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FREQUENCY CONTROL ANALYSIS FOR BIOCHEMICAL OSCILLATORS

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Abstract. Direct application of the control coefficients employed in metabolic control assessments of stable states to dynamic systems is not possible. Here, we demonstrate a workaround for this restriction based on the determination of the Jacobian's eigenvalues for the relevant system of dynamical equations. It is suggested that the relationship between frequency and rate constants is homogenous. The validity of summation theorems for frequency tested on the various examples proves this assumption. We here discus the phenomena of temperature compensation of frequency in clockwise biochemical oscillations from the point of view of metabolic control analysis and conclude that some of reaction stages in the temperature-compensated oscillations must have a negative frequency control coefficients.

Keywords: *biochemical oscillations, frequency control, control analysis.*

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1. Introduction

In biological networks, periodic occurrences are common (Patke *et al*., 2020; Partch *et al.*, 2014). Periodic signals can be a function of time, space, or both, depending on the oscillator's mechanism. Some of these periodic events are critical to the functioning of the living system in which they occur. As a result, identifying the pathways that determine these oscillations should be vital, if only to understand which molecular abnormalities result in pathological oscillations. The intricacy of what causes biological oscillations is sometimes overestimated by attributing complete control to a single pacemaker. It has recently been demonstrated that the frequency of biological oscillations can be modulated by more than one enzyme (Reijenga *et al.*, 2001). All of these traits may be influenced by the system's molecular biological processes.

The functional significance of oscillatory occurrences may be found in any of these traits or in their combinations. Because of the relevance and inherent complexity of controlling biochemical oscillations, a systematic approach to understanding this control may be beneficial.

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Metabolic Control Analysis (MCA) is a systematic method for assessing steady-state control. It measures the amount to which any parameter, but especially all molecular activities, controls any steady-state variable within a metabolic circuit.

Because oscillations are dynamic, they do not exist in a steady state. Standard MCA cannot be used to analyze transitory oscillations. Acerenza et al. (1989) proposed an operational definition of a timedependent control coefficient as the relative change in a system variable at time t after a perturbation of a parameter at time zero, divided by the relative change in that parameter. However, it appears that this time-dependent control coefficient is ineffective for characterization of autonomously oscillating systems because its value diverges with time. Neither standard MCA nor its extensions proposed by Acerenza et al. (1989) and Heinrich and Reder (1991) can be used to continuously fluctuating concentrations in a limit cycle oscillation (Kholodenko *et al*., 1997; Demin *et al*., 1999). In contrast, the frequency of such oscillations is time independent, which should allow for the development of an MCA-like technique for those features. The theoretical foundations of a more generic approach have been advanced (Kholodenko *et al*., 1997), but elaborations on how it should be applied in actual systems have been absent. The metabolic control study of steady-state systems revealed principles that control coefficients should obey, which was a significant benefit. The summation theorems of these laws have a corollary in the control analysis of oscillatory systems. The summation theorems had been demonstrated by numerical experiments employing Fourier transformations for frequency control (Reijenga *et al.*, 2002). This summation theorem is quite important. First, when intuition may have suggested that control should be limited to a single pace-making step, the theorem demonstrates the correct formulation of the intuition. The frequency control should be one, but it could be distributed among all participating catalytic activities.

The strongly proof of summation theorems and its application conditions, as well as practical determination method of control coefficients for self-oscillating biochemical networks are not proposed yet. Furthermore, it is not clear, that for what kinetically parameters are true summation theorems.

This investigation directed to solve these problems. Therefore, results of this approach will allow to understand a molecular mechanism of generating and regulation of such extremely important biological process, as biodynamical information, encoded by frequency of biochemical autonomously oscillations.

2. Theory and definitions

Let us consider sustained-oscillatory system which consists of two independent variables *x* and *y*. Let the system be described by system of two autonomous differential equations:

$$\begin{cases} \frac{dX}{dt} = v_1(x,y) \\ \frac{dY}{dt} = v_2(x,y) \end{cases} \quad (1)$$

It is obvious that if the system (1) is in a sustained oscillatory mode, then the linear velocity of a representing point on the phase plane (2) won't depend on time

$$v_f = \sqrt{v_1^2(x,y) + v_2^2(x,y)} \neq f(t), \quad (2)$$

On the other hand, it is known that the cyclic frequency of oscillations ω is connected with phase velocity by a simple ratio:

$$\omega = 2\pi \frac{v_f}{l} = \frac{2\pi}{l}\sqrt{v_1^2 + v_2^2}, \quad (3)$$

where *l*-length of the closed phase limit trajectory. In steady self-oscillations the length of the closed phase trajectory (a limit cycle) remains to a constant on time, therefore the frequency of oscillations is directly proportional with phase velocity and doesn't depend on time.

