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OSCILLATIONS IN VESICULAR COMPARTMENTS

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The oscillatory phenomena which occur in metabolic processes are of great interest to chemists and biologists for a better understanding of far-from-equilibrium behavior that exists in biological systems. In this paper, we present a model for metabolic oscillations in a vesicular compartment and show how oscillations of the components involved in an energy transducing system may arise from our model under certain external conditions.

1. Introduction

Cellular respiration is a membrane bound process involving the electron transfer system and phosphorylation site. A long train of virtually undamped oscillation of H^+ and K^+ concentrations, of the rate of O_2 uptake, of the volume of the mitochondrion, and of the redox state of cytochrome b, pyridine nucleotide, and flavoprotein has been observed during cellular respiration [1–3]. The requirements for generating the oscillation are [4]: (a) an energy source, derived from either substrate oxidation or ATP hydrolysis; (b) alkali cations, which have been made permeable to the inner membrane by EDTA treatment or by presence of ionophores; (c) weak acid anions, such as acetate or phosphate; (d) an osmotic support, such as sucrose, which is impermeable to the inner membrane; (e) an optimum *pH*: and (f) intact, coupled mitochondria.

It appears that the adenine nucleotide system and the rate of ion transport are the controlling factors for the oscillations, whereas a proton gradient across the membrane serves as a positive feed-back [2]. A crossover point between flavoproteins and cytochrome b suggests that a carrier or a site enzyme [5,6] located between the two is responsible for the oscillation in the ion movement. The oscillations of ADP/ATP ratio in the mitochondrial matrix, however, suggest that the reverse ATPase is also involved in generating the oscillation.

The phenomenon of oscillatory transport of ions in mitochondria is valuable for elucidating bioenergetic aspects of cellular metabolisms. Although oscillations in mitochondria have attracted a great deal of attention, no theoretical attempt to explain them has been made to the present time. In this note we present a model of a coupled enzyme system in a vesicular membrane, which generates oscillations in *pH* and metabolic concentrations.

2. Model

Let us consider a small vesicle of volume V, with three kinds of systems, an enzyme system, a proton-carrier system, and a product–substrate exchange system, embedded in a vesicular membrane (see fig. 1). Each of these systems behaves independently of the rest except for the fact that the enzyme system transports protons from outside (or inside) to inside (or outside), whereas the proton-carrier system transports protons by a simple diffusional process. The product produced inside by an enzyme system is exchanged with the substrate coming from outside by the product–substrate exchange system.

In order to simplify a mathematical formulation, let us make the following plausible assumptions for the properties of these complexes: (i) An enzyme system

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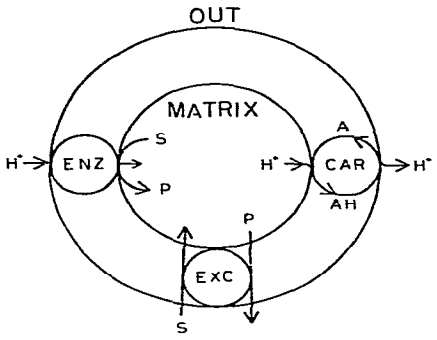

Fig. 1. A schematic representation of the main events occurring in a vesicular compartment, e.g. in a mitochondrion. Here, ENZ, CAR, and EXC stand for an enzyme system, a proton-carrier system, and a product–substrate exchange system.

contains a Bohr proton such that its substrate has a preferential binding to a protonated form of the enzyme. That is, the apparent dissociation constant for the substrate depends on the pH inside. The substrate binding step is assumed to occur very fast, whereas the release of a proton to the matrix occurs slowly. In a formal way, this process may be expressed as

