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Sensitivity analysis of a pathway with respect to fast and small temperature change

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ABSTRACT

Temperature affects all enzymes simultaneously in a metabolic system. The enzyme concentration in a biochemical system can be considered as invariant under fast and small temperature change. Therefore, the total sensitivity of a steady state flux through a pathway with respect to the temperature can be expressed as: the apparent activation energy of a steady state pathway flux equals the sum of weighted activation energies of the individual reactions contributing to the flux, where the weighting factors are the flux control coefficients of these reactions in the context of the network. Correspondingly, since the elasticity of any enzyme with respect to temperature is always nonzero, only the reactions with a nonzero flux control coefficient contribute accordingly to the temperature sensitivity of the pathway.

1. Introduction

Temperature represents a major environmental factor that influences any living organism (Stephanopoulos et al., 1998; Nielsen et al., 2003; Roels, 1983; Zakhartsev et al., 2015; Esener et al., 1981). The temperature undergoes natural ir/regular long-term and significant variations: epoch, seasonal or circadian. In the same time a fast, random, small and short-term deviation of current temperature from its average value (*i.e.* fluctuations) always occur. The regulation of enzyme amounts through gene expression allows compensating the long-term effect of temperature variation thereby achieving homeostasis of a metabolic function (Ruoff et al., 2007). However, under the fast, small and short-term [shorter than the regulation of enzyme concentrations through the gene expression loop] temperature variation the enzyme concentration in a biochemical system can be considered to be invariant because its adjustment takes longer time during which the temperature variation occurs. Nevertheless, without any exception, temperature exerts a direct pleotropic effect on the rate of all chemical reactions in a biochemical system. In a network context, the effect of temperature variations can be partially compensated through the kinetic regulatory (feedback/feedforward) mechanisms, which, nevertheless, are also temperature dependent and therefore the steady state flux through a pathway responds to the rapid temperature variations. Thus, the question arises how the temperature sensitivity of the steady state pathway flux can be understood in a network context. The aim of this research is to describe the temperature response of a steady state pathway flux to fast, small and short-term temperature change on the base of Metabolic Control Analysis (MCA).

2. Assumptions

The following assumptions were accepted for the analysis presented in this study:

- a biochemical system is in a steady-state
- the kinetic mechanism of all enzyme catalysed reactions in a biochemical system is considered as the pseudo-first order unsaturated kinetics
- temperature variations are considered to be small, approximately 1% of current temperature
- temperature variations are considered to be faster than the regulation of enzyme concentrations via gene expression mechanisms, *i.e.* the enzyme concentration is considered to be invariant in course of the temperature variation
- the response and elasticity coefficients are assessed with respect to the fast and small temperature variations

3. Theoretical background

From the theory of Metabolic Control Analysis (MCA) follows, that in an arbitrary biochemical system consisting of N metabolites (X_1, X_2, …, X_N; $i = 1, 2,..., N$) reacting via K enzyme catalysed reactions (r_1, r_2, …, r_K; $j = 1, 2,..., K$) the steady-state flux through a metabolic pathway (J) can be influenced by external signals from the environment, which impacts enzyme activity or steady-state rate of jth step (r_j). These influences are formally described as changes in external parameters (p), such as concentrations of external metabolites

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(substrates or effectors) or physical parameters, *e.g.* temperature (T), pH, ionic strength (Kholodenko, 1988). In order to describe it, Kacser and Burns (1973) (Kacser and Burns, 1973) have introduced a response coefficient (R), which quantifies the sensitivity of the steady-state pathway flux (J) with respect to the external parameter/effectors:

$$R_p^J = \frac{p}{J}\frac{\partial J}{\partial p} \tag{1}$$

The external parameter can influence the behaviour of the system by modulating the enzyme activity, but not its concentration. If the external parameters (p) can be held constant after it has been altered while the system reaches a new steady state, then its impact on J through the pathway can be expressed through individual impacts onto enzymes of the pathway. If the external parameter p acts on J through being an effector for the jth pathway enzyme, the response coefficient with respect to the effect of p is composed by two factors:

- the sensitivity of a pathway flux J to the activity of the jth enzyme (r_j) that is the target for the effector p, *i.e.* the enzyme's flux control coefficient ($C_{r_j}^J$). The flux control coefficient is the global entity, which measures relative steady state change in pathway flux (J) in response to a relative change in a parameter (p), *e.g.* enzyme activity of steady state rate (r_j) of jth step:

$$C_{r_j}^J = \left(\frac{p}{J}\frac{\partial J}{\partial p}\right)\Big/\left(\frac{p}{r_j}\frac{\partial r_j}{\partial p}\right) = \frac{r_j}{J}\frac{\partial J}{\partial r_j} \tag{2}$$

