The Mathematical Model of Self-oscillations of Kai Proteins in Incubation Solution

Shahin K. Bayramov1* and Kamandar M. Yaqubov2

1Department of Biochemistry, Azerbaijan Medical University, Baku, AZ1022, Azerbaijan.
2Department of Pharmacology, Azerbaijan Medical University, Baku, AZ1022, Azerbaijan.

Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

An autocatalytic mathematical model of self-oscillations in in vitro solutions of Kai proteins (KaiA, KaiB, KaiC) and ATP is offered. The model describes the main processes in the solution of Kai proteins, namely the process of a phosphorylation / defosphorillyation of KaiC protein which is accelerated by influence of KaiA and is inhibited by KaiB protein influence. By the method of the metabolic control analysis it is shown that the frequency (period) and amplitudes of self-oscillations of the components of Kai-proteins are temperature compensated.

Keywords: Mathematical model; biochemical oscillation; KAI proteins.

1. INTRODUCTION

Circadian rhythms, endogenous oscillations of physiological activities with a period of ~24 h, are found in a wide spectrum of organisms and enhance their fitness in a day/night alternating environment [1]. Among these organisms, cyanobacteria are the simplest organisms that
exhibit circadian rhythms [2,3]. Three clock genes KaiA, KaiB and KaiC have been identified in the cyanobacterium, Synechococcus elongatus PCC 7942 as essential timekeeping components. A transcription/translation-based autoregulatory loop of KaiBC gene expression has been proposed to drive circadian rhythms [4]. In this model, KaiA and KaiC are proposed as positive and negative regulators of KaiBC expression, respectively [4].

However in it is recently established [5] that the elongation of a gene of PCC 7942 in Synechococcus microorganisms for generation of oscillations in the Kai - proteins solutions isn't required. It is revealed that the oscillatory behavior is observed in in vivo conditions without formation of KaiBC - mRNA of a complex and without presence of inhibitors of the translation and/or a transcription. In works [6] it is shown that in in vitro solution of the cleared proteins KaiC, KaiA, KaiB, and ATP (Adenosine Three Phosphate) there are not damping self-oscillations of concentration of components. It is established that KaiC is enzyme with autokinase and autophosphatase activities [7,8], and KaiA supports the KaiC [9] functions, at the same time, KaiB reduces influence of KaiA on KaiC [8,10]. Authors of work [11] established that Kai - proteins interact among themselves in all possible combinations.

2. RESULTS AND DISCUSSION

Considering the above data there is an interest about the possible scheme of interactions of Kai proteins which can generate self-oscillations and did not involve transcription or translation. It is well established that in incubation solution a hexamers of KaiC, dimers of KaiA and tetrarsers of KaiB occur [10]. Phosphorylation of KaiC hexamers occurs by ATP, and the process is accelerated by a KaiA dimer, and suppressed by KaiB tetramer. In this model we propose that (i)-KaiC hexamers can exist in three states: unphosphorylated, low (partially) phosphorylated and associated with KaiA dimers, and high (full) phosphorylated and associated with KaiA dimers states, (ii)- the associated with KaiA dimers high phosphorylated KaiC hexamers enhance the phosphorylation of low phosphorylated KaiG, (iii)- unphosphorylated KaiC hexamers enhance dissociation of cluster of Kai A,B,C proteins and (iii)-KaiB attenuates KaiA-stimulated KaiC phosphorylation.

In this model KaiA2HC6p, indicates the cluster of fully phosphorylated KaiC6 hexamer and KaiA dimer (KaiA2). KaiC6p denotes partially phosphorylated KaiC hexamer and KaiC6-unphosphorylated KaiC hexamers. In process R1, KaiC6 binds KaiA2 (KaiA dimer), forming KaiA2C6p. Since KaiA dimers facilitate the autokinase activity of KaiC6 [9], KaiA2C6 rapidly is convert to the low phosphorylated form KaiA2C6p. In the process R2, KaiA2C6p is convert to high phosphorylated cluster KaiA2HC6p and as in [12], we propose that KaiA2HC6p adopts such a conformation so that it can facilitate the phosphorylation of low phosphorylated KaiA2C6p into KaiA2HC6p by process R3. In the next step, R4, KaiB tetramer associates with KaiA2HC6p to form the cluster KaiA2B4HC6p. This is consistent with experimental data that demonstrate that KaiB interacts with KaiC only after KaiC has bound to KaiA [8,11]. The process R5 express the dissociation of cluster KaiA2B4HC6p by interaction with hexamer KaiC6. In the results of this process occurs KaiC dephosphorylation by exchange of subunits among KaiC6 and KaiA2B4HC6p and KaiA and KaiB dissociates from KaiC hexamers. In the process R6 it is simulate the diminishing influence of KaiB tetrarsers to phosphorylation process of KaiC hexamers by a manner, that KaiB tetrarsers interact with the low phosphorylated KaiA2C6p complex and therefore, the inactive complex KaiA2B4C6p is form. This reaction is reversible.

