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Influence of Ca^{2+} Oscillatory Influx on Membrane Ca^{2+}-ATPase Activity: a Kinetic Model

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Abstract—A kinetic model for the membrane Ca^{2+}-ATPase is considered. The catalytic cycle in the model is extended by enzyme auto-inhibition and by oscillatory calcium influx. It is shown that the conductive enzyme activity can be registered as damped or sustained Ca^{2+} pulses similar to observed experimentally. It is shown that frequency variations in Ca^{2+} oscillatory influx induce changes of pulsating enzyme activity. Encoding is observed for the signal frequency into a number of fixed levels of sustained pulses in the enzyme activity. At certain calcium signal frequencies, the calculated Ca^{2+}-ATPase conductivity demonstrates chaotic multi-level pulses, similar to those observed experimentally.

Key words: Ca^{2+}-ATPase, oscillations, signal coding, kinetic model

Erythrocyte membrane Ca^{2+}-ATPase transports calcium ions from the cell (against the concentration gradient) using the free energy of ATP hydrolysis [1]. This enzyme, which has been studied, belongs to the family of transporting enzymes (pumps), P-type ATPases, characterized by similar catalytic mechanisms [2, 3]. The catalytic mechanism of this enzyme family involves an enzyme phosphoryl-intermediate that participates in catalysis.

The kinetic mechanism for all these enzymes is usually interpreted on the basis of the (E_1-E_2) model, i.e., the model of two conformers with different affinity for Ca^{2+} binding sites (higher in E_1 and lower in E_2) [4-6]. The calcium ion pathway through the internal part of the enzyme molecule is discussed in the literature taking into account the known spatial structure of the sarcoplasmic Ca^{2+}-ATPase (the most studied member of the family) [7, 8].

Recent works [9-11] have shown that membrane pumps and channels, traditionally considered as drastically different, can differ only in the mechanism of opening/closing of their gates. Pumps can function as channels under certain conditions [10, 11] (violated alternative opening/closing of the gates). Internal and external pump gates should be interconnected to determine the ion movement mechanism against the concentration gradient [11].

The first paper in this series [12] discussed the kinetic oscillatory mechanism that can explain the alternative opening/closing for gates located in these enzymes at the opposite membrane surfaces. The kinetic model explained reciprocal damped oscillations for different enzyme states (E_1 and E_2). Calculated oscillations for E_1 concentration were of pulse character similar to experimental pulses observed [10]. Pulses of different levels for calcium conductivity (usually pulses of three levels) were repeated chaotically in experiments with the enzyme incorporated into small azolectin vesicles (liposomes) [10].

We investigate now the influence of fluctuations and other variations of calcium ion levels possible in small liposome or cell volumes on the Ca^{2+}-ATPase activity.

Our analysis shows that additional periodic changes in Ca^{2+} levels in a small volume can induce modifications and repeats in time of the damped pulse activity for the Ca^{2+}-ATPase in a way similar to that experimentally observed [10].

Additional changes in Ca^{2+} level in cells (liposomes) can be induced not only by noise (fluctuations) but also by some oscillatory signals directed to the Ca^{2+}-ATPase.

Such oscillatory signals can strongly influence the self-oscillatory kinetic behavior of the pump. It has been

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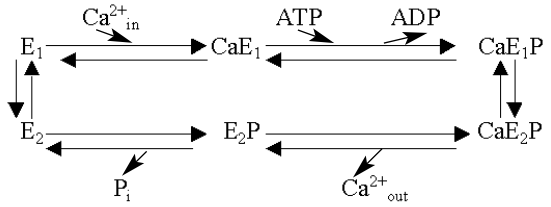
recently shown [13] that noise influence on calcium levels can enhance robust intracellular Ca^{2+} oscillations.

This paper interprets experimental results [10, 14] and analyzes the influence of additional oscillatory signals on frequency, amplitude, and form of self-oscillatory activity for P-type ion transporting ATPases.

We introduce the disturbance of internal Ca^{2+}_{in} concentrations in a form of simple harmonic oscillations that can simulate various disturbances (including noise of ion concentration in a small volume of cells or liposomes).