- the strength of the effect of p on the jth enzyme, *i.e.* elasticity coefficient of r_j with respect to p ($\varepsilon_p^{r_j}$). The elasticity coefficient is the local entity, which deals with the response of any reaction rate (r_j) (when the enzyme is 'isolated' from the rest of the system) towards a change in an external parameter p:

$$\varepsilon_p^{r_j} = \frac{p}{r_j}\frac{\partial r_j}{\partial p} \tag{3}$$

Thus, the response coefficient of the steady-state pathway flux (J) with respect to the external parameter p which affects a single (or target) enzyme in a pathway is:

$$R_p^J = C_{r_j}^J \varepsilon_p^{r_j} \tag{4}$$

If p affects more than one enzyme, the net response coefficient R_p^J will be given by the sum of the responses from each enzyme, *i.e.* to the sum of the weighted elasticity coefficients with respect to p for all the affected enzymes, $\varepsilon_p^{r_j}$, where the weighting factor is the flux control coefficients $C_{r_j}^J$ of corresponding enzymes in a network:

$$R_p^J = \sum_{j=1}^{K} C_{r_j}^J \varepsilon_p^{r_j} \tag{5}$$

Contributions to the sum come only from the enzymes for which both terms $C_{r_j}^J$ and $\varepsilon_p^{r_j}$ are nonzero to be able to affect the pathway (Fell, 1997). Even if the elasticity of an enzyme with respect to p is large, the response of the flux is only significant if the corresponding flux control coefficient is nonzero (Stephanopoulos et al., 1998).

4. Response coefficient with respect to temperature

Temperature (T) is a pleiotropic factor that simultaneously influences all enzymes in a biochemical system (Stephanopoulos et al., 1998; Nielsen et al., 2003; Roels, 1983; Zakhartsev et al., 2015; Esener et al., 1981). Therefore, the response coefficient of a pathway (equation (5)) can be derivatized with respect to the temperature as the environmental parameter. According to the MCA methodology, the temperature should change rapidly and to small values and be held at a new value while the steady state pathway flux reaches a new steady state.

5. Local response to temperature

Obviously, each biochemical reaction (r_j) that contributes to the pathway flux being isolated from the system has a unique elasticity with respect to temperature due to temperature sensitivity of the reaction rate constant (k_j) expressed by the Arrhenius equation. Thus, in the simplest form, the forward net-rate of an irreversible first-order biochemical reaction is:

$$r_j = k_j \prod_{i=1}^{N} [X_i] = A_j \exp\left(-\frac{E_a^{r_j}}{RT}\right) \prod_{i=1}^{N} [X_i] \tag{6}$$

where: k_j is the rate constant of the jth reaction $[s^{-1}]$; T is the absolute temperature $[K]$; R is the gas constant $[J/(mol \times K)]$; A_j is the frequency of collisions in the correct orientation $[s^{-1}]$; $E_a^{r_j}$ is the activation energy for the jth reaction $[J/mol]$; $[X_i]$ is the concentration of ith metabolite participating in jth reaction. From the Collision Theory applied to the pseudo-first order unsaturated kinetic mechanism of enzyme catalysed reaction follows that A_j includes its own temperature sensitivity as well as an enzyme (*i.e.* catalyst) concentration. However, for the small temperature change the change of A_j is negligibly small. Therefore, under the assumption of the invariance of enzyme concentrations under the fast and small temperature variations the A_j can be considered as the constant.

Therefore, if the external affecting factor is the temperature T, then the elasticity coefficient (equation (3)) of the jth reaction rate (equation (6)) with respect to temperature can be written as:

$$\varepsilon_T^{r_j} = \frac{T}{r_j}\frac{\partial r_j}{\partial T} = \frac{T}{r_j}\frac{\partial}{\partial T}\left(A_j \exp\left(-\frac{E_a^{r_j}}{RT}\right)\prod_{i=1}^{N}[X_i]\right) = \frac{T}{r_j} r_j \frac{E_a^{r_j}}{RT^2} = \frac{E_a^{r_j}}{RT} \tag{7}$$

Thus, the elasticity of the reaction rate r_j with respect to temperature does not depend on the concentrations of metabolites X_i and it is proportional to the activation energy of the reaction and the temperature.