$$\begin{array}{ccccc} & & \mathrm{EH} & \overset{K_S}{\rightleftharpoons} & \mathrm{EHS} \overset{k}{\longrightarrow} \mathrm{EH} \\ \mathrm{H^+_{out}} + \mathrm{S_{in}} + & & \Big\uparrow\Big\downarrow K_a \quad K'_a & & \Big\uparrow \quad \Big\downarrow \\ & & \mathrm{E} & \rightleftharpoons & \mathrm{ES} \overset{k}{\longrightarrow} \mathrm{E} \end{array} + \mathrm{P_{in}} + \mathrm{H^+_{in}}, \qquad (1)$$

where S_{in} and P_{in} stand for the substrate and product in the compartment, respectively, and H^+_{out} and H^+_{in} stand for the hydrogen ion outside and inside the compartment, respectively. Note that we are concerned here with a particular case where the enzyme system transports a proton from outside to inside. It should, however, be pointed out that the derivations and results for the other case (i.e. a proton transport from inside to outside) are essentially the same as those for the case presented here. It should also be pointed out that, for the substrate-supported or ATP-supported oscillations in mitochondria [7], the other case is of more interest.

(ii) There is a proton binding site in each proton-carrier system. The protonation step occurs very fast; however, transport of a proton by a carrier across the membrane is a slow process. The process may be summarized as

$$\begin{array}{ccc} & \mathrm{A_{in}} \overset{\beta}{\underset{\beta}{\rightleftharpoons}} \mathrm{A_{out}} & \\ \mathrm{H^+_{in}} \Big\downarrow\Big\uparrow & K_A \quad K_A & \Big\downarrow\Big\uparrow \mathrm{H^+_{out}}. \\ & \mathrm{AH_{in}} \overset{\beta}{\underset{\beta}{\rightleftharpoons}} \mathrm{AH_{out}} & \end{array} \qquad (2)$$

Here, β is a jumping rate constant [8,9], and K_A is the acid dissociation constant.

(iii) The product inside and substrate outside are exchanged by a third kind of membrane system with the following process:

$$\mathrm{S_{out}} + \mathrm{P_{in}} \overset{\alpha}{\rightarrow} \mathrm{S_{in}} + \mathrm{P_{out}}. \qquad (3)$$

Oxidative phosphorylation in mitochondria provides an example for our model: The vesicular interior is the mitochondrial matrix, an enzyme system is ATPase (that is, in the case of ATP-supported oscillation [7]), a proton-carrier system is the P_i carrier or a permeant acid anion, and the ATP–ADP exchange is accomplished by the ADN carrier.

3. Mathematical expressions based on the model

Since a detailed mathematical derivation and a discussion on the condition for metastability on related problems appeared elsewhere [8,9], we omit details but discuss how the following expressions come from the processes (i)–(iii) above.

According to the processes (i) and (iii), the change of the substrate and product concentrations with respect to time may be expressed by the following equations:

$$\mathrm{d}[\mathrm{S_b}]_{in}/\mathrm{d}t = \alpha[\mathrm{S}]_{out}[\mathrm{P}]_{in} - \gamma Y, \qquad (4a)$$

and

$$\mathrm{d}[\mathrm{P}]_{in}/\mathrm{d}t = -\alpha[\mathrm{P}]_{in}[\mathrm{S}]_{out} + \gamma Y, \qquad (4b)$$

where the first term in eqs. (4a) and (4b) is due to the process (iii) and the second term is due to the enzyme–substrate reaction shown in eq. (1). In the above equations, γ is equal to ke_0, e_0 is equal to the number of enzyme molecules embedded in the inner membrane divided by V, and $[\mathrm{S_b}]_{in}$ is $[\mathrm{S}]_{in}$ plus bound substrate.

