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Analysis and characterization of transition states in metabolic systems

Transition Times and the Passivity of the Output Flux

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In this paper we study the transitions between steady states in metabolic systems. In order to deal with this task we divided the total metabolite concentration at steady state, σ, into two fractions, δ (the Output Transient Mass) and β (the Input Transient Mass). These masses allow us to define two new Transition Times, τ_δ (Output Transition Time) and τ_β (Input Transition Time), which are related with the course of output and input mass to the system respectively. We show the equivalence between these terms and the Total Transition Time, τ_T, previously defined [Easterby (1986) Biochem. J. **233**, 871–875]. Next, we define a new magnitude, the Output Passivity of a transition, ρ, which quantifies a new aspect of the transition phase that we call the passivity of the output progress curve. With these magnitudes, all of them being experimentally accessible, several features of the transient state can be measured. We apply the present analysis to (*a*) the case of coupled enzyme assays, which allows us to reach conclusions about the progress curves in these particular transitions and the equivalence between τ_σ and τ_δ, and (*b*) some experimental results that allow us to discuss the biological significance of the Output Passivity in the transition between steady states in metabolic systems.

INTRODUCTION

Traditionally studies on metabolic regulation have been focused on the steady state, but less attention has been paid to the study of the transitions between those steady states. Studies in this field have dealt with the time scale of metabolic transitions towards steady state in a coupled enzymic assay. The approach to this problem has followed two paths. The first one is coping with the quantification of the total duration of the transition by measuring the t_{99}. This t_{99} has the physical meaning of the Relaxation Time of the system, which is the time necessary to reach 99% of the final steady state (Storer & Cornish-Bowden, 1974; Easterby, 1981; Brooks *et al.*, 1984). The second one refers to the problem by using a relative magnitude such as the Transit Time, Transition Time or Transient Time, τ (Hess & Wurster, 1970; Barwell & Hess, 1970; Reich & Sel'kov, 1981; and more specifically, Easterby, 1981, 1984, 1986). τ is generally defined as the ratio at steady state of the total metabolite concentrations, σ, of a given system over its flux, J:

$$\tau = \sigma/J$$

Being defined in this way, τ is not the absolute time needed to reach the steady state but reflects a temporal characteristic of the system as it evolves towards the steady state.

Acerenza *et al.* (1989) have extended the Metabolic Control Analysis to the study of transition states. Our group has described the Control of Transition Time in a reconstituted glycolytic system (Torres *et al.*, 1989) and has developed Control Analysis to account for the general description of the Control of Transition Time in metabolic systems (Meléndez-Hevia *et al.*, 1990).

There is another important aspect of metabolic motions, namely the shape of the progress curves (Reich & Sel'kov, 1981), which has remained practically unexplored. The shape is represented by the appearance of the dynamics of the metabolic system. In fact two processes with the same or different t_{99} values can have very different shapes, this being a crucial distinction in the response of metabolic systems.

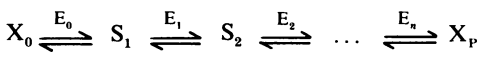
Our aim in the present paper is to cope with this aspect of transitions between steady states. In order to describe it we define two new Transition Times, τ_δ and τ_β. These Transition Times are respectively associated with the course of the final product of the system and the input of mass to the system, during the transition towards the steady state. By using these terms we introduce a new concept, the Output Passivity of a transition, ρ, which gives information about this aspect of the transitions between steady states. Finally, we apply this approach to the case of coupled enzyme assays and to the study of transitions between two different steady states in a reconstituted rabbit muscle glycolytic system described by us elsewhere (Torres *et al.*, 1989).

THEORY

Transition Masses

Let us consider a pathway bounded by two metabolites, X_0 and X_P, containing any number of enzymes and pools between them as illustrated in Scheme 1.

The following analysis is performed under the assumption that all metabolite transformations are monomolecular or pseudomonomolecular and take place with a stoichiometry of 1:1. The mechanism of each enzyme is unspecified, allowing any degree of reversibility and saturation. The constancy of the concentrations of X_0 and X_P might be thought of as being based on the same assumptions as apply to an 'ordinary' enzyme assay generating an initial rate; this could include the concentration of X_P being zero, so that the last step is virtually irreversible, but this is not a necessary feature of the system. Alternatively, the concentrations of X_0 and X_P can be maintained by some other mechanism outside the system under consideration.