This reasoning applies equally to reversible reactions, which, in the context of the biochemical system, either can be in equilibrium or displaced from the equilibrium. The temperature dependence of the forward and reverse rate constants likely differs. Therefore, the net-rate of reversible reaction can be further decomposed to the component steps with own temperature dependencies and corresponding flux control coefficients. Moreover, for the reactions significantly displaced from the equilibrium one of the component process can be disregarded.

6. Global response to temperature

A biochemical system is comprised of a network of enzyme catalysed metabolic reactions, through which the flux of matter is performed (Stephanopoulos et al., 1998; Nielsen et al., 2003; Roels, 1983). Thus, many reactions contribute simultaneously, but in different way, into the steady state flux through a pathway (J). The pathway flux can be estimated by the activity of the terminal output reaction. For example, ethanol production gives estimate of the overall metabolic flux through fermentative pathway in anaerobic conditions in yeast. When the yeast culture is subjected to the fast temperature change, then the output ethanol flux shows exponential dependence on the temperature (exemplified at Fig. 1). This gives the reason to conclude that under the fast and small temperature change the temperature dependence of the steady state pathway flux (J) also can be described through the Arrhenius equation:

$$J = A_J \exp\left(-\frac{E_a^J}{RT}\right)\prod_{i=1}^{N}[X_i] \tag{8}$$

Where: E_a^J is the apparent activation energy of the pathway flux J; $[X_i]$ is the concentration of output ith metabolite excreted from a pathway, *i.e.* external metabolite *e.g.* ethanol, *etc*. Here, the A_J is also

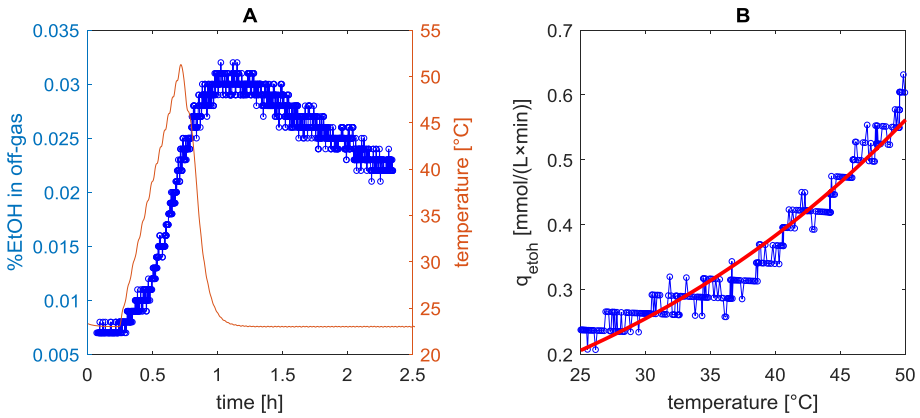

Fig. 1. An example of the temperature dependency of output pathway flux under fast temperature change.A. The evolution of ethanol content in off-gas from bioreactor (blue dots) in course of the fast temperature change (1 °C/min; red line) which has been monitored (with photoacoustic gas monitor INNOVA 1313) in anaerobic glucose unlimited batch culture of yeast *Sacchromyces cerevisiae* CEN. PK 113-7D growing in anaerobic CEN. PK medium (Zakhartsev et al., 2015) (pH = 5.0) at 23 °C. On the moment of the beginning of the temperature change, the culture was in the exponential growth phase with the biomass concentration 1.2 $[g_{dw}/L]$. g_{dw} – gram of dry weight of biomass.B. The temperature dependence of the rate of ethanol formation in gas phase in course of the temperature change (panel A). The volumetric rate of ethanol formation in the gas phase was calculated as $q_{etoh} = (V_g P\alpha)/(V_r RT)\ [mol/(L \times min)]$; where: $V_g = 0.109\ [L/min]$ – flow of carrier gas (pure nitrogen); $P = 101.4\ [kPa]$ – barometric pressure; α – vapour fraction in off-gas (blue dots at panel A); $V_r = 0.15\ [L]$ – volume of head-space in bioreactor; $R = 8.31445\ [(L \times kPa)/(mol \times K)]$; T – temperature $[K]$. The data were fit to the Arrhenius equation (red curve) with following parameters: $A = 85970\ [mol/(L \times min)]$; $E_a = 32060\ [J/mol]$. The goodness of fit is $R^2 = 0.949$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

considered as the constant (for details see the previous chapter).

