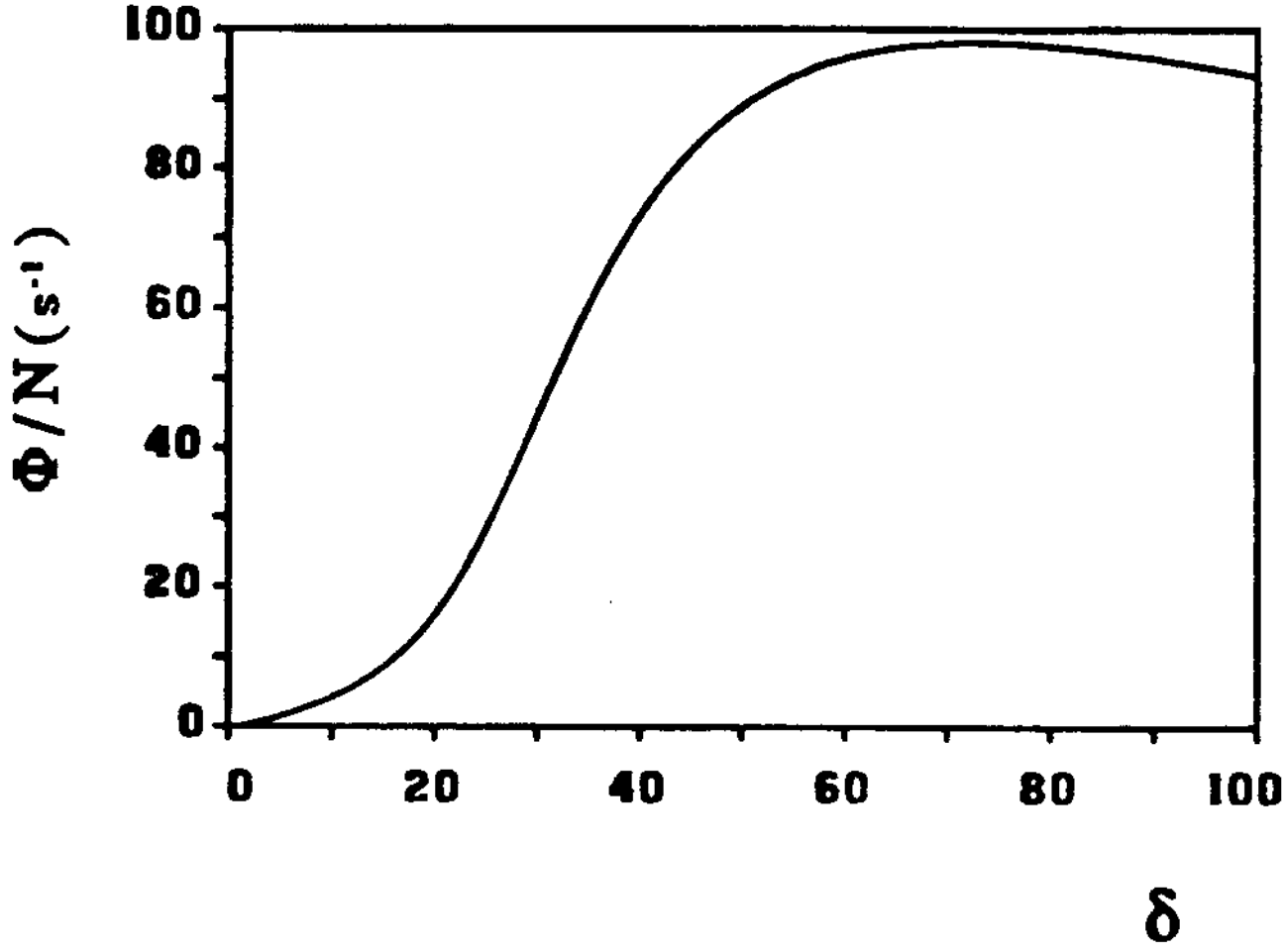

Fig.3

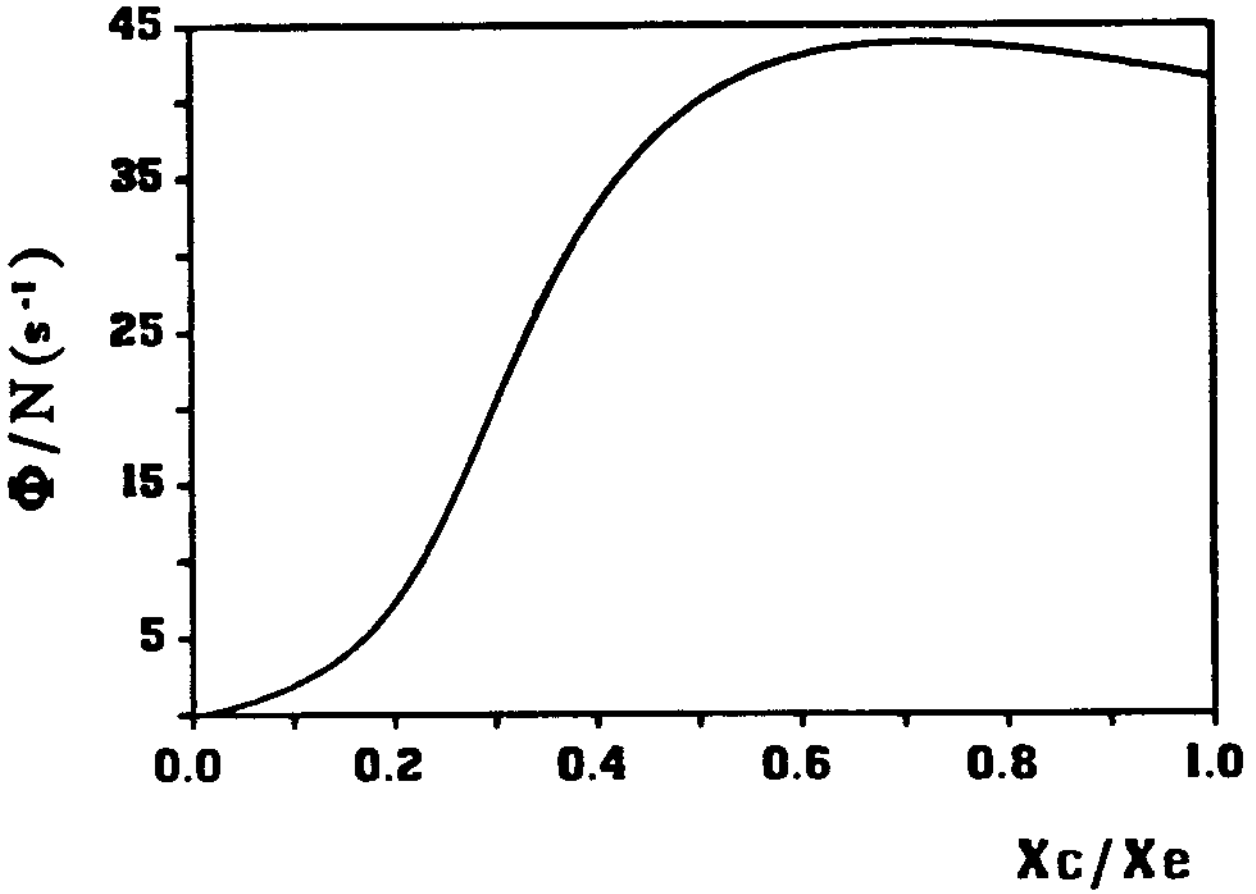

