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Oscillatory Activity of P-Type Membrane Adenosine Triphosphatases: a Kinetic Model

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Abstract—A kinetic model for membrane P-type adenosine triphosphatases is considered, the main application being to the erythrocyte Ca^{2+}-ATPase. It is shown that a simple modification of the known catalytic mechanism of the ATPase by addition of a self-inhibition step and the steady calcium influx leads to damped oscillations in the system discussed. In this way, the model can explain the kinetic experimental results obtained for the purified enzyme in solution as well as for the enzyme incorporated into liposome membranes. The estimated kinetic parameters are close to the experimental ones. Alternative changes in time, demonstrated by the kinetic model for the conformational enzyme states, E_1 and E_2, confirm the model of two alternatively functioning gates in the ion pumping Ca^{2+}-ATPase.

Key words: oscillations, P-type adenosine triphosphatases, kinetic model

The P-type ATPases are well known as the family of enzymes that transduce energy from ATP to active ion transport across cell membranes [1-3]. These enzymes are intensively studied, especially during recent years after details of the structure for one of the enzymes of this family have been determined on the atomic level [4, 5]. The enzymes of the family follow similar kinetic mechanisms with partial steps clearly characterized [6-8]. The enzymes are studied both in solution and incorporated into membranes using different experimental techniques. Two conformational states, E_1 and E_2, having different affinity for transported ions, are known. The ion binding site in these states is exposed to the opposite sides of the membrane [9, 10].

The important role of a phosphoryl intermediate in the catalytic mechanism is emphasized by the name of these transporting enzymes (P-type) and is discussed in the literature [11].

This paper considers a kinetic model applicable mainly to plasma membrane Ca^{2+}-ATPase of erythrocytes, but qualitatively this model can be applied to various enzymes of the family.

The purified Ca^{2+}-ATPase of erythrocytes showed an initial burst of phosphorylation activity in the pre-steady-state kinetics under certain conditions [12]. Moreover, this enzyme, incorporated into azolectin liposomes, showed short infrequent pulses of different levels for the transported Ca^{2+} ions [13, 14]. The pulsating activity has been shown [13] to be independent of the membrane potential.

Two alternative modes of functioning have been discovered for this ATPase under changed conditions: the ion pump mode, and the mode of the B-type channel [13, 14].

This paper interprets the pulse-like functioning of the ATPase using a kinetic model. The model explains how external and internal gates can be open/closed in counter-phases (E_1 and E_2 can oscillate in counter-phases). In this sense, the kinetic model confirms the structural model considered recently for such enzymes [15].

KINETIC MODEL FOR Ca^{2+}-ATPase

This paper analyzes the kinetic behavior of the Ca^{2+}-ATPase using a widely accepted scheme for its catalytic cycle [8], but with a small modification. The simple modification takes into account recent experimental data [16] and allows us to interpret damped oscillations in the enzyme activity and in the level of transported Ca^{2+}, similar to the multi-level activity pulses observed [13, 17].

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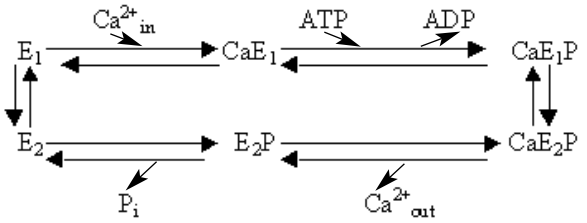
All investigations of the kinetic mechanism for membrane Ca^{2+}-ATPase confirm the following principal kinetic scheme:



(1)

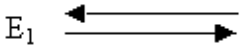
We simplify the scheme slightly, combining rapid ATP binding with ADP release and with the phosphoryl-enzyme, CaE_1P, formation.

Plasma membrane Ca^{2+}-ATPase (PMCA) links ATP hydrolysis to Ca^{2+} transport from the cytoplasm across the plasma membrane of most cells. Two conformational enzyme states, E_1 and E_2, are of great importance in the kinetic mechanism (1). The affinity of E_1 for Ca^{2+} is much greater than that of E_2 [6].

For simplicity, we do not consider the stoichiometry, possibly complicated for Ca^{2+} ions, because this stoichiometry is not of principal importance for our kinetic analysis.