Scheme 1

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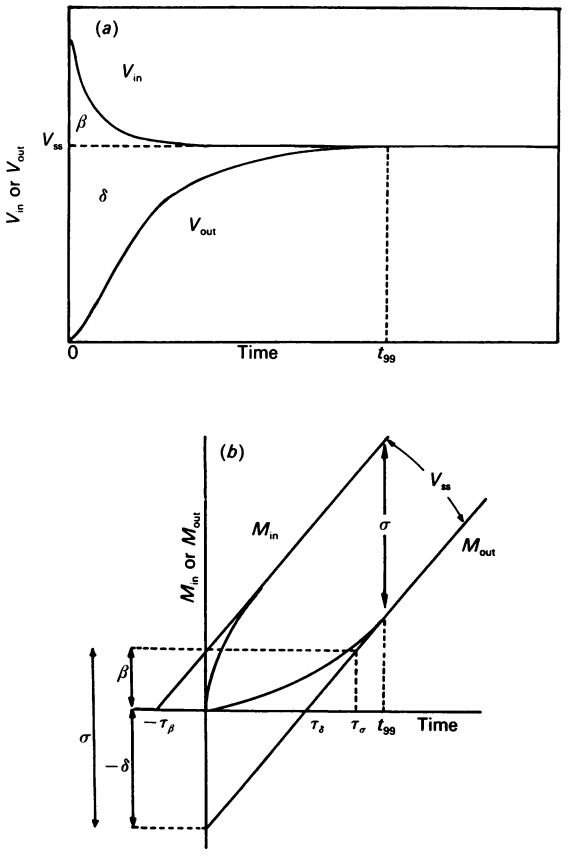

Fig. 1. Evolution curves of input and output rates in a transition from 'empty' system to steady state

The system is as that represented in Scheme 1. The initial conditions are all enzymes present and all metabolites absent. (*a*) At $t = 0$ a fixed concentration of X_0 is applied and its rate of disappearance, $V_{in}(t)$, is recorded as well as the rate of production of X_P, $V_{out}(t)$ (see the text). At $t = t_{99}$ the system reaches the steady state where $V_{in}(t_{99}) = V_{out}(t_{99}) = V_{ss}$. At this moment $\beta(t_{99}) = \beta$ and $\delta(t_{99}) = \delta$. These curves were obtained by computer simulation of a system as represented in Scheme 1. (*b*) Progress curves of the system are represented. Conditions were as for panel (*a*) (see the text).

Starting with an 'empty' system, i.e. with the concentration of all intermediate metabolites, S_j, zero at $t = 0$, the present treatment will apply to a system that evolves towards the steady state asymptotically: the system tends to an asymptotically stable node. Initially the rate of input of X_0 to the system, $V_{in}(0)$, will have a certain value different from zero, and $V_{out}(0)$ will be zero owing to the absence of substrate of the last enzyme. Fig. 1(*a*) shows the typical evolution of the input and output rates along the transition phase towards the steady state. $V_{in}(t)$, owing to the reversibility or feedback, decreases with time, while $V_{out}(t)$ increases, both approaching asymptotically to V_{ss}, which is the flux of the system at steady state. When $t = t_{99}$ all the rates of the system (V_{ss}) and the metabolite concentrations remain practically unchanged.

The total mass having entered during an interval of time t, $M_{in}(t)$, is given by:

$$M_{in}(t) = \int_0^t V_{in}(t) \cdot dt$$

This may be written in the alternative form:

$$M_{in}(t) = V_{in}(t) \cdot t - \int_{V_{in}(0)}^{V_{in}(t)} t \cdot dV_{in} = \alpha(t) + \beta(t) \qquad (1a)$$

where

$$\alpha(t) = V_{in}(t) \cdot t$$

and

$$\beta(t) = -\int_{V_{in}(0)}^{V_{in}(t)} t \cdot dV_{in} \qquad (1b)$$

Both $\alpha(t)$ and $\beta(t)$ have a graphical and physical meaning that can be observed in Fig. 2(*a*). $\alpha(t)$ is the area of the inner rectangle defined by the value of $V_{in}(t)$ and t. Physically it represents the total mass having entered the system until a given time t if the input rate takes the constant value $V_{in}(t)$. Accordingly $\beta(t)$ represents the additional mass that has entered the system in comparison with $\alpha(t)$ and that we must add to $\alpha(t)$ to have $M_{in}(t)$.