Reaction scheme:

\[
\begin{align*}
R1: & \quad \text{KaiA2} + \text{KaiC6} + \text{ATP} \rightarrow \text{KaiA2C6p} \\
R2: & \quad \text{KaiA2C6p} + \text{ATP} \rightarrow \text{KaiA2HC6p} \\
R3: & \quad \text{KaiA2HC6p} + \text{KaiA2C6p} + \text{ATP} \rightarrow 2 \text{KaiA2HC6p} \\
R4: & \quad \text{KaiB4} + \text{KaiA2HC6p} \rightarrow \text{KaiA2B4HC6p} \\
R5: & \quad \text{KaiA2B4HC6p} + \text{KaiC6} \rightarrow 2 \text{KaiC6} + \text{KaiA2} + \text{KaiB4} + \text{P} \\
R6: & \quad \text{KaiB4} + \text{KaiA2C6p} \rightarrow \text{KaiA2B4C6p}
\end{align*}
\]
The corresponding to reaction processes (R1–R6), system of differential equations (see formulas 1) consist from the rate equations of the stages R1–R6 in the proposed model:

$$\begin{align*}
\frac{dKaiC6}{dt} &= v_5 - v_1 \\
\frac{dKaiA2}{dt} &= v_5 - v_1 \\
\frac{dKaiA2}{dt} &= v_5 - v_1 \\
\frac{dKaiA2}{dt} &= v_5 - v_1 \\
\frac{dKaiB4}{dt} &= v_5 - v_4 - v_6 \\
\frac{dKaiA2C66p}{dt} &= v_1 - v_6 - v_3 \\
\frac{dKaiA2HC6p}{dt} &= v_2 + v_3 - v_4 \\
\frac{dKaiA2B4HC6p}{dt} &= v_4 - v_5 \\
\frac{dKaiA2B4C66p}{dt} &= v_6
\end{align*}$$

Here

$$\begin{align*}
v_1 &= k_1 \times KaiA2 \times KaiC6 \\
v_2 &= k_2 \times KaiA2C66p \\
v_3 &= k_3 \times KaiA2HC6p \times KaiA2C66p \\
v_4 &= k_4 \times KaiB4 \times KaiA2HC6p \\
v_5 &= k_5 \times KaiA2B4HC6p \times KaiC6 \\
v_6 &= k_6 \times KaiB4 \times KaiA2C66p - k_6 \times KaiA2B4C66p
\end{align*}$$

and $k_i (i=1-6)$ rate constants.

In the model there are 3 conserved entities:

$$\begin{align*}
[KaiA2C66p]+[KaiB4]+[KaiA2]+[KaiA2HC66p]= const_1 \\
-[KaiA2]+[KaiC6]= const_2 \\
[KaiB4]+[KaiA2B4HC66p]+[KaiA2B4C66p]= const_3
\end{align*}$$

Consequently this means, that size of corresponding stochiometric matrix of reaction scheme is $7 \times 6$, and rank of matrix is 4, i.e. the characteristic polynomial of Yakobian of system (1) is a polynomial in the fourth degree (2):

$$\lambda^4 + a_1\lambda^3 + a_2\lambda^2 + a_3\lambda + a_4 = 0, \quad (2)$$

where, $a_i$ - coefficients of a characteristic polynomial.

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Fig. 1. A dynamical model of in vitro rhythmic KaiC6 phosphorylation in incubation solution (See text for description)
If all coefficients of a characteristic polynomial (2) are positive at stationary values of concentration of reagents, the steady state is steady. If at least one of coefficients of, $a_i$ of a characteristic polynomial of reactionary system changes a sign from plus to minus at change of concentration of reagents, the steady state of system becomes unstable.

It is well known that autocatalytic stages in dynamic systems destabilize a steady state. From this point of view, processes R3 and R5 draw attention, since they are autocatalytic and can serve as sources of sustained oscillations. The bifurcation analysis of the model carried out by us shows that the reaction R3 of the high phosphorylated KaiC6–KaiA2 complex (KaiA2C6p, Fig. 1) gives a negative contribution to coefficient $a_i$ and therefore is a source of the sustained oscillations.