KINETIC MODEL FOR Ca^{2+}-ATPase

Here we consider a kinetic model that is a modification of our model analyzed in [12]. The widely accepted kinetic scheme with reasonable simplifications, involving known steps of the kinetic mechanism for the erythrocyte membrane Ca^{2+}-ATPase [4], is considered:



(1)

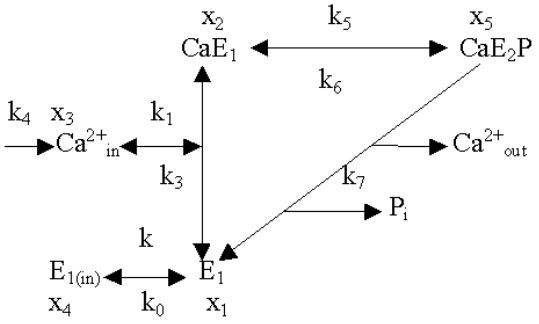
Scheme 1 shows the catalytic cycle for ATP hydrolysis, which enables active transport to occur from the cell plasma against the concentration gradient, $Ca^{2+}_{in} \rightarrow Ca^{2+}_{out}$. All known kinetic models for this enzyme family include two conformational enzyme states, and the transition $E_1 \rightarrow E_2$ induces changes in the affinity of Ca^{2+}-binding sites from higher (E_1) to lower (E_2) [4-6]. Ca^{2+} ions flow out of the cell plasma, going through the transmembrane binding sites during the phosphorylation of an aspartyl residue. The phosphorylation is interconnected with the conformational change, $E_1P \rightarrow E_2P$ (P means the phosphorylated enzyme) [7, 8].

Autophosphorylation is a necessary property of this enzyme family (P-type ATPases) and is the impelling force for the active transport.

Clarification of all steps important for the catalytic mechanism has evoked a criticism of the so-called E_1-E_2 model [15]; however, the conformational changes (more complicated) are not criticised. Consequently, the principal scheme (1) is accepted [7, 8] as taking into account the main kinetic characteristics of the catalysis.

For our analysis, however, it is necessary to include the enzyme auto-inhibition recently observed [16]. We have included it in our preceding work [12] as the additional step, $E_{1(in)} \leftrightarrow E_1$. We also include Ca^{2+}_{in} influx [16].

As a result, we consider the following modified kinetic scheme:



(2)

Kinetic equations for scheme (2) are:

$$\begin{aligned}
dx_1/dt &= kx_4 - k_0x_1 + k_3x_2 - k_1x_1x_3 + k_7x_5;\\
dx_2/dt &= k_1x_1x_3 - k_3x_2 - k_5x_2 + k_6x_5;\\
dx_3/dt &= k_4 - k_1x_1x_3 + k_3x_2;\\
dx_5/dt &= k_5x_2 - k_6x_5 - k_7x_5.
\end{aligned} \tag{3}$$

Similarly to [12], Eqs. (3) were solved numerically for the following initial conditions (4):

$$\begin{aligned}
x_1 = 0;\ x_2 = 0.24;\\
x_3 = 200;\ x_5 = 0.15
\end{aligned} \tag{4}$$

and for the following parameter values (5):

$$\begin{aligned}
k = 0.01\ \text{msec}^{-1},\ k_0 = 1\ \text{msec}^{-1},\ k_1 = 500\ \text{msec}^{-1},\\
k_3 = 0,\ k_4' = 50\ \text{msec}^{-1},\ k_5 = 100\ \text{msec}^{-1},\\
k_6 = 10\ \text{msec}^{-1},\ k_7 = 150\ \text{msec}^{-1}.
\end{aligned} \tag{5}$$

Kinetic parameters were chosen to obtain infrequent damped pulses of calcium conductivity. The time scale and the order of the values for kinetic constants were obtained in this way close to the experimental ones (taking into account variable experimental conditions [4-6]).

However, calcium conductivity induced by the Ca^{2+}-ATPase incorporated into membranes of small liposomes was observed as multiple repeated pulses of various levels [9, 10].

To interpret these experiments, in this paper we include an additional oscillatory variation of Ca^{2+} influx, which can be induced by noise inside the vesicles. The additional variation in Ca^{2+} influx is represented by the following sine-function:

$$k_4 = k_4' + aSin(wt). \quad (6)$$

Frequency w, when changed, induces changed kinetic behavior for the Ca^{2+}-ATPase, including kinetic behavior similar to that experimentally observed [10, 14].

