29 Can metabolic control analysis be applied to hierarchical regulated metabolism? MCA versus HCA

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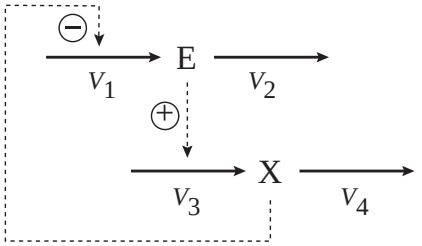
Introduction

Metabolic control analysis, MCA, (Kacser and Burns, 1974; Heinrich and Rappoport, 1975) has been applied to many metabolic systems and has been proven to be a useful tool in the quantitative description of these systems. In the living cell the level of metabolism is connected to other cellular systems, such as signal-transduction and transcription/translation, which control metabolic reactions via regulatory interactions. Control is therefore not distributed amongst the enzymes in the metabolic pathway only, but also amongst the reactions that occur in these regulatory systems. An extension of MCA, hierarchical control analysis, HCA, (Kahn & Westerhoff, 1991) does take regulatory interactions between different cellular systems into account. Usually these two types of control coefficients do not have the same value. Does this imply that MCA does not provide us correct information of how a set of metabolic reactions is controlled in a living cell? This question will be addressed in this article. Using a model that consists of metabolic reactions and reactions on the transcription/translation level, we show that in a certain time span, a metabolic system can behave in the same way as if it were isolated from the global system. In other words, descriptions of MCA are valid in this time span. On a longer time scale, descriptions with HCA are required. Two properties of the systems, i.e., the eigenvalues and the transient times (Easterby, 1981), seem to indicate the time separation. The larger the dif-



$$v_1 = k_1 - k_i[X] \quad (29.1)$$
$$v_2 = k_2[E] \quad (29.2)$$
$$v_3 = k_3[E] \quad (29.3)$$
$$v_4 = k_4[X] \quad (29.4)$$

Fig. 29.1 Schematic presentation of the hierarchical system. See text for details

ference between the two sets of eigenvalues or between the transient times of enzymes and metabolites, the better metabolism is separated in time from the level of transcription/translation.

Materials and methods

The model

We used a mathematical model (Fig. 29.1, eqs. 29.1 to 29.4) and carried out simulations using the modelling program Gepasi 3 (Mendes, 1997). The model describes two levels within the cell, metabolism and the level of transcription/translation. The level of transcription/translation influences the metabolic level by providing enzyme and the metabolic level regulates the level of transcription/translation by inhibition. The rate equations are defined with linear kinetics for reasons of simplicity; using this type of kinetics makes it possible to analyse the system analytically. In reaction 1, enzyme is produced at a constant (steady state transcription/translation) rate k_1. This rate is decreased by metabolite X with an inhibition rate constant k_i. The degradation of the enzyme is proportional to the enzyme concentration. The production rate of metabolite X is determined by the concentration of enzyme at a rate constant k_3. Metabolite X is degraded at a rate proportional to the concentration of X. The concentration of the enzyme of this reaction is assumed to be constant. All substrates, enzymes and effectors that are assumed constant in the model are not explicitly stated in the rate equations.

MCA and HCA

If we ignore the interactions with the level of transcription/translation we can apply MCA to the metabolic level. For the control of the X producing enzyme on the steady-state concentration of X we find

$$c_{k_3}^{[X]_{ss}} = \frac{1}{\varepsilon_X^{v_4} - \varepsilon_X^{v_3}} = 1 \qquad (\varepsilon_X^{v_3} = 0,\ \varepsilon_X^{v_4} = 1) \quad (29.5)$$

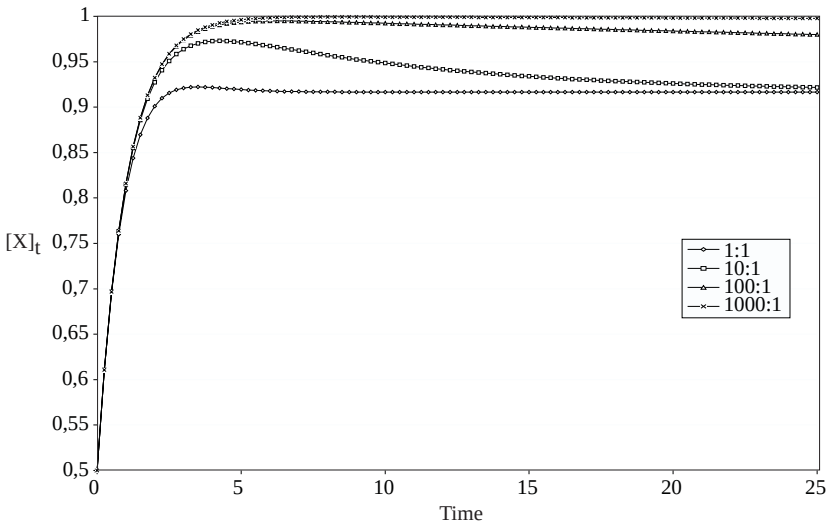


Fig. 29.2 Results of several simulations. In the legend the ratio between the rate constants of metabolism and transcription/translation are indicated.