The negative contribution of autocatalytic step R5 which describes autocatalytic dephosphorylation of the complex KaiC6 by the "exchange" interaction of nonphosphorylated KaiC6, suggested in [13-15] to the coefficient of a characteristic polynomial is compensate by positive contributions other reaction stages and therefore in the absence of the autocatalytic step R3, no sustained oscillations appear possible.

The model’s differential equations (1) were solved numerically using the software programs SBW (authors H. Sauro et al., http://www.sbml.org) and results of numerical solution of (1) with following parameter values:

$$k_1 = 0.01 \mu M^{-1} h^{-1}, \quad k_2 = 0.001 \mu M^{-1}, \quad k_3 = 8.60 \mu M^{-2} h^{-1}, k_4 = 6.25 \mu M^{-1} h^{-1}, \quad k_5 = 0.2 \mu M^{-1} h^{-1}, \quad k_{\text{de}} = 0.0005 \mu M^{-1} h^{-1}, \quad k_{\text{ph}} = 0.01 \mu M^{-2} h^{-1}, \quad [\text{KaiA2}] = 0.703 \mu M, \quad [\text{KaiB4}] = 0.337 \mu M, \quad [\text{KaiC6}] = 1.436 \mu M, \quad [\text{KaiA2C6p}] = 0.255 \mu M, \quad [\text{KaiA2B4HC6p}] = 0.027 \mu M, \quad [\text{KaiA2HC6p}] = 0.005 \mu M, \quad [\text{KaiA2B4C6p}] = 0.152 \mu M$$

are illustrated in Figs. 2a, b, c.
It should be noted that expansion of model by additional reactions of formation of complexes KaiA2, KaiB4, and KaiC6 from respectively monomers KaiA, KaiB, and KaiC doesn't change a dynamic picture of oscillations, for this reason we offer more simplified model.

2.1 Frequency and Amplitude Control Analysis and Temperature Compensation

Temperature compensation of frequency of oscillations is an essential property of clockwise biochemical oscillations, which means that in some living oscillatory biochemical processes the frequency (or period) remains as constant (more exactly, approximately constant) in physiologically allowed range of the temperature change [16]. For the verification of the temperature compensation of above described model, it is necessary metabolic control analysis for frequency in model. From the point of view of metabolic control analysis, temperature compensation means, that a response coefficient of frequency relatively to temperature change must be equal or near to zero. Consequently, this fact may be a very good criteria for the verification of oscillatory model for the temperature compensability.

It is well known, that each rate constant depends on temperature and this dependence obeys the Arrhenius equation,

\[ k_i = A_i e^{-\frac{E_i}{R T}} \]

where \( E_i \) is the activation energy, \( R \) is the gas constant, and \( T \) is the temperature in Kelvin. \( A_i \) is the pre-exponential factor, which is also treated as a constant. Therefore a change of temperature of reaction system exponentially influences on rate constant, namely, the increase of temperature holds an exponentially increase of all rate constants.

For the quantitatively determine a response of frequency on change of temperature, we present cyclic frequency as complex function from temperature as:

\[ \omega(T) = \omega(k_i(T)) \]

\[ \frac{\partial \omega}{\partial T} = \sum_{i=1}^{n} \frac{\partial \omega}{\partial k_i} \frac{\partial k_i}{\partial T} \]

From here

\[ \frac{T \, \partial \omega}{\omega \, \partial T} = \sum_{i=1}^{n} k_i \frac{\partial \omega}{\partial k_i} \frac{\partial k_i}{\partial T} \] (3)

Here,

\[ C_{\omega i}^k = \frac{k_i \, \partial \omega}{\omega \, \partial k_i} \] is control coefficient for frequency on rate constant. Then

\[ C_{\omega i}^T = \frac{T \, \partial \omega}{\omega \, \partial T} \] will be a responcity coefficient of frequency on the temperature and

\[ C_{k_i}^T = \frac{T \, \partial k_i}{k_i \, \partial T} \] will be a responcity coefficient of \( i \)-th rate constant on the temperature.

