Control analysis of transit time for free and enzyme-bound metabolites: physiological and evolutionary significance of metabolic response times

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Control analysis of transit time, defined as $\tau = \sigma/J$, has previously been considered with the constraint of low enzyme concentrations compared with free pools of metabolites [Meléndez-Hevia, Torres, Sicilia and Kacser (1990) Biochem. J. **265**, 195–202]. One of the conclusions was that the sum of the control coefficients of the transition time with respect to enzyme concentration was −1. Here we demonstrate that, if the enzyme-bound pools are taken into consideration (which would be important at high enzyme concentrations and high affinities), the sum lies between 0 and −1. The transition time between two steady states, which are frequent physiological events, is mainly governed by time constants involved in changing the enzyme concentrations. Some physiological and evolutionary aspects are discussed.

INTRODUCTION

The capacity of fast response to reach a given steady state after a specific stimulus is an interesting property of living cells, with a clear physiological significance. Several authors [1–3] have proposed expressions to quantify it, as transit time, transition time or transient time, τ. In this paper we will use Easterby's definition [3] for transit time, τ, which is based on mass conservation, and is defined as the ratio of the total mass of metabolites inside the system, σ, to the flux, J, at steady state:

$$\tau = \frac{\sigma}{J} \quad (1)$$

τ can therefore also be obtained from the progress curve (see Figure 1), and its physical meaning is the mean time taken by one molecule to cross (transit) the system at steady state, from the time it is taken up by the first enzyme as the initial substrate until it is released by the last enzyme of the sequence as the end product. Easterby's approach was carried out under the strong constraint that the first step is irreversible; this would make trivial the analysis of control, because under these conditions the first enzyme is absolutely 'rate-limiting'. This restriction was removed in the analysis of Meléndez-Hevia et al. [4] as Figure 1 shows. Easterby in a subsequent paper [5] did consider variable input and bound pools, as they could affect the overall transition time, but no attempt to relate these to the control characteristics of a system was made. This is the subject of the present paper. The value of τ can be determined from steady-state analysis [eqn. (1)] without following the transient in both the progress curves. Nevertheless, under the most general cellular conditions, it reflects the metabolic response time. In effect, if we compare two metabolic systems at steady state with the same flux but with different concentrations of intermediate pools (see Figure 2), the one that has less mass inside is usually the faster to reach the steady state. This can be easily understood as follows: if the system starts from an empty state (with no intermediate metabolite mass inside), then the speed at which the steady state is reached will be the higher the smaller the value of σ for a given flux. As will be discussed below, the transit time so defined is not really of physiological interest, as no system starts 'empty' of pools. It is the transit time between two different steady states that should be considered. This is given by the formula $\tau_{ab} = \tau_b - \tau_a(J_a/J_b)$ [3] which gives the transit time of a system that passes from one steady state a to another b and where τ_a and τ_b can be defined in terms of the 'empty to steady state' operation. This is the physiologically important transit time as there are

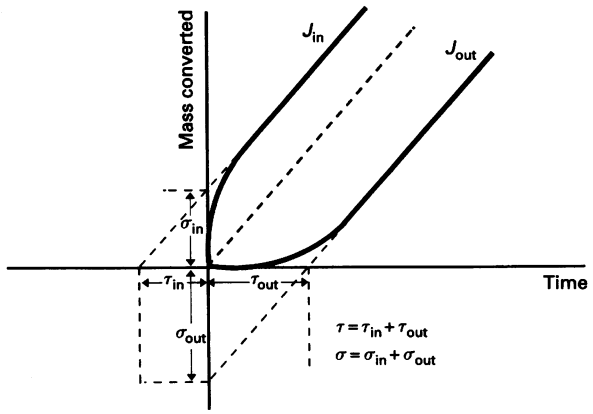

Figure 1 Plot showing the transient of a metabolic system to reach a stable steady state asymptotically

Intersections of the asymptotes of input and output fluxes with the ordinate axis give the total mass of intermediates inside the system, and intersections of these asymptotes with the abscissa give the transit time. Each of these variables includes two fractions, σ_{in}, τ_{in} and σ_{out}, τ_{out}, according to which progress curve (input or output) is due [4]. The dotted line represents what the input flux would be if the first step was irreversible; in that case, σ and τ would be equal to output values, as σ_{in} and τ_{in} would be zero.