Fig.4

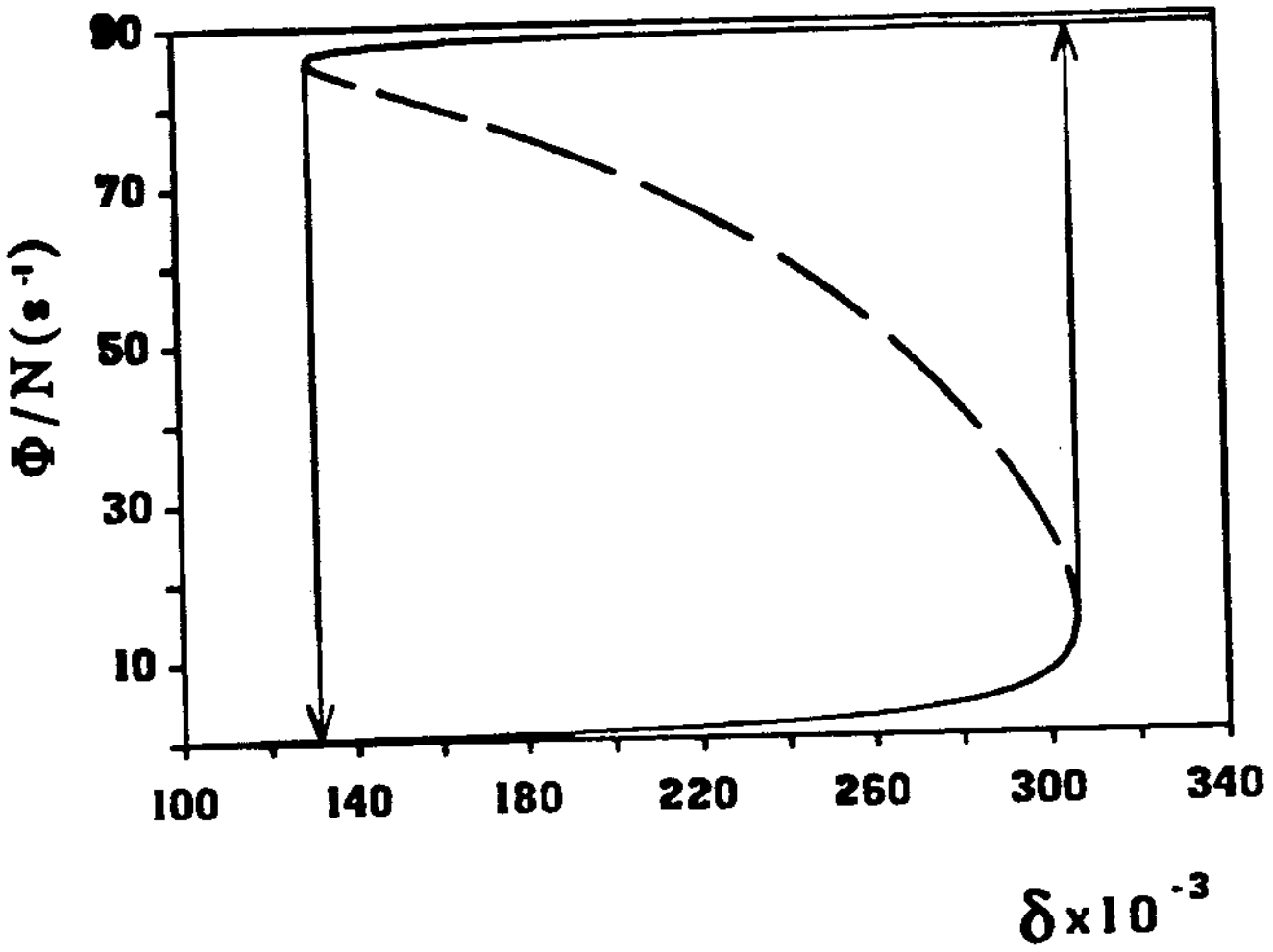

Fig.5