We add the steady Ca^{2+} influx to scheme (1) in our kinetic model.

Moreover, we take into account the self-inhibited enzyme form, $E_{1(in)}$, studied in [16], that equilibrates with the active enzyme form, E_1:



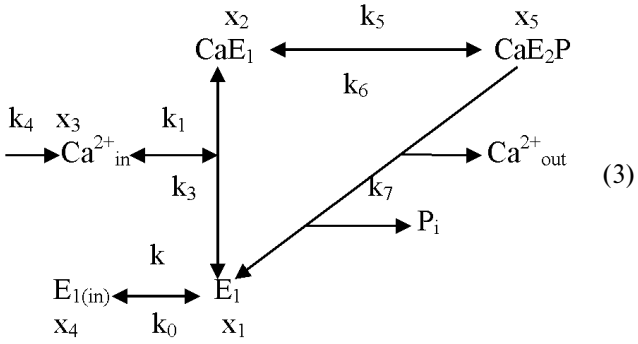
(2)

Enzyme self-inhibition demonstrated in [16] is a result of movements in the C-end of the ATPase molecule in the calmodulin-binding domain. We take for the simplicity of calculations some steps to be approximately irreversible, directed in the preferential direction, as was done in simpler models [8, 12].

Simpler kinetic models for the ATPase (without Ca^{2+} influx and without inactive enzyme form, $E_{1(in)}$) analyzed in [8, 12] show at certain initial conditions the initial burst of the phospho-enzyme level during the pre-steady-state kinetics followed by the steady state.

Our model with simple and realistic modifications of the known scheme can describe qualitatively new behavior, the damped activity oscillations (instead of a single initial burst) during the pre-steady-state kinetics.

After simplifications and modifications, we consider the following simple scheme, conserving kinetically important steps:



(3)

Enzyme concentrations of different enzyme states are constrained in scheme (3) by the following balance:

$$x_4 = 1 - x_1 - x_2 - x_5 . \quad (4)$$

Kinetic equations for scheme (3) are normalized divided by the total enzyme concentration E:

$$\begin{aligned}
dx_1/dt &= kx_4 - k_0x_1 + k_3x_2 - k_1x_1x_3 + k_7x_5;\\
dx_2/dt &= k_1x_1x_3 - k_3x_2 - k_5x_2 + k_6x_5;\\
dx_3/dt &= k_4 - k_1x_1x_3 + k_3x_2;\\
dx_5/dt &= k_5x_2 - k_6x_5 - k_7x_5 .
\end{aligned} \quad (5)$$

Various k_i here represent the rate constants shown in scheme (3), x_i represent normalized concentrations of the reaction participants as shown in scheme (3).

Ca^{2+} influx (shown in scheme (3) by constant k_4) is not detailed.

RESULTS

The system of kinetic equations (4) and (5) for model (3) was solved numerically using a computer program. We used the DBSolve program (creator I. I. Goryanin, Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences). Parameter values were chosen to result in damped oscillations similar to those observed experimentally [13, 17]. Ca^{2+} pulses observed in a small volume of artificial vesicles [13] possibly were modified by Ca^{2+} concentration noise (additional oscillations). Our next paper [18] is devoted to effects of calcium noise (additional oscillations). Ca^{2+} influx,

when changed by side fluxes, can induce repeats in damped oscillations produced by Ca^{2+}-ATPase as observed in [13, 14].

Other kinetic schemes, similar to scheme (3), were studied earlier [19, 20], but they were applied to other enzyme systems.

Kinetic equations for scheme (3) are nonlinear due to comparable values of enzyme and Ca^{2+}_{in} concentrations. This nonlinearity is necessary for oscillations to arise.

Enzyme self-inhibition, studied in [16] and added to the catalytic cycle in our model, is also necessary for damped oscillations to arise in system (3). Self-inhibition branch, going out of the catalytic cycle, produces the enzyme states along the catalytic cycle not interconnected by the balance constraint. This branch assimilates scheme (3) with substrate-inhibition schemes, for which oscillatory kinetic behavior is well known. Analysis shows that the branch, going out of the catalytic cycle in our model, induces complex values for the roots of the characteristic polynomial for the linearized kinetic equations, if the branch step is slow. Detailed theoretical analysis for similar kinetic schemes is presented in [20]. Oscillations can arise in scheme (3) at slow Ca^{2+}_{in} influx and slow transition from the inactive enzyme to the active enzyme, that is the case in the system with the participation of Ca^{2+}-ATPase [16].