Further, if the external variable factor p is the temperature T, then the response coefficient (defined in equation (1)) of the steady state pathway flux J with respect to temperature (equation (8)) can be written as:

$$R_T^J = \frac{T}{J}\frac{\partial J}{\partial T} = \frac{T}{J}\frac{\partial}{\partial T}\left(A_J \exp\left(-\frac{E_a^J}{RT}\right)\prod_{i=1}^{N}[X_i]\right) = \frac{T}{J}\left(J\frac{E_a^J}{RT^2}\right) = \frac{E_a^J}{RT} \tag{9}$$

Thus, the response coefficient of the pathway flux with respect to fast and small temperature change does not depend on the concentrations of metabolites (including biomass) and proportional to the apparent activation energy of the pathway and temperature.

7. Response coefficient with respect to temperature

Correspondingly, to re-formulate equation (5) in terms of the response coefficient of a steady state pathway flux J with respect to temperature T (as the external parameter p) the following equation is derived for all contributing reactions:

$$R_T^J = \sum_{j=1}^{K} C_{r_j}^J \varepsilon_T^{r_j} \tag{10}$$

Substituting equations (7) and (9) into equation (10) gives the following solution:

$$\frac{E_a^J}{RT} = \sum_{j=1}^{K} C_{r_j}^J \frac{E_a^{r_j}}{RT} \tag{11}$$

$$E_a^J = \sum_{j=1}^{K} C_{r_j}^J E_a^{r_j} \tag{12}$$

Thus, equation (12) shows that the apparent activation energy of a steady state pathway flux is equal to the sum of weighted activation energies of the individual reactions contributing to the steady state pathway flux, where the weighting factor is the flux control coefficients of this reaction in the context of the metabolic network. Correspondingly, since the elasticity of any enzyme with respect to temperature is always nonzero, only the reactions with a nonzero flux control coefficient will contribute to the temperature sensitivity of the steady state pathway flux. Moreover, it is important to note that some reactions might have positive or negative flux control coefficients with respect to the output flux, therefore they correspondingly contribute to the apparent activation energy of a pathway.

Change of the temperature necessarily results in change of r_j (equation (6)), however, if the proportional change in steady state pathway flux (J) also occurs, then it is not necessary that $C_{r_j}^J$ (equation (2)) changes under this circumstances.

Nevertheless, the flux control coefficient can change in course of fast and significant temperature change through affecting elasticities of the reaction rates with respect to the metabolite concentrations ($\varepsilon_{X_i}^{r_j}$) (Fell, 1997; Cornish-Bowden, 2012) due to variation of the concentrations of the intermediate metabolites in a pathway. This would have corresponding consequences onto flux ($C_{r_j}^J$) and the concentration ($C_{r_j}^S$) control coefficients because they can be expressed through $\varepsilon_{X_i}^{r_j}$ as it follows from the summation and the connectivity theorems (Fell, 1997; Cornish-Bowden, 2012). Thus, temperature induced change in $C_{r_j}^J$ would have the corresponding contribution to equation (12). Whereas, the expected variations in $C_{r_j}^S$ might be large in magnitude, but it has no impact on the temperature sensitivity of the pathway.

8. Discussion and conclusion

Temperature (T) affects simultaneously all enzymes in a biochemical system. Under fast and small temperature variations, the enzyme concentration is invariant, because under this assumption the change in enzyme concentration via gene expression is slower than the temperature change. Total response of steady state pathway flux with respect to the temperature will be the sum of the individual responses from each enzyme affected, but only those that have nonzero flux control coefficient in the network. Correspondingly, if a certain reaction in a complex network has a negative flux control coefficient with respect to the output pathway flux, then it contributes negatively to the temperature response of the output flux (equation (12)). In fact, equation (12) additionally contributes to the understanding of the compensation mechanism of temperature effects in metabolic systems as previously demonstrated (Ruoff et al., 2007; Aasen and Ruoff, 2008). Equation (12) is only valid for a fast and small temperature changes, because the response coefficient is defined as a first order approximation, which is true only for small temperature variations. For a large change in T, the total effect will not be the sum of the effects on each enzyme because of the non-linear nature of the kinetics of metabolic systems. Also, this view (equation (12)) is only valid for pathways without genetic regulation of the enzyme concentration. Thus, valid only for the fast and small temperature variations when the enzyme concentration is invariant and the observed change in the enzyme activity is exclusively due to the temperature effects.

The equation (12) decomposes onto elemental components the temperature sensitivity of the steady state pathway flux with respect to a fast and small temperature change on the base of MCA when the only varying parameter is the temperature.

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