In the same equations, Y is the fraction of substrate-bound enzyme and takes the following form:

$$Y = [S]_{in}/(K_m + [S]_{in}), \tag{5a}$$

where K_m is the apparent dissociation constant of substrate. From eq. (1) we find

$$K_m = K_s(1 + K_a/[H^+]_{in})/(1 + K'_a/[H^+]_{in}). \tag{5b}$$

According to the process (ii), the fraction of carriers facing the matrix, $[C]_{in}$, may be obtained from the following equation:

$$d[C]_{in}/dt = \beta[C]_{out} - \beta[C]_{in} = \beta C_0 - 2\beta[C]_{in}, \tag{6a}$$

where

$$[C]_{in} = [A]_{in} + [AH]_{in}, \tag{6b}$$

C_0 is the number of carriers (embedded in the membrane) divided by V (i.e. N_B/V), $[A]_{in}$ is the fraction of unprotonated form of the carrier multiplied by N_B/V. The rapid protonation step yields the expression for $[A]_{in}$ as

$$[A]_{in} = \frac{K_A/[H^+]_{in}}{1 + K_A/[H^+]_{in}}[C]_{in}. \tag{6c}$$

The time change of the charge density q of ions which can protonate may be written as

$$dq/dt = \beta[A]_{in} - \beta[A]_{out} + \gamma Y, \tag{7a}$$

where q is given by

$$q = [H^+]_{in} - K_w/[H^+]_{in} - [A]_{in} - e_0(1 - Y)\bar{r}_E - e_0 Y\bar{r}_{ES}. \tag{7b}$$

Here, K_w is the water dissociation constant, and $\bar{r}_E$ and $\bar{r}_{ES}$ are, respectively, the average number of protons dissociated from the substrate-free and substrate-bound enzymes. If the matrix contains buffers whose pK_a lies in the pH oscillatory range, then an additional term, $[B]\bar{r}_B$, should be subtracted from eq. (7b), where $[B]$ is the buffer concentration and $\bar{r}_B$ is the average number of protons dissociated from the buffer. The concentration of H^+ in the matrix may be obtained from eq. (7a) by use of a chain rule,

$$dq/dt = (d[H^+]_{in}/dt)dq/d[H^+]_{in}. \tag{8}$$

Note that eq. (7a) is highly non-linear in $[H^+]$. At the steady state limit, i.e. $dq/dt = 0$, this equation yields instabilities in the phase diagram of pH_{in} versus $[S]_{in}$ [10]. Thus, eq. (7a) together with eq. (4a) gives rise to oscillations in the transient state.

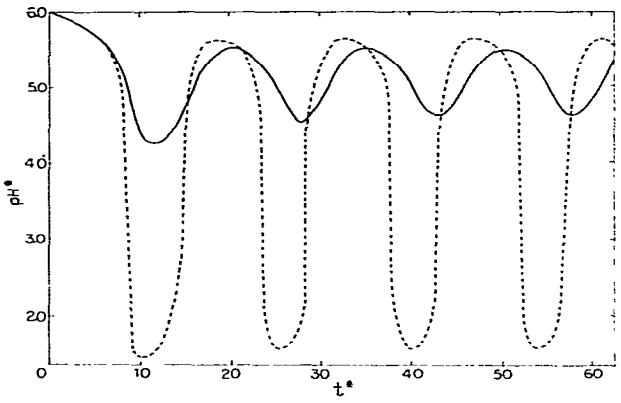

Fig. 2. Oscillations in pH^* with respect to t^*. The initial conditions used for computation are: $pH^*_{in} = 6$, $P^*_{in} = 1$, $S^*_{in} = 0$, $C^*_{in} = 0.1$, $e^*_0 = 0.01$, and $C^*_0 = 0.2$. The external conditions $pH^*_{out} = 6$ and $S^*_{out} = 0.2$ were imposed for our computation. Other parameters used are $\alpha^* = 0.2$, $\gamma^* = 1$, $pK^*_a = 5$, $pK'^*_a = 10$, $pK^*_A = 6$, and $pK^*_w = 14$. The solid line was computed with the same parameters as those of the dashed line, except that the matrix contains a buffer having $pK^*_a = 4$ with the concentration of $B^* = 0.1$.