If we do take account of the interactions with the level of transcription/translation we find the global control of this enzyme on the steady state concentration of X by using HCA (note that Global Control Coefficients are indicate by a capital rather than a lower case c):

$$C_{k3}^{[X]_{ss}} = \frac{1}{\varepsilon_X^{v_4} - \varepsilon_X^{v_3} - \frac{\varepsilon_X^{v_1}}{\varepsilon_E^{v_2} - \varepsilon_E^{v_1}}} = \frac{1}{1-\varepsilon_X^{v_1}} \quad (\varepsilon_X^{v_3} = 0,\ \varepsilon_X^{v_4} = 1,\ \varepsilon_E^{v_1} = 0,\ \varepsilon_E^{v_2} = 1,\ \varepsilon_X^{v_1} = \frac{-k_i[X]_{ss}}{k_1 - k_i[X]_{ss}} = -\frac{k_i k_3}{k_2 k_4}) \tag{29.6}$$

Results

Global versus metabolic control

Eq. 29.6 shows that the global control of enzyme 3 can differ greatly from its metabolic control.

It is assumed by many authors that control coefficients derived with MCA describe correctly the response of the system to a change in a parameter, because the reactions on the level of metabolism are much faster than those of transcription/translation, i.e., the levels are separated in time. We here survey the correctness of this assumption and see which prerequisites have to be fulfilled for this assumption to be safe. We performed computer simulations with the model (Fig. 29.1) and followed the evolution of X in time after a perturbation in rate constant k_3, at different choices of system parameters. We investigated whether it was possible to calculate the Metabolic Control Coefficient from these simulations.

Table 29.1 Comparison of the 'measured' (i.e., calculated from the simulations) metabolic control coefficient with the analytically found metabolic control coefficient at different ratios of rate constants. The ratio between eigenvalues and between transient times are also indicated.

k_{trans}/k_{met}	λ_1/λ_2	τ_E/τ_X	'Measured' Control Coefficient	Analytical Control Coefficient
1		1	0.916	1
0.1	7.17	10	0.927	1
0.01	76.4	100	0.986	1
0.001	768.8	1000	0.998	1

First we varied all the rate constants at the level of transcription/translation ($k_1 = k_2 = 10k_i$) whilst keeping the rate constants at the metabolic level constant at 1. In this way the global steady state was the same for all simulations. To measure the control of enzyme 3 on steady state concentrations of X, k_3 was changed to 2 (small changes should be made to measure control coefficients, but with the kinetics used in this model the system responds in the same way to large changes). The Metabolic Control Coefficient was obtained analytically and was equal to 1 for all parameter choices (eq. 29.5). The Global Control Coefficient was calculated using eq. 29.6 and was 0,9091 for the parameter values used. Also the eigenvalues and transient times were obtained using their analytical expressions (not shown here). Metabolic Control Coefficients were 'measured' (i.e., calculated from the numerical time integrations) by taking the highest concentration of X as the apparent metabolic steady-state level. Results are presented in Fig. 29.2 and Table 29.1.

We see that at higher ratios between rate constants on the different levels, metabolism on this time span is not constrained by the level of transcription/translation. As the kinetics of metabolic and transcription/translation became more distinct, the 'measured' control coefficient changed from the value of the Global Control Coefficient to the value of the Metabolic Control Coefficient.

In these simulations the value of the Global Control Coefficient was about 90% of that of the Metabolic Control Coefficient. It should be better to choose the parameters in the simulations in a way that the value of the Metabolic Control Coefficient and the Global Control Coefficient are more different, and to examine whether such a system is able to show metabolic behaviour. To this aim and to vary the ratio between the eigenvalues and between transient times, the rate constants for the degradation reactions, k_2 and k_4 were varied inversely proportionally (motivated by eq. 29.6). The metabolic control coefficient is again equal to 1 for all parameter choices. The global control coefficient was 0,5. Again we see that the behaviour of metabolism was less constrained by the level of transcrip-