The transition from inactive to active form of the purified enzyme in solution can be spontaneous [8]. In this case the kinetic manifestation of the transition should depend on the total enzyme concentration, as was demonstrated in [8]. It is known also that the enzyme is activated by calmodulin [8, 16]. The mechanism of this activation is well studied [16].

The following initial values have been taken for solution of Eqs. (5):

$$x_1 = 0;\ x_2 = 0.24;$$
$$x_3 = 200;\ x_5 = 0.15\ . \qquad (6)$$

The values of kinetic parameters in Eqs. (5) were varied to analyze their influence upon the form of the kinetic curves.

Figures 1-3 show the curves for the following parameter values.

The reversible activation/inhibition of the enzyme: $k = 0.01$ msec^{-1}, $k_0 = 1$ msec^{-1} (in the real system these parameters strongly depend on the experimental conditions).

Ca^{2+} influx: $k_4 = 50$ msec^{-1} (commonly k_4 depends on calmodulin level and/or other modifiers).

Binding of Ca^{2+}_{in} to high-affinity cites (E_1) does not limit the rate of the catalysis: $k_1 = 500$ msec^{-1}.

The reverse constant was varied; it is rather small, and we take it as zero: $k_3 = 0$, for $k_3 > 100$ msec^{-1} we have no oscillations.

The constant k_5 is the effective constant for rapid ATP binding, ADP release, and slow $E_1 \rightarrow E_2$ transition. This constant is mainly determined by the rate of the conformational transition, $E_1 \rightarrow E_2$, correlated with the phosphorylation. We use $k_5 = 100$ msec^{-1}.

We take the reverse constant $k_6 = 10$ msec^{-1} (this constant slightly depends on the ADP level).

The effective constant k_7 is taken as 150 msec^{-1}.

In addition to these parameter values (close to experimental ones [6-8]), we have analyzed also the influence of the parameter changes upon the kinetic behavior (data are not shown). Strong influence of the Ca^{2+} influx (constant k_4) on the time scale for the damped oscillations was observed.

Figures 1-3 demonstrate the results of the computer solution of Eqs. (4) and (5).

Figure 1 shows the time dependence of the relative E_1 level that binds Ca^{2+}_{in}. Infrequent short damped pulses are seen. The pulses are induced after a rather long lag-phase.

These pulses characterize the opening of internal gates for Ca^{2+}_{in}. Similar pulses have been observed using electrode techniques [13, 14] in azolectin liposomes.

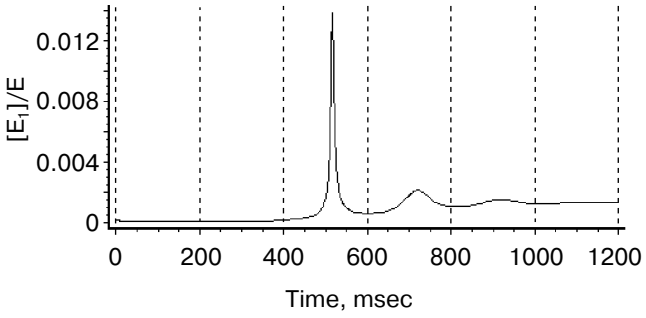


Fig. 1. Damped pulses of calcium conductivity (relative E_1 concentration) calculated according to Eqs. (4) and (5). Parameter values are shown in the text.

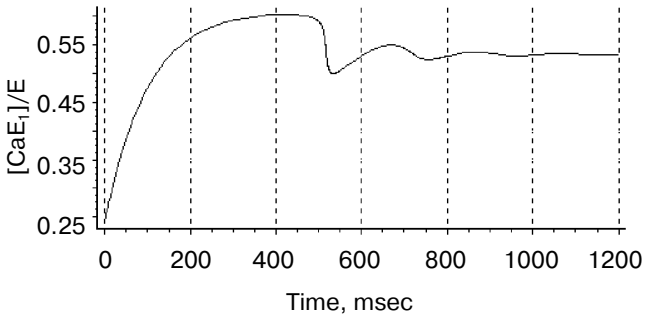


Fig. 2. Damped oscillations of the phosphorylating activity (relative CaE_1 concentration) calculated according to Eqs. (4) and (5). Parameters as in Fig. 1.