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** With great sadness we announce that Henrik Kacser died suddenly on 13 March 1995.

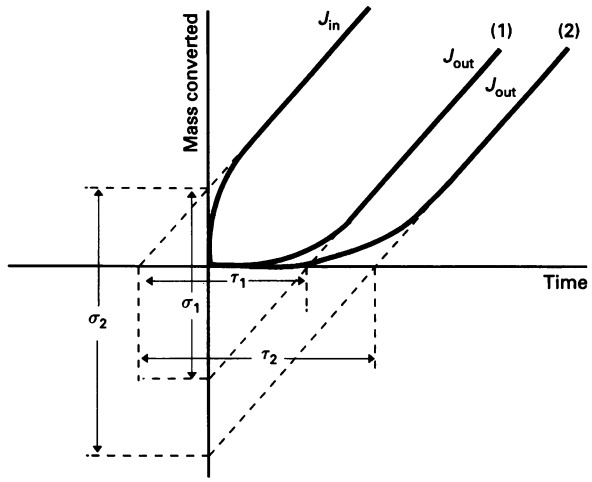

Figure 2 Plot showing the transient of two metabolic systems that reach a steady state with the same flux but have different intermediate concentrations and thus different responses

System (1) has less mass of intermediates inside at steady state, σ, as the ordinate shows, and its transit time, τ, is also smaller than system (2), in reaching the steady state. In order to simplify the Figure, it has been assumed that the transient of input flux is the same for both cases. In general, however, this is not so.

many known instances in which metabolism switches (e.g. aerobic to anaerobic, glycolysis to gluconeogenesis, etc.). These changes are always initiated by an environmental alteration which sets into motion fairly large changes in some enzymes and fluxes. The transition time, and any changes in this time, then follow from these changes in flux.

We shall take a single unbranched pathway as a model system for analysis. The operation on this pathway that we shall consider is the simultaneous change in the concentrations of all the enzymes, however brought about. We shall first consider how the individual values τ_a and τ_b and their control coefficients are affected and how the amount of enzyme complex affects these values. We shall then consider these effects on the important τ_{ab}.

Control of transit time has been analysed both theoretically and experimentally [6] under the constraint of low enzyme concentrations, and therefore assumes that most of the metabolite mass inside the system is due to free pools, and that therefore the concentrations of enzyme-bound intermediates are small. Subsequent to the publication of our paper [4], another publication appeared [7] which came to identical conclusions. The main conclusion of such analysis was the summation theorem for control coefficients (C) of transit time:

$$\sum_{i=1}^{n} C_{e_i}^{\tau_F} = -1 \tag{2}$$

where e_i is the concentration of i^{th} enzyme and τ_F is the free transit time, i.e. the ratio of the total concentration of free intermediates, σ_F, and the flux at steady state (J):

$$\tau_F = \frac{\sigma_F}{J} \tag{3}$$

The meaning of eqns. (2) and (3) is clear. When all enzyme concentrations are simultaneously increased by a factor $\alpha = de_i/e_i$, not necessarily 'small' [8,9], the flux is increased by the same factor

$$dJ = \alpha \cdot J_a;\ J_b = J_a\,(\alpha+1) \tag{4}$$

and

$$\Sigma C_{e_i}^{J} = 1$$

whereas the concentration of the free pools remains unaltered:

$$\sigma_F^a = \sigma_F^b$$

The transit time is decreased by $(\alpha+1)$ (Figure 3a)

$$\tau_F^b = \frac{\sigma_F}{J\cdot(\alpha+1)} = \frac{\tau_F^a}{\alpha+1} \tag{5}$$

It is easily seen that this applies to τ_F^a and τ_F^b, where τ_F^b is now the transition time after the α operation, so that:

$$\tau_F^{ab} = \frac{\sigma_F^b}{J_b} - \frac{\sigma_F^a}{J_a}\left(\frac{J_a}{J_b}\right) = \frac{\sigma_F^b - \sigma_F^a}{J_b} \tag{6}$$

because:

$$\sigma_b = \sigma_a,\quad \tau_{ab} = 0 \tag{7}$$

The transition is therefore instantaneous. *In vivo*, however, this will not be observed because the synthesis of the new protein on induction of the pathway enzymes will take some time.