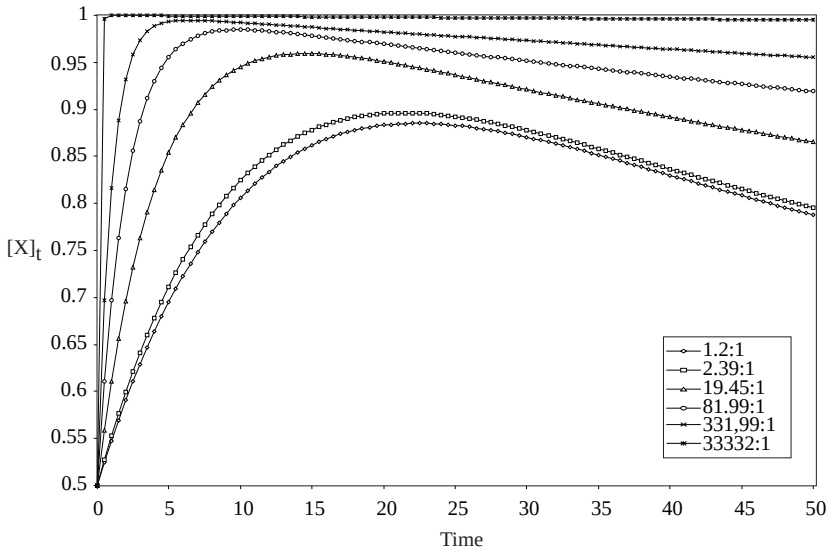


Fig. 29.3 Results of several simulations. In the legend the ratio between the rate constants of metabolism and transcription/translation are indicated.

Table 29.2 Comparison of the 'measured' metabolic control coefficient with the analytically found metabolic control coefficient at different ratios of rate constants. The ratio between eigenvalues and between transient times are also indicated.

k_2, k_4	λ_1/λ_2	τ_E/τ_X	Measured	Analytical
0.01, 0.1	1.2	10	0.77	1
0.009, 0.111111	2.4	12.4	0.79	1
0.004, 0.25	19.5	62.5	0.82	1
0.002, 0.5	82	250	0.969	1
0.001, 1	332	1000	0.990	1
0.0001, 10	33332	100000	0.999	1

tion/translation at an increasing ratio between transient times and between the eigenvalues (Fig. 29.3 and Table 29.2).

Fitting eigenvalues

The system analyzed here exhibited short-term (metabolic) behaviour as well as global behaviour. It should be possible to determine these two types of behaviour experimentally in a living cell. Here we focus on measurements of the eigenvalues of the model system. The 'experiments' were done on computer. A simulation was run and 'samples' were taken at different intervals. The eigenvalues were obained by curve fitting and compared to the values found analytically.

The total behaviour of X in time is described by the equation

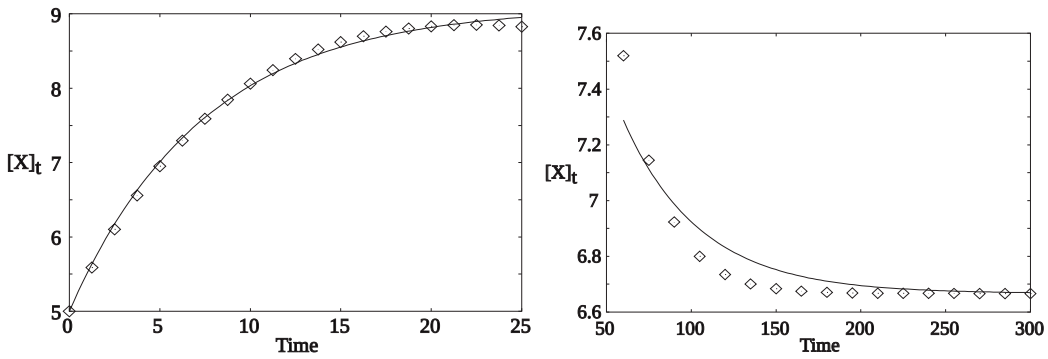


Fig. 29.4 Fitting eqs. 29.8 and 29.9 to 'experimental' measurements of metabolite concentrations to obtain the eigenvalues of the system.

Table 29.3 Comparison of the eigenvalues found by fitting the 'experimental' data to obtain the analytically found eigenvalues.

	λ_1	λ_2	λ_1/λ_2
Analytical	−0.06	−0.05	1.2
Measured	−0.135091	−0.0220431	

$$[X]_{(t)} = [X]_{ss} + Av_{11}e^{\lambda_1 t} + Bv_{21}e^{\lambda_2 t} \tag{29.7}$$

where $[X]_t$ is the change of metabolite X in time, $[X]_{ss}$ the steady state value, A and B are constants, v_{11} and v_{21} are components of the eigenvectors, t is time and the λ's are the eigenvalues.