RESULTS

Figures 1-4 show the results of numerical solution of Eqs. (3) implemented by the computer program DBSolve (I. I. Goryanin, Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences). The solution involves the constraint for total enzyme concentration:

$$x_1 + x_2 + x_4 + x_5 = 1. \quad (7)$$

All concentrations x_i for the reactants are normalized by the total enzyme concentration E. Reaction participants, their normalized concentrations, x_i, and kinetic constants are shown in scheme (2).

Calcium conductivity, $Ca^{2+}_{in} \rightarrow Ca^{2+}_{out}$, is proportional to concentration E_1, which characterizes opening of the internal gate in the membrane Ca^{2+}-ATPase. This conductivity, independent of the membrane potential, as demonstrated in [10], under certain conditions was registered in the form of short infrequent pulses.

We observed large changes in the calculated calcium conductivity through the Ca^{2+}-ATPase when the frequency of oscillations for Ca^{2+} influx in formula (6) was changed.

Figure 1 shows the result of solution of kinetic equations (3) at frequency $w = 0.01$ msec^{-1}. The constant amplitude for calcium influx, as shown in formula (6), was not varied and was equal to $a = 20$ msec^{-1}.

Figure 1 shows that opening of the internal gate (proportional to E_1 concentration) looks like periodic pulses of the same level (the same amplitude).

The greater value of $w = 0.05$ msec^{-1} induces two-level periodic pulses as shown in Fig. 2.

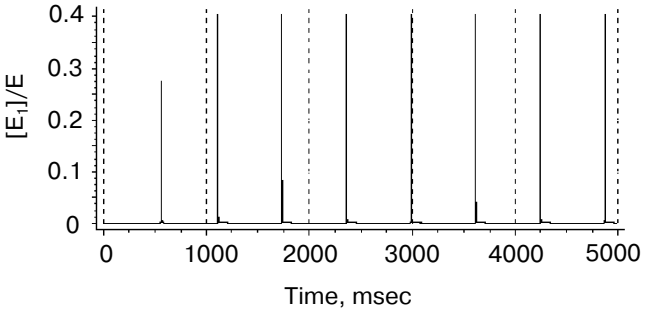


Fig. 1. Computed periodic pulses of a single level for the input enzyme activity. Frequency of the influx for the calcium signal $w = 0.01$ msec^{-1}; other parameters as in the text.

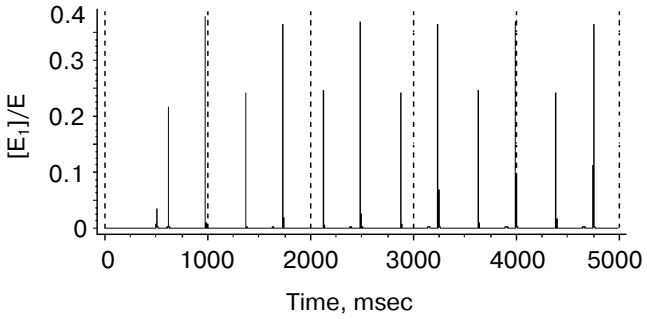


Fig. 2. Computed periodic pulses of two levels for the input enzyme activity. Frequency of the influx for the calcium signal $w = 0.05$ msec^{-1}; other parameters as in Fig. 1.

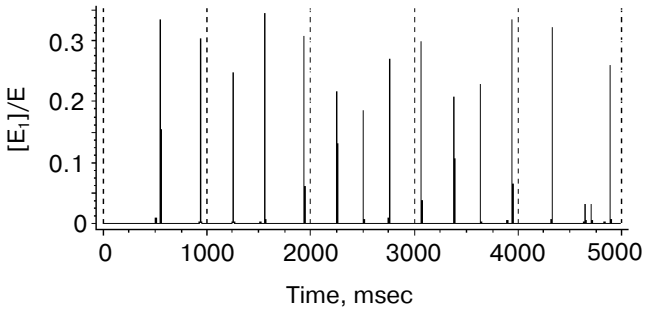


Fig. 3. Computed stochastic pulses of a number of levels for the input enzyme activity. Frequency of the influx for the calcium signal $w = 0.1$ msec^{-1}; other parameters as in Fig. 1.

The higher frequency $w = 0.1$ msec^{-1} induces multilevel spontaneous pulses, as shown in Fig. 3. Similar pulses have been observed in the experiment [10] for the input calcium conductivity of the Ca^{2+}-ATPase incorporated into liposome membrane.

Finally, Fig. 4 shows that further increase in w up to $w = 0.3$ msec^{-1} induces damped pulses of five different levels. Similar calcium oscillations have been observed in experiments [14].