The same analysis can be done considering the rate of output $V_{out}(t)$ (see Fig. 2*b*). Accordingly we reach the equivalent expression:

$$M_{out}(t) = V_{out}(t) \cdot t - \int_0^{V_{out}(t)} t \cdot dV_{out} = \gamma(t) - \delta(t) \qquad (2a)$$

where

$$\gamma(t) = V_{out}(t) \cdot t$$

and

$$\delta(t) = \int_0^{V_{out}(t)} t \cdot dV_{out} \qquad (2b)$$

where $M_{out}(t)$ and $V_{out}(t)$ are the total mass having come out from the system and the rate of output of X_P from the system respectively.

At any time, t, $\gamma(t)$ is the area of the rectangle defined by the value of $V_{out}(t)$ and t. On their own $\delta(t)$ is the area inside the rectangle $\gamma(t)$ situated above the progress curve of $V_{out}(t)$. The physical meaning of these magnitudes has a direct translation in terms of mass, as was shown in the case of $M_{in}(t)$.

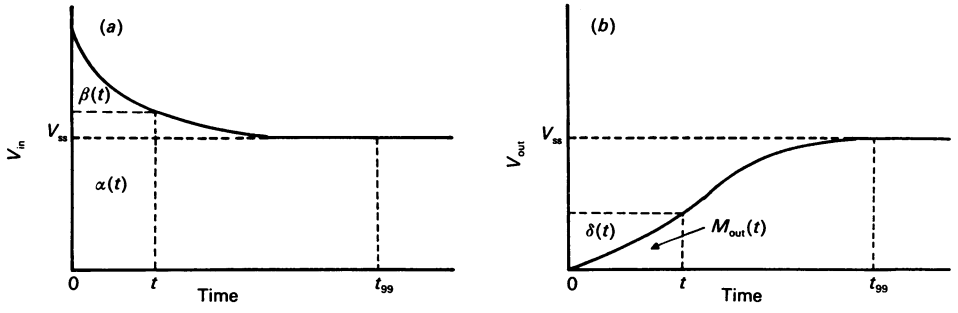

Fig. 2. Graphical analysis and physical interpretation of the input and output evolution curves and their corresponding progress curves

(*a*) Negative variation of $V_{in}(t)$ is represented. At any time $\beta(t)$ is equal to the total area under the curve, $M_{in}(t)$, minus $\alpha(t)$. Once the system reaches the steady state $\beta(t)$ remains constant and equal to β. Physically $M_{in}(t)$ represents the total mass having entered the system and β the inner mass associated with the decreases in $V_{in}(t)$ along the transition. (*b*) $V_{out}(t)$ increases with time from zero at $t = 0$ to V_{ss} at $t = t_{99}$. At any time $\gamma(t)$ is equal to $\delta(t)$ plus $M_{out}(t)$, the total area under the $V_{out}(t)$ curve. Physically $M_{out}(t)$ represent the total mass having left the system at any time. After the steady state $\delta(t) = \delta$, remaining constant.

Mass partition

Mass conservation requires that at any time the total mass input, $M_{in}(t)$, is accounted for either as intermediate, free or enzyme-bound, $\alpha(t)$, or as a product, $M_{out}(t)$. In our system, where the rate of input is allowed to vary, this condition is met by the following equation:

$$M_{in}(t) = M_{out}(t) + \sigma(t) \quad (3)$$

where $\sigma(t)$ represents the total mass inside the system at any time t.