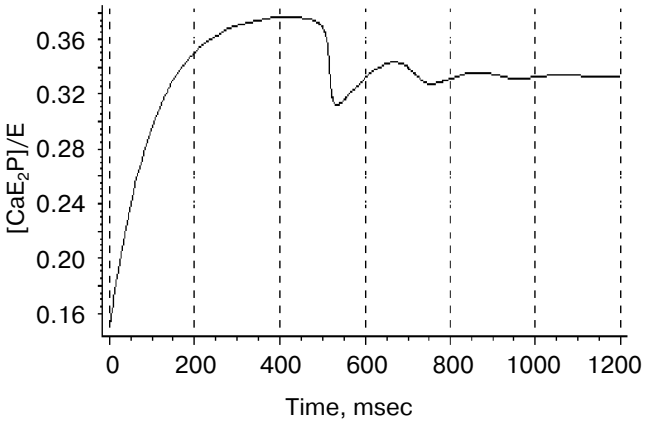


Fig. 3. Damped oscillations of the dephosphorylation (relative CaE_2P concentration) calculated according to Eqs. (4) and (5). Parameters as in Fig. 1.

Parameter values can be chosen to describe the monotonously increased E_1 level up to the steady state, as was observed under certain conditions [13]. However, this paper models the damped oscillations only.

Figure 2 demonstrates the time dependence of the relative CaE_1 level that characterizes the rate of the phosphorylation and the conformational transition, $E_1 \rightarrow E_2$. Similar kinetic curves were observed when the pre-steady-state phosphorylation of the purified enzyme was registered [7]. The authors of paper [7] supposed that they observed only a single initial activity burst. However, we can suppose that some of their experimental data [7] are better interpreted by a kinetic curve with the damped oscillations like in Fig. 2.

Figure 3 shows the time dependence of the relative CaE_2P level that characterizes the rate of Ca^{2+}_{out} release and the enzyme dephosphorylation. The kinetic curve in Fig. 3 practically repeats the curve in Fig. 2. This kinetic curve represents the opening of external gates for Ca^{2+}_{out}. Comparison of the figures leads to a conclusion that the opening of internal and external gates proceeds in counter-phases.

In this way, the model considered can interpret the coordination of internal and external gates, as is necessary for correct functioning of these enzymes in the mode of an ion pump.

DISCUSSION

A kinetic model for the membrane Ca^{2+}-ATPase that is qualitatively valid for the family of P-type ATPases shows the possibility to generate damped activity oscillations. Enzyme activity pulses of different amplitudes can be repeated after some time intervals in a more complicated system (a number of interacting pumps, the Ca^{2+} noise in a small volume, and other complicating factors).

The analysis of the kinetic model in this paper shows the possibility to generate pulses of calcium conductivity of different levels (amplitudes), which are similar to the observed ones [13, 17].

The kinetic model of this paper uses the known consecutive steps in the catalytic cycle [6-8] and uses the Ca^{2+}_{in} influx. Moreover, the model involves the recently discovered enzyme self-inhibition [16].

A phenomenon of damped oscillations seems interesting from various points of view.

First, it seen from Figs. 1 and 3 the relative levels of E_1 and E_2 change in counter-phases. This means that the enzyme in a membrane can open the gates at the two membrane sides alternatively, as postulated for enzymes of this type [15].

Under certain conditions (at certain parameter values), a marked ion flux can exist not only against the gradient (well-known kind of enzyme functioning as a pump) but also in the opposite direction (into the cell). This means that the enzyme under these conditions changes the pump functioning into channel functioning, as recently discovered [13, 15].

Finally, the oscillatory functioning of ion-transporting systems is interesting in their ability to modulate oscillatory ionic signals coming from other systems to the cell. The problems of coding/decoding of oscillatory Ca^{2+} signals have recently become of growing interest [21]. This paper can add new information to understand the problem of coding/decoding for cellular signals.

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