One of fundamental assumptions in the theory of metabolic control is that rate of different stages in a metabolic network are directly proportional with rate constants *p* (parameters). It means that if all rate constants simultaneously are change by *t* - times, then reaction rate also will change by *t* times, i.e.

$$v(tp) = tv(p) \quad (4)$$

Therefore, rates of reactions are homogeneous of degree 1 functions of parameters, consequently the frequency of the periodic decision of dynamic system (1) also is homogeneous of degree 1 function from rate parameters:

$$\omega(tp) = t\omega(p) \quad (5)$$

For the following analysis the definition of homogeneity is needed.

A function, $f(x_1, x_2, \dots x_n)$ is called homogeneous of degree *h* in $x_1, x_2, \dots x_n$, if

$$f(tx_1, tx_2, \dots tx_n) = \mathrm{t}^{\mathrm{h}} f(x_1, x_2, \dots x_n)$$

for all $t \neq 0$. Pertinent to metabolic control analysis is a theorem on homogeneous functions derived by Leonard Euler (1707- 1783). This theorem establishes a relation between a homogeneous function $f(x_1, x_2, \dots x_n)$ its arguments $\boldsymbol{x_i}$, and the partial derivatives, $\partial f/\partial x_i$: if $f(x_1, x_2, \dots x_n)$ is homogeneous of degree h in $x_1, x_2, \dots x_n$ then

$$x_1 \frac{\partial f}{\partial x_1} + x_2 \frac{\partial f}{\partial x_2} + \cdots x_n \frac{\partial f}{\partial x_n} = hf$$

Applying this theorem to function of frequency (5) we will receive:

$$p_1 \frac{\partial \omega}{\partial p_1} + p_2 \frac{\partial \omega}{\partial p_2} + \cdots + p_n \frac{\partial \omega}{\partial p_n} = \omega \quad (6)$$

The formula (6) can be rewritten as:

$$\frac{p_1}{\omega}\frac{\partial \omega}{\partial p_1} + \frac{p_2}{\omega}\frac{\partial \omega}{\partial p_2} + \cdots + \frac{p_n}{\omega}\frac{\partial \omega}{\partial p_n} = 1 \quad (7)$$

On terminology of the modern theory of metabolic control, coefficients of metabolic control are defined as the relation of a relative increment of system or local characteristics of metabolic process (∂Y/Y) on a relative increment of a regulating parameter of defiant this increment ($\partial Z/Z$):

$$C_z^Y = \frac{\partial Y/Y}{\partial Z/Z} = \frac{Z}{Y}\frac{\partial Y}{\partial Z}$$

According to this terminology, it is obvious that terms in (7) are frequency control coefficients:

$$C_{\mathrm{p}_i}^{\omega} = \frac{\mathrm{p}_i}{\omega}\frac{\partial \omega}{\partial \mathrm{p}_i} \quad (8)$$

and

$$\sum_{i=1}^{n} C_{\mathrm{p}_i}^{\omega} = 1 \quad (9)$$

This result is the summation theorem for frequency control coefficients. This summation theorem for frequency control coefficients is obtained on the basis of assumption of homogeneity relation for frequency in internal parameters. For the testing of validity of this theorem it is necessary to find a way of calculation of these control coefficients.

3. A way of calculation of frequency control coefficients

It is well known that the frequency of sustained oscillation process which is described by system of the kinetic equations as (1) is determined by complex part of the eigenvalues of corresponding Jacobian of system (1). These eigenvalues are defined by values of parameters of system (1). If the obvious analytical view of complex part of eigenvalues as function from parameters is known, then it is easily possible to find frequency control coefficients by formula (8).

In many cases the obvious analytical type of complex part of these eigenvalues are unknown and therefore are calculated by means of computing programs. In such cases we offer the following way for calculate the control coefficients: Let us rewrite the formula (8) as

$$C_{\mathrm{p}_i}^{\omega} = \frac{\mathrm{p}_i}{\omega}\frac{\partial \omega}{\partial \mathrm{p}_i} \approx \frac{\mathrm{p}_i}{\omega}\frac{\Delta \omega}{\Delta \mathrm{p}_i} \tag{10}$$

and give enough small increment to the considered parameter Δp_i. This increment will cause corresponding small change in complex part of eigenvalue $(\Delta\omega)$. Further by replacing these small increments in (10) we can calculate the frequency control coefficients.