In experiments performed *in vitro*, on the other hand, an almost instantaneous transition depending only on the mixing is observed if new enzymes are added to the system.

However, this constraint of the small bound pools is strongly determining because, as we shall demonstrate here, the control properties of transit time are quite different in systems without this constraint. The feature of high enzyme concentrations in cells in a number of cases (see e.g. [10–13]) makes this study necessary to understand how the most general case of transition works in living cells. We will assume in this analysis that intermediate pools bound to each enzyme are an important fraction of the total intermediate mass inside the metabolic system. We shall use the term 'bound intermediate', B_i, in the most general sense, as the whole set of different molecular species bound to the enzyme E_i. This includes all intermediates of the enzyme catalysis sequence, from the original substrate taken by the enzyme until the product is released. Let $\sigma_B = \Sigma B_i$ be the total concentration of all these enzyme-bound intermediates. The ratio of this total concentration (σ_B) and the steady-state flux (J) will be called bound transit time, τ_B:

$$\tau_B = \frac{\sigma_B}{J} \tag{8}$$

We also define (total) transit time τ as the total concentration of intermediates ($\sigma = \sigma_B + \sigma_F$) over the flux at steady state [4]:

$$\tau = \tau_B + \tau_F = \frac{\sigma_B + \sigma_F}{J}$$

Bound transit times have been considered previously (see e.g. [3]) but treatment was in terms of special assumptions about the kinetics of the steps and these effects on the control coefficients were not addressed. In the following, we shall develop an approach that is independent of special kinetic assumptions.

THEORY

Consider a metabolic system that is a chain of enzyme reactions, all of which are reversible and sequentially operate (Scheme 1) to convert the initial free substrate X_0 into the free end product X_p.

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} \cdots \overset{E_{n-1}}{\rightleftharpoons} S_{n-1} \overset{E_n}{\rightleftharpoons} X_p$$