4. Computation and results

It is convenient to introduce the following unitless quantities:

$$\alpha^* = \alpha K_s/\beta, \quad \gamma^* = \gamma/\beta K_s, \quad t^* = t\beta.$$

$$K^*_a = K_a/K_s, \quad K'^*_a = K'_a/K_s, \quad K^*_A = K_A/K_s,$$

$$K^*_w = K_w/K_s^2, \quad S^* = [S]/K_s, \quad P^* = [P]/K_s,$$

$$H^* = [H^+]/K_s, \quad e^* = e_0/K_s, \quad AH^* = [AH]/K_s$$

and $C^*_0 = C_0/K_s$. (9)

With these definitions, the differential equations developed above were solved using a two-time step procedure. Eq. (6a) was eliminated by imposing the initial condition $C_0 = 2[C]_{in}$, which forces $d[C]_{in}/dt = 0$ to hold at all times. As a boundary condition, we

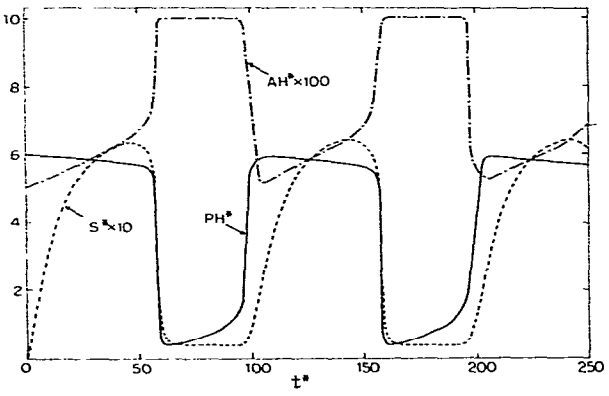

Fig. 3. Elongation in the oscillatory periods due to the steepness of a *pH*-activity curve. The conditions used for computation are the same as the dashed line of fig. 2, except that the enzyme contains two Bohr protons.

let the external concentrations of H^+ and substrate be constant at all times.

As shown by fig. 2, our simple system containing the main features of the mitochondrial compartment [11] may generate oscillations, if certain external conditions were met. Note that the amplitude of *pH* oscillations may be greatly reduced if the matrix contains buffers (the pK_a of which lies in the *pH* oscillatory range) as demonstrated by the solid line in fig. 2.

As shown by fig. 3, the period may be elongated considerably by making the *pH*-activity curve steeper. That is, fig. 3 was obtained by modifying K_m of eq. (5b) to

$$K_m = K_s(1 + K_a/[H^+]_{in})^2/(1 + K'_a/[H^+]_{in})^2, \qquad (10)$$

which is equivalent to the assumption that an enzyme system contains two Bohr protons instead of one. Fig. 3 also shows the oscillations in the concentrations of protonated form of the carrier in the membrane and of substrate in the matrix.

It might be worthwhile to mention that the period can be made much longer if larger values of K_s are used for the computation [10]. It should also be mentioned that the parameters such as K^*_w and K'^*_a do not play any role in generating the oscillations (as long as their magnitude is smaller than pH^*_{out}).

5. Discussion

According to our model, the requirement for metabolic oscillations in a vesicular compartment may be summarized as follows: First, a proton is pumped into the matrix from outside by an enzyme system, which is believed to possess Bohr protons [12]. The proton thus pumped inside activates the enzyme activity. Second, a proton inside is picked up by the carrier and is carried outside by a simple diffusion process. Upon arrival at the external medium, the carrier releases the corresponding proton. This process (i.e. diffusion of protons to outside) results in inhibition of the enzyme activity. When these two processes become rate-limiting, oscillations in *pH*, substrate, product and enzyme activity may arise in the vesicular compartment.

A proof that the oscillation obtained here is not a result of artifacts in computer solutions has been worked out [10] by means of a phase plane diagram [13] and also by means of a linear stability analysis [14]. It is unlikely that the observed mitochondrial oscillations are due to instrumental artifacts since such oscillations have been observed in many different laboratories [3,7]. Furthermore, the quantitative proof presented here reinforces a belief that the observed oscillations cannot be instrumental artifacts.

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