From Arrhenius equation we receive that

\[ C_{k_i}^T = \frac{E_i}{RT} \] (4)
By regarding (4) in (3)

\[ C_i^\omega = \frac{1}{RT} \sum_{i=1}^{n} C_k^\omega E_i \]  

(5)

Formula (5) shows, that temperature compensation requires that one or several of the frequency control coefficients need to be negative, because R, T and activation energies are positive. Consequently, the in the temperature compensated oscillators should be a some reactions that have opposing effects on the frequency relatively to temperature changes. Temperature compensation can occur within a certain temperature interval, whenever the activation energy \((E)\) weighted sum of the control coefficients is near to zero.

The frequency control coefficients in (5) we calculate by a manner as described in [17,18] and obtained values are listed in Table 1.

As it is visible from Table 1, model has temperature -compensate mechanism and may be temperature-compensated. Really, in model the process R4 (rate constant \(k_4\), (In process R4, KaiB4 associates with KaiA2HC6p to form the ternary complex KaiA2B4HC6p) has a negative frequency control coefficient.

Such result reasonably from the point of view of molecular kinetics as the increase in temperature has to complicate formations of a complex.

From the Table 1 it is visible, that in model there is one more stage with negative frequency control coefficient. This is reaction of inhibition of complex KaiA2C6p phosphorylation by protein KaiB, (process R6) that also corresponds to the fact that KaiB diminishes the effect of KaiA on KaiC [8,10], though has considerably small value in comparison with frequency control coefficient of stage R4.

From the theory of oscillations it is known that values of an amplitude of self-oscillations are defined by internal parameters (the rate constants in our case) self-oscillatory system. Since the change of temperature influences to rate constants on Arrhenius formula, it is arise an interesting question about the influence of temperature change on the amplitude of oscillations of Kai proteins. In order to answer to this question we carried out the control analysis of amplitudes of oscillations on the way described in [18] and the received results are listed in Table 1.

As it is in high mentioned expansion of model by addition of reactions of formations of complexes KaiA2, KaiB4, and KaiC6 from respectively monomers KaiA, KaiB, and KaiC doesn’t change a dynamic picture of oscillations. However, the advantage of “minimal” models like the one presented here is that they are easily applied and extendable.

### Table 1. Control coefficients of the reaction steps on the model

| \(k\) | \(C^\omega_{ki}\) | \(C^A_{KaiA2}\) | \(C^A_{KaiB4}\) | \(C^A_{KaiC6}\) | \(C^A_{A2B4HC6p}\) | \(C^A_{A2HC6p}\) | \(C^A_{A2B4Cp}\) | \(C^A_{A2C6p}\) |
|---|---|---|---|---|---|---|---|---|
| 0.376 | 6.32 | 6.73 | 6.53 | 6.74 | 6.64 | 6.71 | 6.56 |
| 0.017 | -6.07 | -6.15 | -6.11 | -6.02 | -6.07 | -5.71 | -4.73 |
| 0.778 | -5.40 | -5.14 | -5.25 | -5.69 | -5.45 | -5.85 | -5.27 |
| -0.361 | 7.51 | 6.82 | 7.25 | 6.64 | 6.78 | 6.29 | 6.36 |
| 0.187 | -2.84 | -2.54 | -2.84 | -3.12 | -2.01 | -2.58 | -3.10 |
| -0.029 | 0.481 | 0.46 | 0.76 | 1.36 | 0.53 | 1.14 | 0.53 |
| 0.029 | -0.002 | -0.18 | -0.34 | 0.09 | -0.42 | 0.00 | -0.35 |
| 0.997 | -0.001 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.0001 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

*All kinetic parameters are increased simultaneously by 5% and the total change of frequency \(\omega\) and amplitudes \(A_i\) is determined.
3. CONCLUSION

In this work an autocatalytic oscillatory model of interaction of Kai - proteins is offered. The model describes the most important processes in solution of Kai - proteins, (KaiA, KaiB, and KaiC), namely process of a phosphorylation / dephosphorylation of KaiC protein which is accelerated by influence of KaiA and is inhibited by KaiB protein influence. In the model the transcription-translation negative feedback is simulated by autocatalytic stages R3, which describes the phosphorylation of low phosphorylated KaiA2C6p into high phosphorylated KaiA2HC6p.

The phenomenon called "temperature compensation" - the most important distinctive property of biochemical "clock" which, in our opinion, has to take place in biochemical systems for the reason that oscillations in biological processes are also carriers of the biophysical information coded by characteristics of oscillation. Protection of this information against distortion by casual temperature fluctuations is necessary for live systems. Here we conclude that at temperature compensated biochemical reaction there have to be stages which are opposite reacting on influence of temperature on self-oscillation frequency.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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