Strong control on the transit time in metabolic channelling

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Abstract A suite of different characteristic times is used to describe the temporal behavior of a metabolic pathway. Here we focus on the 'transit' time, that is the average time it takes for a molecule, entering the steady-state pathway as a substrate, to exit the pathway as a product. We show that metabolic channelling results in dramatic changes in control exerted by pathway enzymes on the transit time. In an 'ideal' pathway a doubling of the enzyme concentrations halves the transit time. In a dynamic channel such an increase can reduce the transit time by a factor of four or more.

1. Introduction

In the cytoplasm of living cells, high concentrations of enzymes and macromolecular crowding drive the formation of specific enzyme-enzyme complexes, which may involve the direct transfer ('channelling') of intermediate metabolites. The paradigm of 'metabolic channelling' refers to mechanisms in which the reaction product of one enzyme is transferred to the next enzyme in a pathway without mixing with the bulk-phase pool (for reviews see [1,2]). So-called ideal pathways, in which enzymes (catalysts) interact solely via bulk-phase metabolites, lack such mechanisms. Different possible mechanisms of channelling have been discussed and the terms 'dynamic' and 'static' channelling have been coined. Recent studies [3–14] revealed significant differences in the control of pathway flux, metabolite concentrations and in transient-time behavior between channelled and ideal systems.

Temporal aspects of the behavior of a metabolic pathway are described by a suite of characteristic times. Here, we discuss the 'transit' time, defined as the average time it takes for a molecule entering the steady-state system as a substrate to reach the exit point and leave the system as a product (cf. [15]). In the case when the entry step is irreversible the transit time can be measured in radioactive tracer studies by adding a pulse of labelled substrate to the system at a steady state, without significant perturbation of the total substrate concentration, and estimating the average time for the label to flow through the system.

In the present paper we studied the control over the transit time of a molecule passing through a channel formed by two dynamically interacting enzymes. The results show that metabolic channelling can lead to dramatic changes in the total control exerted by pathway enzymes on the transit time.

2. Results and discussion

For a dynamic channel, in which the entry reaction (binding step 1, Fig. 1) and the exit reaction (product releasing step 4) are irreversible, the transit time (τ) equals the ratio of the total amount of intermediates in the pathway (σ) and the total flux (J) at the steady state (cf. [15]):

$$\tau = \sigma/J, \quad \sigma = X + E_1S + E_2P + E_1XE_2 \tag{1}$$

(Here and below the concentrations of substances are designated by the same symbols as the substances themselves.) Eq. 1 admits a simple interpretation of τ as the time required to free the steady-state system of all its intermediates at the constant output flux (J) and zero input flux. However, this interpretation is not quite operational unless one follows a small amount of labelled molecules put into a system in a pulse-chase experiment.

The control exerted by an enzyme over the transit time (τ) can be quantified as a control coefficient with metabolic control analysis. This control coefficient is the fractional change in τ divided by the fractional change in the enzyme concentration (e_i), extrapolated to infinitesimally small change:

$$C^{\tau}_{e_i} = (d\tau/\tau)/(de_i/e_i) = d\ln\tau/d\ln e_i \tag{2}$$

The derivatives are taken at a pathway steady state. It follows from Eqs. 1 and 2 that the control coefficient over τ can be expressed in terms of the corresponding control coefficients over σ and J,

$$C^{\tau}_{e_i} = C^{\sigma}_{e_i} - C^{J}_{e_i} \tag{3}$$

In 'ideal' metabolic pathways the concentrations of enzymes are much smaller than those of metabolites, and the enzyme-bound intermediates can be ignored in the evaluation of the transit time. Since in such pathways the control exerted by the enzymes on any free metabolite concentration adds up to 0, and the control exerted on the flux adds up to 1, the control exerted by all the enzymes on the transit time equals -1 [16,17]. This value increases when the concentrations of enzyme-bound metabolites are taken into consideration [18–20]. Therefore, for any pathway in which direct enzyme-enzyme interactions are absent, the total control over the transit time must be less negative than -1.

It has been shown recently [5,14] that the summation theorems for both flux and concentration control coefficients differ between channelled and ideal metabolic pathways. Since the control over the transit time in Eq. 3 is expressed into the control over the concentrations of the intermediates and over the flux through a channel, one may expect differences in the control of the transit time between ideal and channelled pathways. When the concentrations of the two enzymes in the dynamic channel of Fig. 1 are equal, the total control over the transit time can be expressed in terms of the *elemental* control coefficients of the channelled steps as follows (see Appendix):

$$C^{\tau}_{e_1} + C^{\tau}_{e_2} = (\gamma + (C^{\sigma}_5 + C^{\sigma}_6) - (C^{J}_5 + C^{J}_6))/(1 + E_1XE_2/e),$$

$$\gamma = (E_1XE_2 - X)/\sigma \tag{4}$$

Here e is the total concentration of either enzyme (Fig. 1), C^{σ}_5, C^{J}_5

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and C_6^{σ}, C_6^{J} are the *elemental* control coefficients of steps 5 and 6 over σ, and J defined in terms of identical relative modulation of forward and reverse rate constants [5,21]:

$$C_i^z = \mathrm{d}\ln z/\mathrm{d}\ln k_i^+, \quad k_i^-/k_i^+ = \mathrm{const}, \quad i = 5,6, \quad z = \sigma, J \qquad (5)$$