For an illustration of this way we will consider following examples.

Model 1: Simple oscillation model Volterra

As the first example, we will consider Volterra's simple self-oscillatory model:

$$\begin{cases} \frac{dx}{dt} = k_1 x - k_2 xy \\ \frac{dy}{dt} = k_2 xy - k_3 y \end{cases} \tag{11}$$

This system (11) has a characteristic equation:

$$\lambda^2 + k_1 k_3 = 0 \tag{12}$$

The roots of this equation, i.e. eigenvalues of Jacobioan of the system (11) are

$$\lambda_{1,2} = 0 \pm i\sqrt{k_1 k_3} \tag{13}$$

As it is stated above, the cyclic frequency of the periodic decision of system (11) will be equal:

$$\omega = \sqrt{k_1 k_3} \tag{14}$$

Consequently, frequency control coefficients for this model are easily calculated by formula (9):

$$C_{k_1}^{\omega} = \frac{k_1}{\omega}\frac{\partial \omega}{\partial k_1} = \frac{1}{2}\frac{k_1}{\sqrt{k_1 k_3}}\sqrt{\frac{k_3}{k_1}} = \frac{1}{2}$$

$$C_{k_2}^{\omega} = \frac{k_2}{\omega}\frac{\partial \omega}{\partial k_2} = 0$$

$$C_{k_3}^{\omega} = \frac{k_3}{\omega}\frac{\partial \omega}{\partial k_3} = \frac{1}{2}\frac{k_3}{\sqrt{k_1 k_3}}\sqrt{\frac{k_1}{k_3}} = \frac{1}{2}$$

$$\sum_{i=1}^{3} C_{k_i}^{\omega} = 1$$

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

$C_{k_1}^{\omega}$	$C_{k_2}^{\omega}$	$C_{k_3}^{\omega}$	Sum	Sum*
0.5	0.0	0.5	1	1
*All kinetic parameters are increased simultaneously by 1% and the total change in ω is determined				

Model 2: A core model by Bier et al. (2000)

Bier et al. (2000) describes glycolysis in terms of two variables, i.e., (internal) glucose and ATP. The system is summarized by the following dynamical system:

$$\begin{cases} \frac{dG}{dt} = V_m - k_1 GT \\ \frac{dT}{dt} = 2k_1 GT - k_2 \frac{T}{K_m + T} \end{cases} \quad (16)$$

where G and T denote the internal glucose concentration and the ATP concentration, respectively. V_m is the constant influx of glucose and k_1 is the enzyme activity (or concentration) of PFK. There is a positive feedback, i.e., ATP stimulates its own production. Furthermore, ATP is broken down according to Michaelis–Menten kinetics. In this study, we used the following parameter set as a reference state: $V_m = 0.36$, k_1=0.02, $k_2 = 6$, $K_m = 13$.

Numerical decision of system (16) by software programs SBW (http:// www.sbml.org) gives for the corresponding eigenvalues:

$$\lambda_{1,2} = 0.00664 \pm 0.112374i$$

Consequently, frequency of the periodical decision of (16) is defined by:

$$\omega = 0.112374$$

Let increase kinetic parameters of system by 1% and calculate the corresponding relative change of ω:

$$\text{on } \frac{\Delta V_m}{V_m} = 0.01,$$

$$\frac{\Delta\omega}{\omega} = \frac{0.112858 - 0.112374}{0.112374} = 0.00431, \text{ and } C_{V_m}^{\omega} = \frac{V_m}{\omega}\frac{\Delta\omega}{\Delta V_m} = 0.431;$$

$$\text{on } \frac{\Delta k_1}{k_1} = 0.01,$$

$$\frac{\Delta\omega}{\omega} = \frac{0.112947 - 0.112374}{0.112374} = 0.0051 \text{ , and } C_{k_1}^{\omega} = \frac{k_1}{\omega}\frac{\Delta\omega}{\Delta k_1} = 0.51;$$

$$\text{on } \frac{\Delta k_2}{k_2} = 0.01,$$

$$\frac{\Delta\omega}{\omega} = \frac{0.112436 - 0.112374}{0.112374} = 0.00056 \text{ , and } C_{k_2}^{\omega} = \frac{k_2}{\omega}\frac{\Delta\omega}{\Delta k_2} = 0.056.$$

Table 2. Control coefficients of the reaction steps on the model 2.