Scheme 1

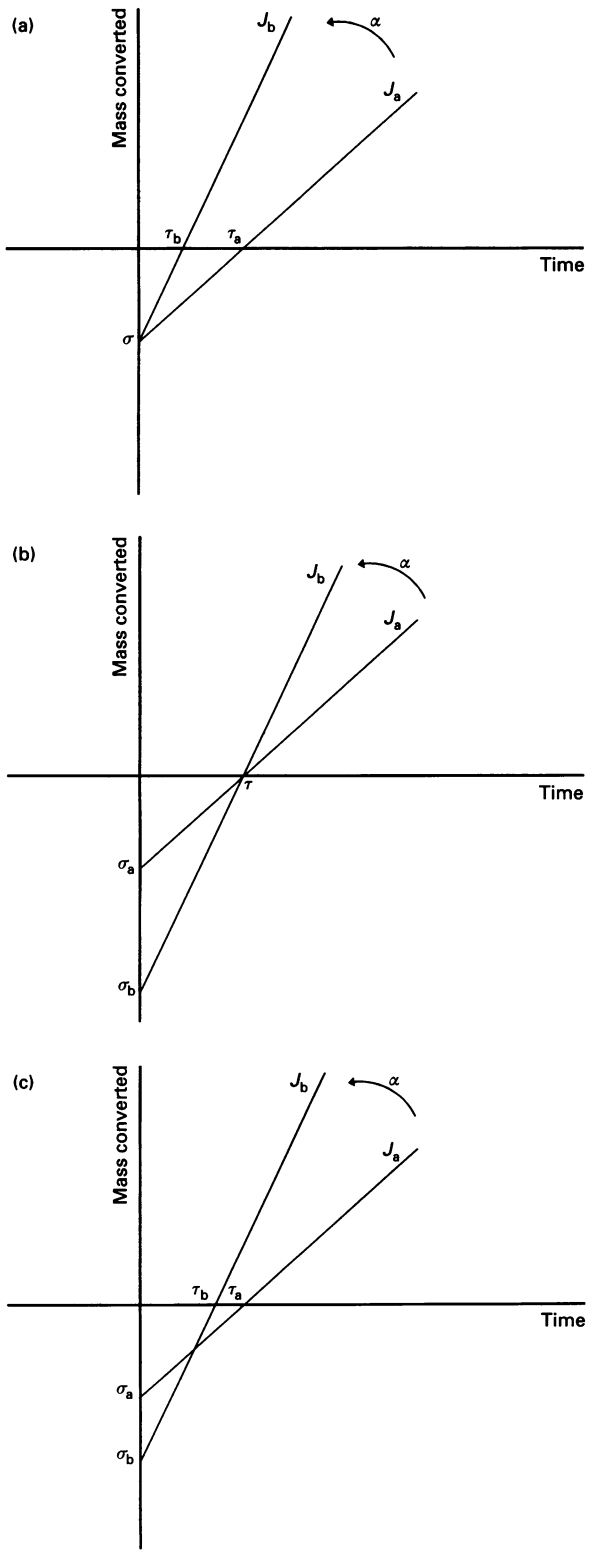

Figure 3 Effect of increasing the concentration of all the enzymes in different metabolic systems, according to their initial enzyme concentration, on the flux, transit time and total intermediate pools

The increase in all enzymes by the same factor, $\alpha = de_i/e_i$, always promotes an increase in steady-state flux by the same factor $dJ/J = \alpha$, it being $J_b = (\alpha+1)J_a$, but its effect on the transit time and intermediate pool is quite different depending on the enzyme concentration of the system. In order to simplify the Figures, only asymptotes to the transient curves are plotted, and only output fluxes are represented. These simplifications do not involve any restriction to the conclusions. (**a**) A system with an extremely low enzyme concentration and therefore virtually all intermediates as free pools; transit time is reduced by the same factor, i.e. $\tau_b = \tau_a/(\alpha+1)$, but there is no variation in the intermediate pools. (**b**) A system with high enzyme concentration, where virtually all intermediates are as enzyme-bound pools; there is also an increase in the total pool concentration $\sigma_b = (\alpha+1)\sigma_a$, but transit time is not affected. (**c**) Effect of the same change on a system with its intermediate metabolites distributed among free and bound pools; the effect on transit time and pools is intermediate between the two preceding cases.

Let us assume (for the moment) that it is under the classical constraints of metabolic control analysis (independence and additivity of single enzyme effects [14,15]). We shall consider here that every step is reversible, as usual, with no loss of generality [3,4]. Also we shall assume that the concentration of each enzyme may have any value including values of the same order as or higher than its substrate. It should be emphasized that the relative concentrations of enzyme and metabolite (so frequently studied and quoted in the literature) are not a sufficient condition for taking bound pools into consideration. It is quite possible that there is a very high concentration of enzyme(s), but with very low affinities etc. the concentration of bound pools σ_B may equally be low, i.e. $\sigma_B \ll \sigma_F$. In such a case the formulations based on low enzyme concentrations discussed above apply. It requires both high concentration of enzyme(s) and high affinities before a significant effect of the bound pools becomes important. Now, with the system working at an asymptotically stable steady-state, let us make a certain fractional change (not necessarily small) in the concentration of all the enzymes by the same factor $\alpha = de_i/e_i$ simultaneously. Once the system reaches its new steady state, it will show the following features. (a) The flux of the system has also been increased by the same factor $dJ/J = \alpha$ [8,9]. (b) All free pools of intermediate S_F remain at the same concentration, i.e. $dS_F/S_F = 0$ as $\Sigma C_e^{S_F} = 0$ [9]. From this last property it follows that the degree of saturation of each enzyme (the fraction of bound enzyme over total enzyme) has not been changed because all are working at the same concentration of free substrate and product [16]. As the concentration of metabolite bound to the enzyme, which we designated B_i, must be equal to the concentration of enzyme bound to the metabolite and because the total concentration has been changed by a factor α, we can write