If the concentration of the bulk phase intermediate (X) exceeds significantly the concentration of enzyme-bound intermediates, σ in Eq. 4 is close to X and γ tends to -1. Whether the pool size (X) and the flux (J) decreases or increases with activities of the channelled steps depends on the elemental rate constants within enzyme mechanisms. We have previously proved [5,22] that the control exerted by the channelled steps on X can be negative ($C_5^X + C_6^X < 0$), at the same time when the control over the flux J is positive ($C_5^J + C_6^J > 0$). In fact, the magnitude of the former expression can be as low as −1, and the magnitude of the latter can be as high as +1, so that (see Eq. 4) the sum of enzyme control coefficients over transit time can be well below −2. In other words, for a dynamic channel an equal relative increase in the enzyme concentrations by a factor α ($\alpha > 1$) may lead to an α^2-fold, or a stronger decrease in the transit time, whereas for a pathway without direct protein interactions, such an increase may lead only to an α-fold or less extensive decrease in the transit time. It is worth mentioning that the sum of the control coefficients can drop below −2 only in a certain range of the enzyme concentrations of a dynamic channel. Numerical simulations confirm these suggestions. Fig. 2 illustrates how the control over the transit time can change with an increase in the total concentrations of enzymes, hence with channelling.

It is important to note that channelling does not necessarily result in a stronger negative control exerted by enzymes on the transit time. Depending on the elemental rate constants within enzyme mechanisms, channelling may cause an increase in the pool size, i.e. the sum ($C_5^{\sigma} + C_6^{\sigma}$) can be positive [22]. In this case, Eq. 4 shows that in a dynamic channel the absolute value of the control over the transit time can be less than in an analogous pathway lacking a channel.

That channelling can lead to an increased enzyme control on the transit time implies that regulators of those enzymes (e.g. those affecting the expression of the genes) may posses a strong control. Consequently, channelling may allow an organism more stringent control on transit time.

A stronger (more negative) control over transit time does not imply that this time is always shorter in channelled pathways. Indeed, the transit time can increase or decrease with channelling, depending on whether the total flux and the pool size increase or decrease. Recent studies showing that at constant flux the free metabolite pool can increase or decrease with channelling [22,23] imply that the same is true for the transit time.

The transit time is just one of the various times that characterise a pathway. This time is only relevant to steady-state properties of the system and not generally to its dynamic properties (however, cf. [16]). The dynamic properties of the system in the vicinity of its stable (in the Lyapunov sense) steady state are characterized by the relaxation times, which have been also called transition times [24]. They indicate how rapidly a system variable such as metabolite concentration or flux relaxes to its steady state value after the system has been perturbed. It is worth noting that the relaxation times for small fluctuations of system variables and small perturbations of parameters are the same, and that these times generally differ from the transit time considered here.

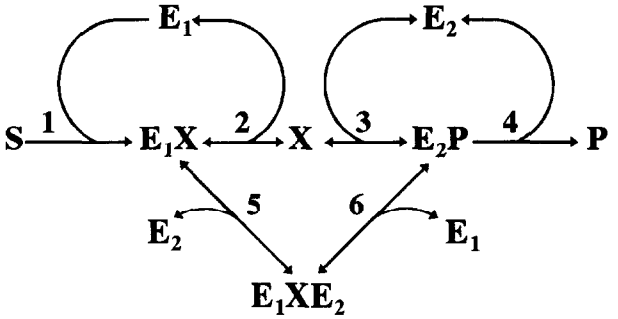

Fig. 1. A dynamic channel. The enzyme-enzyme complex E_1XE_2 is formed after binding the substrate S to E_1. The upper route represents the usual reaction pathway through the bulk-phase intermediate X, catalyzed by free enzymes, and the lower route represents the 'channelling'. The numbering of the elemental steps is shown.