$C_{V_m}^{\omega}$	$C_{k_1}^{\omega}$	$C_{k_2}^{\omega}$	Sum	Sum*
0.431	0.51	0.056	0.997	0.9999
*All kinetic parameters are increased simultaneously by 1% and the total change in ω is determined				

Model 3: The autocatalytic model of Kai-proteins oscillations by Mehra et al. (2006)

In this paper, authors describe a model of in vitro oscillation of cyanobacterial *Kai* proteins (*KaiA, KaiB,* and *KaiC*) in which the *KaiA*- and *KaiB*-assisted autocatalytic phosphorylation and dephosphorylation of *KaiC* are the source for circadian rhythmicity. This model, based upon autocatalysis instead of transcription-translation negative feedback, shows circadian limit-cycle oscillations with *KaiC* phosphorylation profiles and summarized by the following system of differential equations:

$$\begin{cases} \frac{d[KaiA]}{dt} = k_5[KaiABCp] - k_1[KaiA][KaiC] - k_3[KaiACp][KaiA][KaiC] \\ \frac{d[KaiB]}{dt} = k_6[KaiABCp] - k_4[KaiACp][KaiB] \\ \frac{d[KaiC]}{dt} = k_7[KaiCp] - k_1[KaiA][KaiC] - k_3[KaiACp][KaiA][KaiC] \\ \frac{d[KaiCp]}{dt} = k_6[KaiBCp] - k_7[KaiCp] \\ \frac{d[KaiAC]}{dt} = k_1[KaiA][KaiC] - k_2[KaiAC] \\ \frac{d[KaiACp]}{dt} = k_2[KaiAC] - k_4[KaiACp][KaiB] + k_3[KaiACp][KaiA][KaiC] \\ \frac{d[KaiBCp]}{dt} = k_5[KaiABCp] - k_6[KaiBCp] \\ \frac{d[KaiABCp]}{dt} = k_4[KaiACp][KaiB] - k_5[KaiABCp] \end{cases} \quad (17)$$

where *KaiXY* denotes the interaction between *KaiX* and *KaiY* proteins, *KaiCp* indicates fully phosphorylated *KaiC*. In this work we used the following parameters (rate constants) set from the oscillatory region [13]:
$k_1 = 0.0001\mu M^{-1}h^{-1}$, $k_2 = 0.4h^{-1}$, $k_3 = 0.45\mu M^{-2}h^{-1}$, $k_4 = 3.65\mu M^{-1}h^{-1}$, $k_5 = 4.0h^{-1}$, $k_6 = 0.09h^{-1}$, $k_7 = 0.18h^{-1}$.

Values of frequency control coefficients for this model which are calculated by above demonstrated way are listed in Table 3.

Table 3. Control coefficients of the reaction steps on the model 3.

$C^{\omega}_{k_1}$	$C^{\omega}_{k_2}$	$C^{\omega}_{k_3}$	$C^{\omega}_{k_4}$	$C^{\omega}_{k_5}$	$C^{\omega}_{k_6}$	$C^{\omega}_{k_7}$	Sum	Sum*
0.009	0.004	1.598	-1.354	0.087	0.418	0.228	0.99	1.001
*All kinetic parameters are increased simultaneously by 1% and the total change in ω is determined								

The temperature dependence of the frequency and temperature compensation in biochemical oscillations.

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

$$k_i = A_i\, e^{-\frac{E_i}{RT}}, \quad (18)$$

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, a 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: $\omega(T) = \omega(k_i(T))$ and

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

From here

$$\frac{T}{\omega}\frac{\partial\omega}{\partial T} = \sum_{i=1}^{n}\frac{k_i}{\omega}\frac{\partial\omega}{\partial k_i}\frac{T}{k_i}\frac{\partial k_i}{\partial T} \tag{19}$$

As above denoted,

$C_{k_i}^{\omega} = \frac{k_i}{\omega}\frac{\partial\omega}{\partial k_i}$ is control coefficient frequency on rate constant. Then

$C_T^{\omega} = \frac{T}{\omega}\frac{\partial\omega}{\partial T}$ will be a responcity coefficient of frequency on the temperature and

$C_T^{k_i} = \frac{T}{k_i}\frac{\partial k_i}{\partial T}$ will be a responcity coefficient of *i*-th rate constant on the temperature.