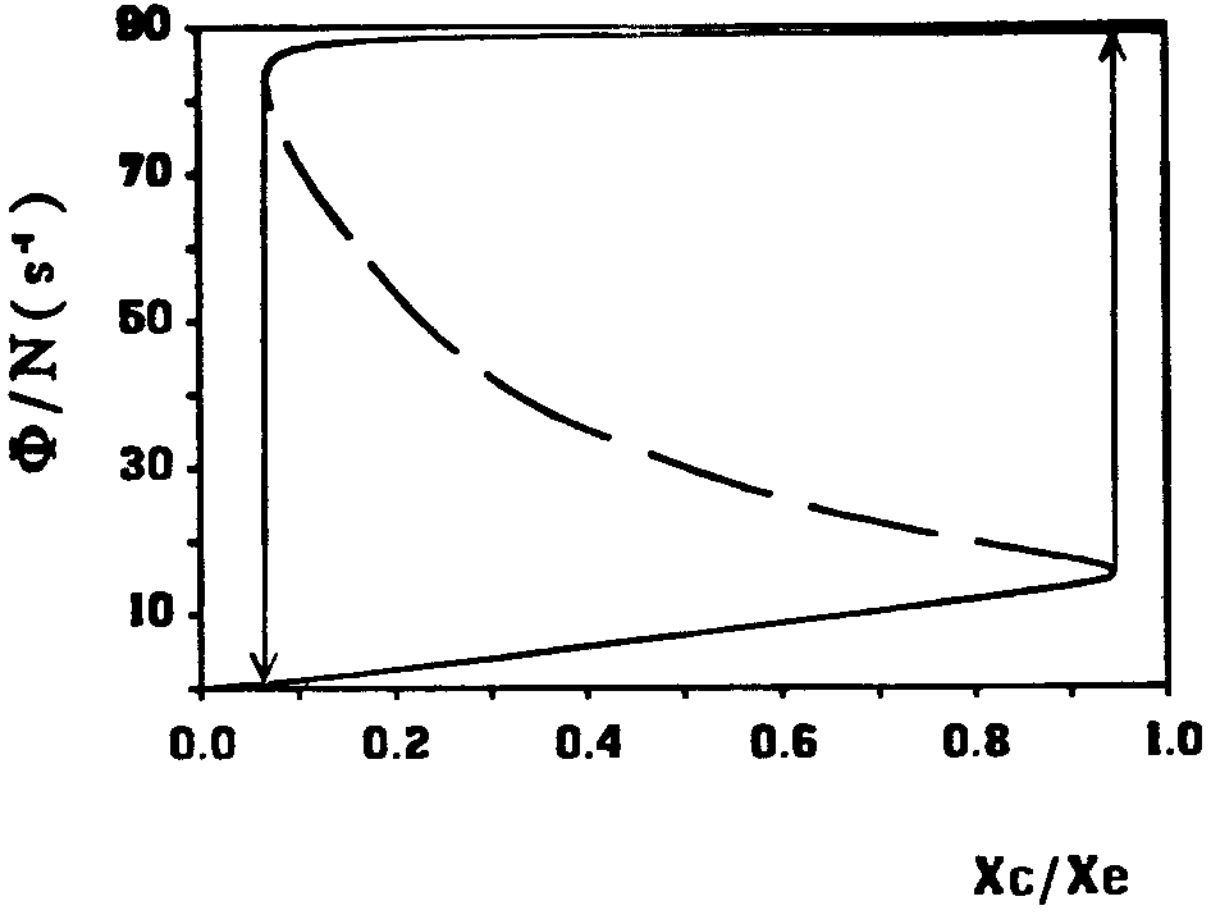

Fig.6

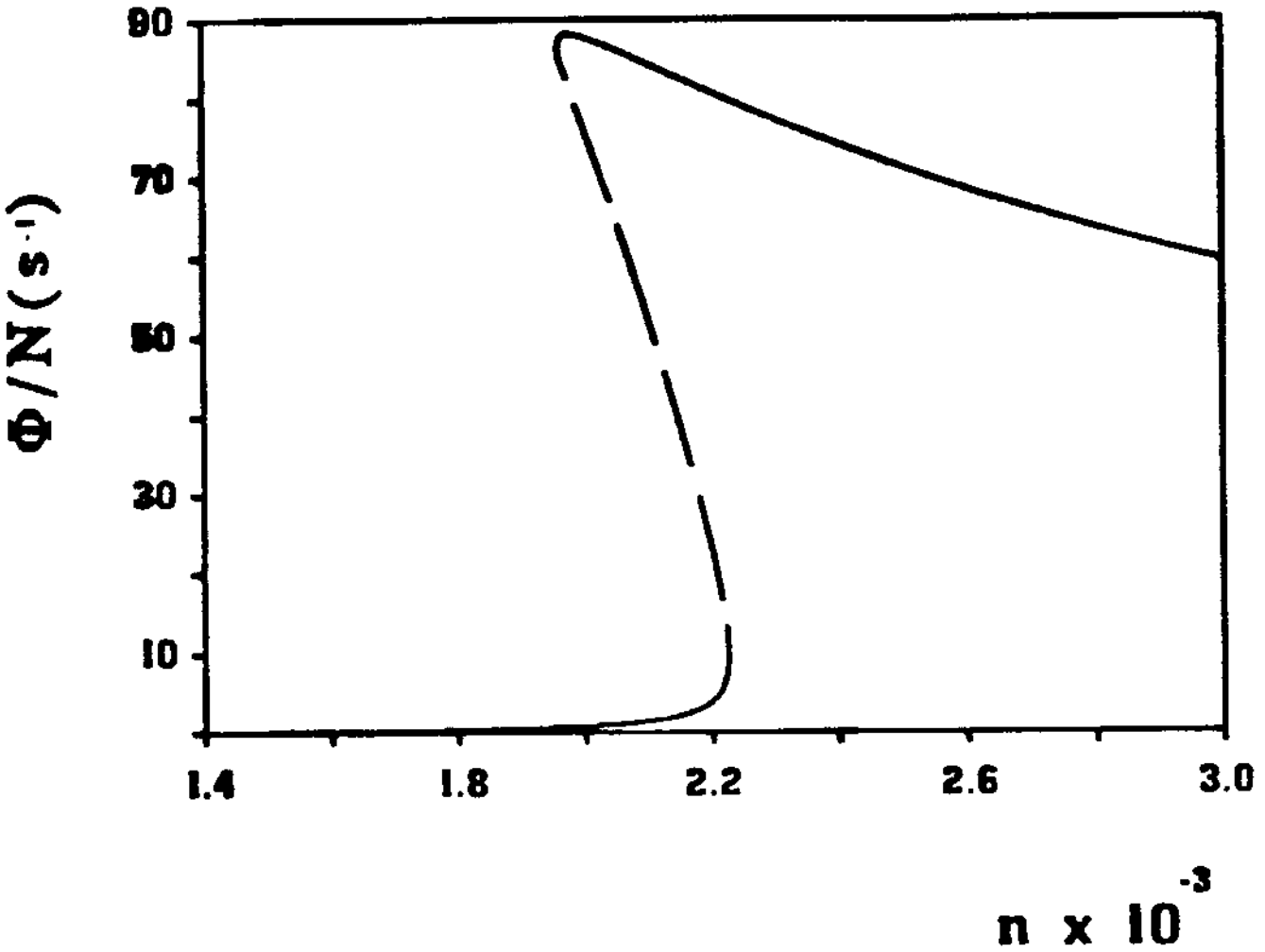

Fig.7

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