$$\frac{dB_i}{B_i} = \alpha \tag{9}$$

The total differential of B_i is:

$$dB_i = \frac{\partial B_i}{\partial e_1}de_1 + \frac{\partial B_i}{\partial e_2}de_2 + \ldots + \frac{\partial B_i}{\partial e_n}de_n$$

Therefore from the last two equations and applying the definition of control coefficient, we derive

$$\frac{dB_i}{B_i} = \alpha = \alpha(C_{e_1}^{B_i} + C_{e_2}^{B_i} + \ldots + C_{e_n}^{B_i})$$

which gives for each B_i

$$\sum_{j=1}^{n} C_{e_j}^{B_i} = 1$$

and for the total pool, σ_B

$$\sum_{j=1}^{n} C_{e_j}^{\sigma_B} = 1 \tag{10}$$

Taking partial derivatives of eqn. (8), and replacing τ_B by its value there, we have:

$$C^{\sigma_B}_{e_j} = C^{\tau_B}_{e_j} + C^{J}_{e_j} \quad (11)$$

Summing all enzymes, and according to the summation theorem of flux control coefficients [8,9], $\Sigma C^J = 1$ and eqn. (10), we find that

$$\sum_{j=1}^{n} C^{\tau_B}_{e_j} = 0 \quad (12)$$

[in contrast with eqn. (2) which sums all free pool coefficients].

This is the summation theorem of control coefficients of bound transit time. Taking into account the theorem for free transit time [eqn. (2)] we can see that the value of the summation of time control coefficients in a metabolic system will depend on the free/bound metabolite ratio (Figure 3). If most of the mass of metabolites inside the system is bound to the enzymes, then most of the transition time will account for time spent by bound pools, and that summation will tend to zero, whereas in a system with most of the intermediates as free pools, they will account for most of the transition time, and consequently that summation will approach -1. Therefore the summation of all control coefficients of total transit time, τ, for free and bound pools will always be between 0 and -1.

$$0 \geqslant \sum_{i=1}^{n} C^{\tau}_{e_i} \geqslant -1 \quad (13)$$

We can demonstrate easily (see the Appendix) the following theorem for the sum of control coefficients of the transit time:

$$\sum_{i=1}^{n} C^{\tau}_{e_i} = -1 + \frac{\sigma_B}{\sigma} \quad (14)$$

This important theorem permits the calculation of the numerical value of the sum of control coefficients if we know experimentally the percentage of metabolite pools sequestered by the enzymes σ_B/σ.

Turning now to the transition time τ_B^{ab} for bound pools, when the system changes from state a to b, where all enzyme concentrations have been increased by a factor $\alpha = de_i/e_i$, we have similarly, as before,

$$\tau_B^{ab} = \tau_B^{b} - \tau_B^{a}\left(\frac{J_a}{J_b}\right)$$

where the τ values are defined as

$$\frac{\sigma_B^b}{J_b} \text{ and } \frac{\sigma_B^a}{J_a} \text{ respectively}$$

and

$$\frac{J_a}{J_b} = \frac{1}{(\alpha+1)}$$

It follows that

$$\tau_B^{b} = \frac{(\alpha+1)\sigma_B^a}{(\alpha+1)J_a}$$

so that

$$\tau_B^{ab} = \tau_B^{a}\left(1 - \frac{1}{(\alpha+1)}\right) \quad (15)$$

This contrasts with eqn. (7) where $\tau_F^{ab} = 0$. For very small increases in enzyme concentration [$(\alpha+1) \to 1$], eqn. (15) gives the same result, $\tau_B^{ab} \to 0$ eqn. (7). For very large values of α, however, $\tau_B^{ab} \to \tau_B^{a}$.