Replacing $M_{in}(t)$ and $M_{out}(t)$ by their values according to eqns. (1a) and (2a) and re-arranging we obtain:

$$\sigma(t) = \alpha(t) + \beta(t) - \gamma(t) + \delta(t) \quad (4)$$

At steady state ($t = t_{99}$):

$$\alpha(t_{99}) = \gamma(t_{99}) = V_{ss} \cdot t_{99}$$

Therefore eqn. (4) becomes:

$$\sigma = \delta + \beta \quad (5)$$

where

$$\sigma = \lim_{t \to t_{99}} \sigma(t),\ \delta = \lim_{t \to t_{99}} \delta(t) \text{ and } \beta = \lim_{t \to t_{99}} \beta(t)$$

Eqn. (5) tells us that the total mass inside the system at steady state, σ, can be expressed as the summation of two terms, δ and β.

So far we have considered the evolution starting from the 'empty' system. However, in conditions when the initial concentration is not zero eqn. (3) becomes:

$$M_{in}(t) = M_{out}(t) + \sigma(t) + \sigma_0$$

From this equation, operating in the same way as above, we reach the equivalent to eqn. (5), but more general:

$$\sigma = \delta + \beta + \sigma_0 \quad (6)$$

where σ_0 represents the inner mass to the system at initial time $t = 0$.

We call δ the 'Output Transient Mass'. This is so because its value expresses the part of the total mass accumulated inside the system along the transition as a consequence of the limitations in the output rate of mass from the system. The term δ thus contains some information about the shape of the output transition. On the other hand β, whose magnitude is related to the shortening of the transient phase owing to the changes in the input rate of mass to the system, will be called the 'Input Transient Mass'. β informs us on the effect of any activation or inhibition that acts on the first enzyme and that provokes the particular transient observed.

The above considerations are based on the assumption that the $M_{in}(t)$ curve was convex (Fig. 1*b*; see also Meléndez-Hevia *et al.*, 1990). The same reasoning is also of application to transitions where the $M_{in}(t)$ curve is concave, i.e. if the interaction increases the $V_{in}(t)$ (positive effector) on the first enzyme. It is worth noting that in these conditions β is negative, which would imply that δ is greater than the inner mass of the system, σ. This reasoning illustrates very well the fact that δ is an ideal mass that does not necessarily have physical existence but has a meaning as a transient feature.

Meaning and equivalence of Transition Times

Starting from the definition of σ as the summation of β and δ, as shown in eqn. (5), we can obtain the corresponding Transition Times associated with β and δ.

Dividing eqn. (5) by V_{ss} we obtain:

$$\tau_\sigma = \tau_\delta + \tau_\beta \quad (6a)$$

where

$$\tau_\sigma = \sigma / V_{ss}, \tau_\delta = \delta / V_{ss} \text{ and } \tau_\beta = \beta / V_{ss} \quad (6b)$$

Eqn. (6a) shows that τ_σ can be decomposed in two terms, each one related to the transition of the input and output mass of the system respectively. The two new terms τ_δ and τ_β are Transition Times, and according to the physical meaning of δ and β we can call τ_δ the Output Transition Time and τ_β the Input Transition Time. As was stated above, the Transition Time, τ_σ, refers to the steady state, giving us limited information about the transition. The important point of eqn. (6a) is that the two new terms τ_δ and τ_β, into which we divide τ_σ, are now related, by definition, to the transition phase and therefore contain information on certain features of the transition. For example, consider in Fig. 1 two transitions that evolve to the final steady-state flux V_{ss} with the same relaxation time, t_{99}. In this case comparison of the τ_δ values now informs us on which output progress curve has a greater convexity, or in other words which system accumulates more mass inside the system along the transition.