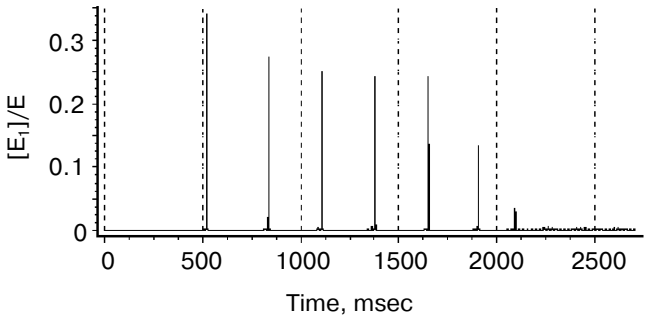


Fig. 4. Computed damped pulses for the input enzyme activity. Frequency of the influx for the calcium signal $w = 0.3$ msec^{-1}; other parameters as in Fig. 1.

Higher frequency *w* leads to a single clearly registered pulse (not shown).

In conclusion, the modeling for kinetics of the reaction catalyzed by the Ca^{2+}-ATPase with changeable in time calcium influx interprets well the experimentally observed changes in levels of calcium conductivity. On the other hand, the modeling shows that periodically changeable calcium influx can be coded by the Ca^{2+}-ATPase into oscillations that are variable in their form and frequency.

DISCUSSION

Intracellular Ca^{2+} oscillations are well studied, and various kinetic models are used for their interpretation (see review [17]). In our work [12], a new model was analyzed for damped calcium oscillations that can be induced by the plasma membrane Ca^{2+}-ATPase. It was demonstrated that such oscillations promote Ca^{2+}-ATPase functioning as the active pump for calcium ions against the concentration gradient, inducing the alternative opening of internal/external pump gates.

Interaction of various oscillators is observed in cells, and it was recently shown [16] that this interaction codes the oscillatory signals in various cell responses and leads to the memory of these signals.

It was shown that the membrane Ca^{2+}-ATPase (plasma membrane calcium pump) also has a memory of calcium spikes [16].

In this paper, we demonstrate various types of coding for input oscillatory calcium signals acting on the plasma membrane Ca^{2+}-ATPase. Figures 1 and 2 illustrate the fact that the input harmonic Ca^{2+} oscillations are transformed into periodic pulses of a single or multiple fixed levels.

Moreover, the input of a certain frequency induces non-periodic (chaotic) pulses (see Figs. 3 and 4). In this case, multi-level pulses are also obtained similar to those observed experimentally for the Ca^{2+}-ATPase [10]. The number of levels for repeated pulses is determined (coded) by the frequency of the input signal.

Various transformations of the input oscillatory signals are explained here by the ability of the system to generate damped nonlinear oscillations. Nonlinear pulsating oscillations of the enzyme activity (Ca^{2+} conductivity) are induced in our model only at certain amplitudes of the calcium input (see our preceding paper [12]). If the amplitude of the calcium input changes in time, the enzyme activity pulses (conductivity pulses) can be induced or not, depending on the input frequency. Detailed theoretical analysis of the time-dependent Ca^{2+} input into the generator of sustained Ca^{2+} oscillations was presented in [13].

Our model interprets the multi-level pulses in the enzyme activity that arise due to damping in the enzyme activity oscillations. The resulting conductivity pulses are not induced by input oscillations only; therefore, the pulse frequency is not equal to the input frequency.

The ability to generate oscillations in our model depends on the construction of the unstable limit cycle (see detailed analysis of the phenomenon in [13]).

Periodic changes of calcium concentration in small volumes of liposomes and cells can be initiated not only by other oscillators but also by noise. The influence of Gaussian noise on intracellular Ca^{2+} oscillations was studied in [13]. The preferential (average) oscillation frequency was isolated in the chaotic (noise) combination of different frequencies [13]. Therefore, harmonic oscillations can model the preferential oscillations of noise.

It was shown [13] that calcium oscillators become more robust (less sensitive to external influences) with greater noise intensity.

Our paper demonstrates another effect: coding of the frequency of the input oscillatory signals into another frequency and the number of different levels for the pulses of the enzyme oscillator. This kind of coding can be important for the cell, inducing multiple responses to a single signal of a single frequency. Moreover, our paper supports the importance of noise processes at the cell level.

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