The first eigenvalue describes the short-term behaviour and the second the transient to the global steady state. To obtain the two eigenvalues they should be fitted separately. The 'fast' eigenvalue should be fitted with

$$[X]_{(t)} = [X]_{mss} + ([X]_{mss} - [X]_{iss})e^{\lambda_1 t} \tag{29.8}$$

Eq. 29.8 describes the increase of X from the initial steady state, $[X]_{iss}$, to $[X]_{mss}$, the 'apparent' metabolic steady state. The 'slow' eigenvalue should be fit with the equation

$$[X]_{(t)} = [X]_{gss} + ([X]_{gss} - [X]_{mss})e^{\lambda_2 t} \tag{29.9}$$

Eq. 29.9 describes the decrease of X from $[X]_{mss}$ to the global steady state $[X]_{gss}$.

First the parameters were set in a way that the ratio between the two eigenvalues was 1.2; they were nearly equal. The results are presented in Fig. 29.4 and Table 29.3. When the ratio between the eigenvalues was small, the evolution of X in time could not be described by the eigenvalues separately. Then it was not possible to assign an eigenvalue to behaviour of a single variable.

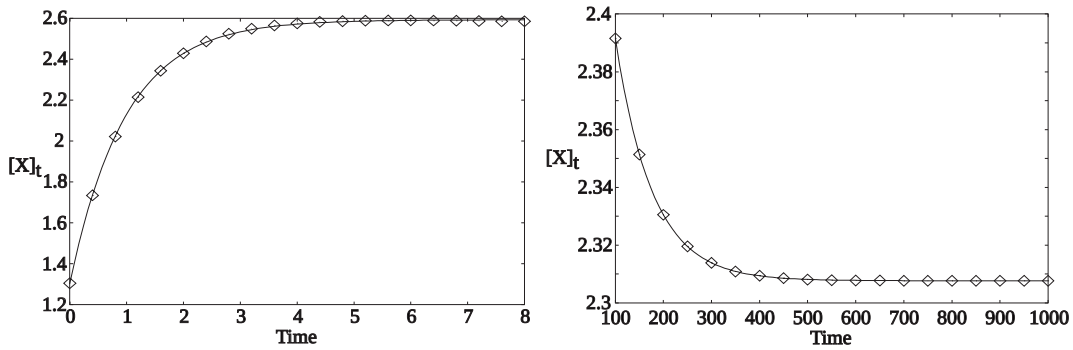


Fig. 29.5 Fitting eqs. 29.8 and 29.9 to 'experimental' data to obtain the eigenvalues of the system.

Table 29.4 Comparison of the eigenvalues found by fitting the 'experimental' data to obtain the analytically found eigenvalues.

	λ_1	λ_2	λ_1/λ_2
Analytical	−0.99696	−0.01304	76.5
Measured	−1.02926	−0.0130297	

At higher ratios, for example, a ratio of 76.5 between the eigenvalues, it was possible to fit each behaviour (metabolic/global) with a separate eigenvalue (see Fig. 29.5 and Table 29.4).

Discussion

We have investigated here a model that consists of metabolic reactions and reactions on the transcription/translation level. We showed that, although in the living cell the global control can differ significantly from the metabolic control, the descriptions of MCA might be valid in a certain time span. The processes on the level of transcription/translation were take to be slow compared to the reactions in metabolism. In the case that the Global Control Coefficient was about 90% the value of the Metabolic Control Coefficient, the deviation of the 'measured' Metabolic Control Coefficient from the analytically found one, was less than 5%, thus within experimental limits, when rate constants at the level of transcription/translation were 100 times smaller than those on the metabolic level. When the Global Control Coefficient had 50% the value of the Metabolic Control Coefficient this limit was reached when rate constants at the level of transcription/translation were 250 times smaller than those on the metabolic level. In most real biochemical systems the rates at the metabolic level are much higher than transcriptional/translational rates. Metabolic reactions occur in the range of seconds, transcription/translation reactions in the range at minutes to hours; we

can thus conclude that, in principle, metabolism and transcription/translation are time-separated in the living cell.

Experimental measurement of the classes (i.e., metabolic and transcriptional/-translational) of eigenvalues in a hierarchical system should be possible if there is a large difference between the eigenvalues. The possibility to measure the eigenvalues indicates that the levels are separated in time.

The results presented in this article were obtained using a simple hierarchical model with linear kinetics determining the rate of reactions. Analogous results were found when the model was defined with non-linear kinetics. Another model consisting of four reactions on the metabolic level and three regulatable enzymes showed the same behaviour at similar parameter choices (results not shown).

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