Easterby (1986) divides the Total Transition Time, τ_T, into various terms, considering the effect of feedback (and reversibility) on the first enzyme in the transition response:

$$\tau_T = \tau_I + \tau_{EI} + \tau_F \quad (7)$$

where τ_I and τ_{EI} refer to the intermediate pools, free and enzyme-bound respectively. On its own, τ_F is a Transition Time associated with the variations in the rate of input of material to the pathway, being defined as:

$$\tau_F = \frac{\int_{V_{in}(0)}^{V_{ss}} t \cdot dV_{in}(t)}{V_{ss}}$$

Note that in eqn. (7) τ_I plus τ_{EI} are just our τ_σ. On the other hand it is clear that τ_F is the same, but with opposite sign, as the above described τ_β (see eqns. 1b and 6b):

$$\tau_F = -\beta / J_{ss} = -\tau_\beta \quad (8)$$

Then by replacing τ_σ by its value according to eqn. (6a) and τ_F from eqn. (8) in eqn. (7), we obtain:

$$\tau_T = \tau_\delta \quad (9)$$

Thus, in conditions in which the input rate is allowed to vary, τ_δ is the Total Transition Time.

These ideas and the meaning of the magnitudes involved in the definition of τ_δ and τ_β can be illustrated in the progress curves of $M_{in}(t)$ and $M_{out}(t)$ (see Fig. 1*b*). At steady state the difference between M_{in} and M_{out} accounts for the mass of all molecular species inside the system, σ. Therefore the asymptote to the $M_{out}(t)$ curve intersects the abscissa at τ_T, which, as was shown in eqn. (9), is τ_δ. By the same reasoning the intercept with the ordinate gives $-\delta$. On the other hand, the asymptote to the $M_{in}(t)$ curve intersects the ordinate in β in such a way that β plus δ gives σ. The intercept with the abscissa gives $-\tau_\beta$; accordingly $\tau_\delta + \tau_\beta = \tau_\sigma$. It is evident that τ_β and τ_δ will be different in general, reflecting in their values the different courses of the input and output progress curves respectively.

An interesting result is obtained when we consider the case where the first enzyme is irreversible. Here the only transition we can observe is that associated with the output flux production, M_{out}. Therefore the Input Transient Mass, β, is zero, and accordingly with eqn. (5):

$$\sigma = \delta$$

Combination of eqns. (6a) and (9) tells us then that:

$$\tau_\sigma = \tau_\delta = \tau_T \quad (10)$$

An experimental consequence of this conclusion is that in such systems the intercept of the M_{out} asymptote with the abscissa gives the τ_σ and intercept with the ordinate the corresponding σ value.

Output Passivity

Characterization of the passivity of transitions. In the above paragraph two transitions with the same relaxation time, t_{99}, were compared by using the Transition Time τ_δ. We concluded that in that case the comparison of the τ_δ values permits us to differentiate between the progress curves. However, in the general case where the Relaxation Times are different, the mere comparison of the τ_δ values does not allow us to distinguish among transitions. In these circumstances it is convenient to introduce a new parameter.

In order to analyse the transitions between steady states we introduce the Output Passivity of a given transition, ρ, defined as:

$$\rho = \frac{\delta}{V_{ss} \cdot t_{99}} = \frac{\tau_\delta}{t_{99}} \qquad (11)$$

The physical meaning of this expression and the concept of passivity involved in it can be easily understood by examining Fig. 1. This dimensionless magnitude, ρ, represents the ratio of δ (the area inside the rectangle delimited by V_{ss} and t_{99} situated above the progress curve of output flux) over the total area $V_{ss} \cdot t_{99}$ (Fig. 1*a*), or alternatively the ratio of the Output Transition Time τ_δ over the total duration of the transition t_{99} (Fig. 1*b*). From its definition it is clear that ρ has values between zero and unity. ρ equal to zero corresponds to a transition in which V_{out} reaches the V_{ss} values at a time $t = 0$, which correspond to a t_{99} of zero. This situation appears when at $t = 0$ the system is exactly full, and accordingly no mass accumulates in the system: $\tau_\delta = 0$. In this case the system has a passivity of zero, i.e. the system reacts instantaneously to the change that provokes the evolution to the new steady state. The opposite extreme situation occurs when ρ is equal to unity: V_{out} remains zero during all the transition, and once the t_{99} is reached V_{out} suddenly increases to V_{ss}. Here the passivity of the transition is maximum and the system only reacts at the end of the transition. In this condition τ_δ equals t_{99}. It can be seen that in both cases τ_δ appears as the minimum value allowed to t_{99}.