From Arrhenius equation (18) we receive that

$$C_T^{k_i} = \frac{E_i}{RT} \tag{20}$$

By regarding (20) in (19)

$$C_T^{\omega} = \frac{1}{RT}\sum_{i=1}^{n} C_{k_i}^{\omega} E_i \tag{21}$$

Temperature compensation 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. From the point of view of metabolic control analysis, temperature compensation means, that a response coefficient C_T^{ω} must be equal or near to zero. Formula (21) 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 temperature-compensated oscillators have to contain reactions that have opposing effects on the frequency and temperature compensation can occur within a certain temperature interval, whenever the activation energy (E_i) weighted sum of the control coefficients is near to zero. The above offered method of calculation of frequency control coefficients allows to conclude, that not all biochemical oscillators are temperature-compensated. Really, frequency control coefficients in Table1 and Table 2 indicate that first two oscillatory models (Model1 and Model2) considered above are not temperature-compensated, since all of that coefficients are positively, but model 3 has temperature - compensate mechanism and may be temperature-compensated. In Model 3 the process R4 (rate constant k_4), (In process R4, *KaiB* associates with *KaiACp* to form the ternary complex *KaiABCp*) has a negative frequency control coefficient (see Table 3) over the entire parameter space for which oscillations are observed.

Formula (17) allows to find the average value of activation energy *(E_a)* for all reaction stages, since $\sum_{i=1}^{n} C_{k_i}^{\omega} = 1$ and if all $E_i = E_a$, then

$$C_T^{\omega} = \frac{1}{RT}\sum_{i=1}^{n} C_{k_i}^{\omega} E_i = \frac{E_a}{RT}\sum_{i=1}^{n} C_{k_i}^{\omega} = \frac{E_a}{RT}$$

and $E_a = RTC_T^{\omega}$.

For example, from experimental data on the temperature-period dependence, graphically demonstrated in Dunlap (1999), we calculate that $C_T^{\omega} \approx 2.6$, and $E_a \approx$ 7kJ/mol.

From the above sensitivity analysis follows that for the temperature-compensated oscillatory system average value of activation energy has to be rather small in comparison with temperature-non-compensated oscillators.

Thus, the way of the frequency analysis demonstrated in this work allows to quantitatively estimate a contribution of different stages of reaction to the frequency of oscillations, to distinguish temperature-compensated oscillatory systems from temperature non-compensated ones and to find out mechanism and core of temperature compensation of oscillation frequency or period.

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References

Patke, A., Young, M.W., & Axelrod, S. (2020). Molecular mechanisms and physiological importance of circadian rhythms. *Nature reviews Molecular cell biology, 21*(2), 67-84.

Partch, C.L., Green, C.B., & Takahashi, J.S. (2014). Molecular architecture of the mammalian circadian clock. *Trends in Cell Biology,* 24(2), 90-99.

Reijenga, K.A., Snoep, J.L., Diderich, J.A., van Verseveld, H.W., Westerhoff, H.V., & Teusink, B. (2001). Control of glycolytic dynamics by hexose transport in Saccharomyces cerevisiae. *Biophysical Journal, 80*(2), 626-634.

Acerenza, L., Sauro, H.M., & Kacser, H. (1989). Control analysis of time-dependent metabolic systems. *Journal of Theoretical Biology, 137*(4), 423-444.

Heinrich, R., Reder, C. (1991). Metabolic control analysis of relaxation processes. *J. Theor. Biol.*, 151, 343–350.

Kholodenko, B.N., Demin, O.V., & Westerhoff, H.V. (1997). Control analysis of periodic phenomena in biological systems. *The Journal of Physical Chemistry B, 101*(11), 2070-2081.

Demin, O.V., Westerhoff, H.V., & Kholodenko, B.N. (1999). Control analysis of stationary forced oscillations. *The Journal of Physical Chemistry B, 103*(48), 10695-10710.

Reijenga, K.A., Westerhoff, H.V., Kholodenko, B.N., & Snoep, J.L. (2002). Control analysis for autonomously oscillating biochemical networks. *Biophysical Journal, 82*(1), 99-108.

Bier, M., Bakker, B.M., & Westerhoff, H.V. (2000). How yeast cells synchronize their glycolytic oscillations: a perturbation analytic treatment. *Biophysical Journal, 78*(3), 1087-1093.

Mehra, A., Hong, C.I., Shi, M., Loros, J.J., Dunlap, J.C., & Ruoff, P. (2006). Circadian rhythmicity by autocatalysis. *PLoS Computational Biology, 2*(7), e96.

Dunlap, J.C. (1999). Molecular bases for circadian clocks. *Cell, 96*(2), 271-290.