For bound pools therefore the transition is not instantaneous but depends on the value of α. For the transition involving both free and bound pools

$$\tau_{Tot.}^{ab} = \tau_F^{ab} + \tau_B^{ab} = 0 + \tau_B^{a}\left(1 - \frac{1}{(\alpha+1)}\right)$$

For the general case, then

$$0 \leqslant \tau_{Tot.}^{ab} \leqslant \tau_B^{a} \quad (16)$$

To this must be added the time taken for the enzyme induction, which may well dominate the process *in vivo*.

As stated above, there is no restriction about the reversibility of the first step of the pathway. Therefore results in eqns. (12) and (13) apply to systems where the ratio τ_{in}/τ_{out}, as well as the ratio τ_B/τ_F, has any value (see Figure 1). In Scheme 1 the stoichiometric ratio between the initial substrate X_0 and the end product X_p is 1, but this simplification involves no loss of generality in our conclusions, as has been previously demonstrated [4].

If not all the enzymes are co-ordinately increased (or decreased), the effect of such a change will depend, apart from the value of α, on the control coefficient of these steps. The limits, as shown in eqn. (13) or (16), cannot, however, be exceeded.

DISCUSSION

The results presented in eqns. (13) and (16) show an interesting property of metabolic systems: the summation of control coefficients of transition time of a metabolic system is between 0 and -1, depending on the concentrations of its enzyme complexes with respect to its intermediates.

Rapid response times of a metabolic system as a consequence of an initial stimulus may well contribute to the fitness of an organism. Our analysis demonstrates that this property and its control is dependent on the concentrations of enzyme complexes and the ratio between these and the free substrates. The fastest response times, $\tau_{Tot.}^{ab} = 0$, are clearly to be found in systems with the lowest saturation of enzymes, all other things being equal. This may be one of the reasons why 'unsaturated' kinetics are generally found in metabolism. The actual response *in vivo* therefore depends only on the speed with which the enzyme concentrations can change. This inevitably can never be instantaneous, and, if rapid responses of enzyme concentration changes produce a selective advantage, then natural selection, one presumes, has made these changes as fast as is compatible with all other functional constraints operating.

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APPENDIX

Demonstration of the summation theorem for the transit time

The whole transit time of the system is obviously the summation of free and bound transit times:

$$\tau = \tau_F + \tau_B = \frac{\sigma_F + \sigma_B}{J}$$

which can be rearranged to give:

$$\sigma_F + \sigma_B = J\tau$$

Taking derivatives with regard to e_i we obtain:

$$(C^{\sigma_F}_{e_i} + C^{\sigma_B}_{e_i})\frac{\sigma_B}{\sigma_F} = \left(\frac{\sigma_F + \sigma_B}{\sigma_F}\right)(C^J_{e_i} + C^\tau_{e_i})$$

Summing all the enzymes e_i:

$$\left(\sum_{i=1}^{n} C^{\sigma_F}_{e_i} + \sum_{i=1}^{n} C^{\sigma_B}_{e_i}\right)\frac{\sigma_B}{\sigma_F} = \left(\frac{\sigma_F + \sigma_B}{\sigma_F}\right)\left(\sum_{i=1}^{n} C^J_{e_i} + \sum_{i=1}^{n} C^\tau_{e_i}\right)$$

From summation theorems:

$$0 + \frac{\sigma_B}{\sigma_F} = \frac{\sigma_F + \sigma_B}{\sigma_F}\left(1 + \sum_{i=1}^{n} C^\tau_{e_i}\right)$$

which can be rearranged to give:

$$\sum_{i=1}^{n} C^\tau_{e_i} = -\frac{\sigma_F}{\sigma_F + \sigma_B} = -1 + \frac{\sigma_B}{\sigma_F}$$

When σ_B is small compared with σ_F

$$\sum_{i=1}^{n} C^\tau_{e_i} \to -1$$