The Output Passivity of a transition, ρ, includes in its definition some responses of the system that change according to the shape of the progress curves of the output flux. Therefore, to evaluate the response shape of a given transition, we must measure the features that participate in the definition of ρ. Experimentally this can be done easily by recording τ_δ and t_{99}.

Transition between steady states. Hitherto the transitions studied have been those from an 'empty' system to one 'full' of intermediate pools. The transitions from one given steady state to another are more interesting and realistic. Consider the situation where a metabolic system in a steady state *a* is modified in the value of a certain parameter, and then a transition occurs from steady state *a* to another steady state *b*. In accordance with the definition of δ, a general expression for this transition can be easily derived:

$$\delta_{ab} = \int_{V_a}^{V_b} t \cdot dV_{out}$$

In this expression δ_{ab} represents the increment in δ when the system evolves from the steady state *a* to steady state *b* (see Fig. 3*a*). On their own V_a and V_b are the corresponding V_{out} at each t_{99} in the steady states *a* and *b* respectively.

The previous definition can be divided in the following way:

$$\int_{V_a}^{V_b} t \cdot dV_{out} = \int_0^{V_b} t \cdot dV_{out} - \int_0^{V_a} t \cdot dV_{out} = \delta_b - \delta_a$$

Dividing by V_b and multiplying the second right term by V_a/V_a we obtain:

$$(\tau_\delta)_{ab} = (\tau_\delta)_b - \frac{V_a}{V_b} \cdot (\tau_\delta)_a \qquad (12)$$

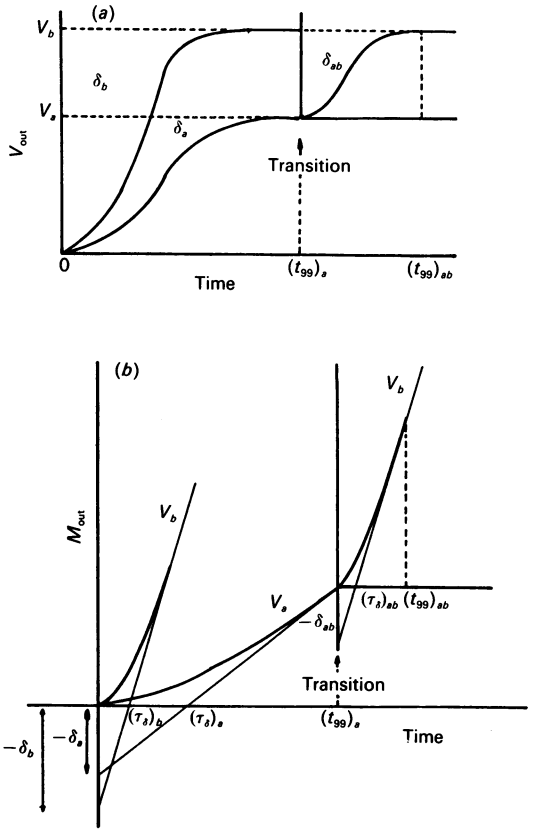

Fig. 3. Graphical interpretation of the Output Transient Mass, δ, and the Output Transition Time, τ_δ, during transition between steady states

System in steady state *a* starts a transition (arrow) to steady state *b*. At the transition point the flux through the pathway is increased, resulting in an increase in the steady-state δ pool concentration. Panel (*a*) shows the plot of $V_{out}(t)$ versus time in accordance with Fig. 1(*a*). Panel (*b*) shows the plot of X_P mass production versus time as in Fig. 1(*b*).

An equivalent equation is derived for $(\tau_\beta)_{ab}$:

$$(\tau_\beta)_{ab} = (\tau_\beta)_b - \frac{V_a}{V_b} \cdot (\tau_\beta)_a \qquad (13)$$