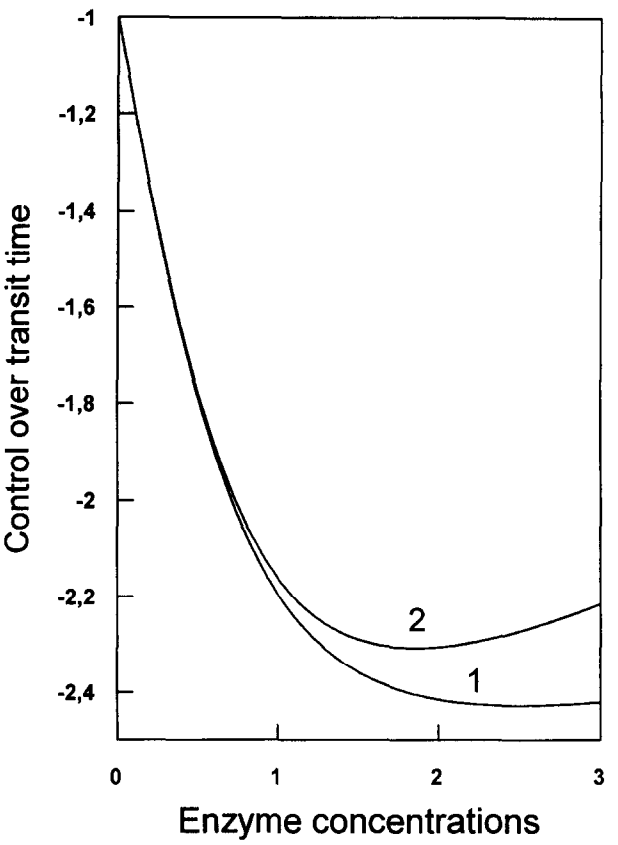

Fig. 2. Variation with protein concentration of the extent to which the enzymes control the transit time in a dynamic channel. For the pathway of Fig. 1 the dependencies of the sum of the enzyme control coefficients ($C_{e_1}^{\tau} + C_{e_2}^{\tau}$) on the total concentration (e) of either enzyme are shown (these equal concentrations, $e_1 = e_2 = e$, are shown in dimensionless units). Either the free intermediate concentration (X) is taken into consideration in the estimation of the transit time (curve 1), or both the free and the enzyme-bound intermediates are taken into consideration (2). The parameter values were (dimensionless units): S=10 , $k_1^+ = 0.2$, $k_1^- = 0$, $k_2^+ = 10$, $k_2^- = 2$, $k_3^+ = 0.001$, $k_3^- = 0.005$, $k_4^+ = 20$, $k_4^- = 0$, $k_5^+ = 0.25$, $k_5^- = 1$, $k_6^+ = 4$, $k_6^- = 1$.

3. Appendix

Using Eq. 3 of the main text the total control over the transit time (τ) can be written as:

$$C_{e_1}^{\tau} + C_{e_2}^{\tau} = (C_{e_1}^{\sigma} + C_{e_2}^{\sigma}) - (C_{e_1}^{J} + C_{e_2}^{J}) \qquad (A1)$$

The enzyme control coefficients over the total pool (σ) can be expressed in terms of the control coefficients over each of the intermediates:

$$C_{e_i}^{\sigma} = (X \cdot C_{e_i}^{X} + E_1X \cdot C_{e_i}^{E_1X} + E_2P \cdot C_{e_i}^{E_2P} + E_1XE_2 \cdot C_{e_i}^{E_1XE_2})/\sigma \qquad (A2)$$

We have previously shown that control exerted by proteins involved in channelling, group-transfer, multi-protein complexes and other protein interactions may be derived by analyzing control at the microlevel of elemental processes and then summing these contributions for a whole protein to

give a macroscopic control coefficient [13]. Expressing in Eqs. A1 and A2 each control coefficient as in Eq. 49 of Ref. [14] and summing the result for either enzyme of a dynamic channel (at equal total concentrations of the enzymes, $e_1 = e_2 = e$) one arrives at:

$$C_{e_1}^{X} + C_{e_2}^{X} = (C_5^{X} + C_6^{X})/(1 + E_1XE_2/e) \quad \textbf{(A3)}$$

$$C_{e_1}^{Z} + C_{e_2}^{Z} = (1 + C_5^{Z} + C_6^{Z})/(1 + E_1XE_2/e), Z = E_2, E_1X, E_2P \quad \textbf{(A4)}$$

$$C_{e_1}^{E_1XE_2} + C_{e_2}^{E_1XE_2} = (2 + C_5^{E_1XE_2} + C_6^{E_1XE_2})/(1 + E_1XE_2/e) \quad \textbf{(A5)}$$

Importantly, the summation theorems for the intermediates bound to the enzyme monomers (E_1X and E_2P) and for the enzyme-enzyme complex (E_1XE_2) differ by the additional terms equal to 1 and 2, respectively, from the control summation theorem for the bulk-pool intermediate (X) [14]. The sum of the control coefficients over the flux through a dynamic channel is given by Eq. 18 of Ref. [14]:

$$C_{e_1}^{J} + C_{e_2}^{J} = (1 + C_5^{J} + C_6^{J})/(1 + E_1XE_2/e) \quad \textbf{(A6)}$$

After substituting Eqs. A2–A6 in Eq. A1 and rearrangement one arrives at Eq. 4 of the main text.

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