Easterby (1986) has obtained a similar expression for Total Transition Time. The important point here is that $(\tau_\delta)_{ab}$ and $(\tau_\beta)_{ab}$ can be estimated from the transitions for the establishment of each steady state from the 'empty' starting point. Consistently, only a limited number of steady states need be known, enabling us to calculate $(\tau_\delta)_{ab}$ and $(\tau_\beta)_{ab}$ in passage between any pair of these states (see Fig. 3*b*). It is worth noting also that the values obtained from eqns. (12) and (13) are different in general from those values $(\tau_\delta)_{ba}$ and $(\tau_\beta)_{ba}$ corresponding to the transition from steady state *b* to steady state *a*. Furthermore it is easy to obtain the relation between them:

$$(\tau_\delta)_{ab} = -\frac{V_a}{V_b} \cdot (\tau_\delta)_{ba} \text{ and } (\tau_\beta)_{ab} = -\frac{V_a}{V_b} \cdot (\tau_\beta)_{ba}$$

Regarding the quantification of the passivity responses in transitions between steady states it is obvious that in these cases this response can be measured by the same procedure as described above. The corresponding equation for the Output Passivity of the transition from steady state *a* to steady state *b* is now:

$$\rho_{ab} = \frac{\delta_{ab}}{V_b \cdot (t_{99})_{ab}} = \frac{(\tau_\delta)_{ab}}{(t_{99})_{ab}}$$

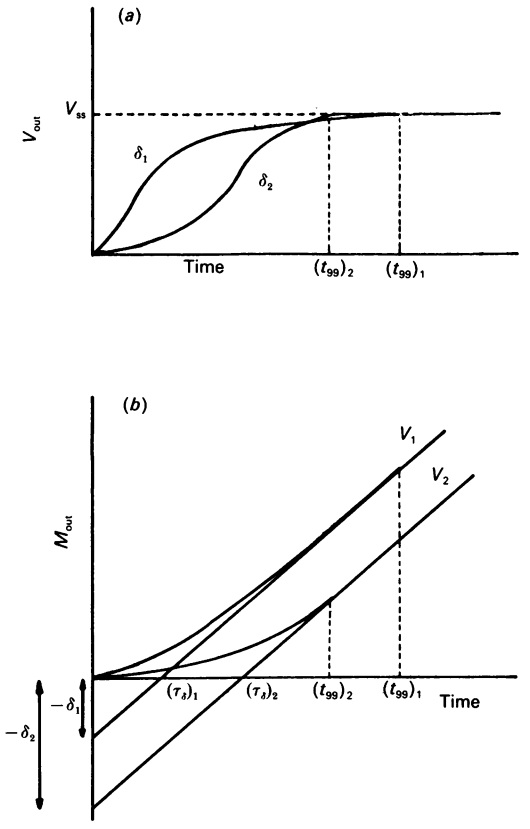

Fig. 4. Comparison of transient response between two different metabolic systems

Panels (*a*) and (*b*) show the evolution curves for two systems that evolve from 'empty' to the same final steady-state flux, V_{ss}, but spending different times in the transition, $(t_{99})_1$ being greater than $(t_{99})_2$. System 1 has a δ less than system 2 and therefore the transition time $(\tau_\delta)_1$ is less than $(\tau_\delta)_2$. According to the definition of the Output Passivity, ρ_2 is greater than ρ_1.

where δ_{ab}, $(\tau_\delta)_{ab}$ and $(t_{99})_{ab}$ represent the δ, τ_δ and t_{99} values of the transition between the steady states *a* and *b* respectively and V_b is the flux at steady state *b*. Since the terms $(\tau_\delta)_{ab}$ and $(t_{99})_{ab}$ are, in general, different from those of $(\tau_\delta)_{ba}$ and $(t_{99})_{ba}$, the Output Passivity of the transition from steady state *a* to steady state *b*, ρ_{ab}, will be different from that corresponding to the transition from steady state *b* to steady state *a*.

Some results concerning the transitions between steady states are discussed in the following section.

RESULTS

Applications of the analysis

The present analysis is applied to two cases that show different behaviour in the response shape.

Coupled enzyme assays. It is a common practice for enzymologists to use auxiliary enzymes as a means to assay the activity of an enzyme when its product is not detectable by conventional techniques. Much work has been done on this subject, dealing with different aspects of the process such as the transient duration of the transition towards the steady state (Easterby, 1973; McClure, 1969) and its optimization (García-Carmona *et al.*, 1981; Brooks *et al.*, 1984). Application of the present analysis to this problem gives us new insight into this question.

Let us consider the simplest example, which is described schematically as:

$$X_0 \xrightarrow{V_0} S_1 \xrightarrow{V_1/K_1} X_P$$

where X_0 is the initial substrate, X_P is the measurable product and S_1 is the single intermediate (product of the reaction under study). V_0 is the rate of the reaction under study and V_1 and K_1 are respectively the maximum rate and the Michaelis constant of the coupling enzyme. The usual assumption is that the first enzyme catalyses a zero-order reaction, i.e. it is an irreversible reaction; V_1 must necessarily be much greater than V_0 and consequently the steady-state concentration of S_1 will be small and less than K_1, so the auxiliary enzyme catalyses a first-order reaction. In this type of system Easterby (1973) and García-Carmona *et al.* (1981) have derived the following equations:

$$V_{out} = V_0 \cdot (1 - e^{(-t/\tau)})$$

$$t_{99} = 4.6 \cdot \tau$$

where τ, the Transition Time, is equal to K_1/V_1 and V_{out} is the rate of production of X_P at any time *t*.

As has been shown above in systems where the first enzyme is irreversible (eqn. 10), $\tau_\sigma = \tau_T = \tau_\delta$, which are the experimentally accessible parameters from the output progress curves.

It is evident from the above expression that as τ_δ becomes smaller (or greater) the Relaxation Time becomes shorter (or longer). However, this fact does not mean that the Output Passivity of the transition changes. In fact an interesting conclusion from this model is that the Output Passivity obtained by using eqn. (11) gives us a constant value, independent of τ_δ and t_{99} and equal to 0.21. Therefore we have a situation in which for different values of V_1 and K_1 both t_{99} and τ_δ have different values but in all cases the passivity of the transition is the same. This illustrates very well that the Output Passivity of a transition measures a different response from the Relaxation Time or the Transition Time.

These coupled enzyme assays therefore show only one type of response passivity in its transitions. But this is not a general behaviour.

Glycolytic transitions. In a previous paper (Torres *et al.*, 1989) Control of Flux and Total Transition Time, τ_δ, were investigated with a reconstructed rabbit muscle glycolytic system *in vitro* as an experimental model. We determined the steady-state flux and Total Transition Time in two conditions of concentration of an external stimulator of the system (fructose 2,6-bisphosphate). The system contained hexokinase, which is affected by a feedback inhibition by glucose 6-phosphate. From these results we can now draw new conclusions regarding the response of the system along the transition. In conditions of low fructose 2,6-bisphosphate concentration, i.e. 2 μM (condition *a*), $(\tau_\delta)_a$ was 11 ± 0.1 min, J_a was 0.025 ± 0.002 μmol of final product/min and $(t_{99})_a$ was 25 ± 0.8 min. In conditions of saturation with this effector, i.e. 10 μM (condition *b*), $(\tau_\delta)_b$ was 7 ± 0.1 min, J_b was 0.047 ± 0.0017 μmol of final product/min and $(t_{99})_b$ was 20 ± 0.3 min. From these data we can calculate the corresponding ρ values, which are $\rho_a = 0.44$ and $\rho_b = 0.35$ for transitions to steady state *a* and steady state *b* respectively. These results inform us that the transition to steady state *a* has more passivity than transition to steady state *b* when both systems start from the 'empty' situation. As can be seen in this case ρ has different values depending on the particular transition, which will be the general behaviour.

DISCUSSION

The terms introduced in the present analysis (σ, δ, β, τ_δ and τ_β), all of which are experimentally accessible, give us information on

either the output or the input progress curves. Thus δ is calculated from τ_δ, which can be obtained from the output progress curves $V_{out}(t)$ (Torres *et al.*, 1989; Meléndez-Hevia *et al.*, 1990). On its own β, because of the experimental difficulties in some cases in establishing the $V_{in}(t)$ curve, can be determined from the values of σ and δ. A simpler way to determine σ would probably be an actual extraction of the intermediates and summing up of their concentration (trichloroacetic acid precipitation would liberate both free and enzyme-bound pools). Usually β will be positive (reversibility or negative feedback on the first enzyme) but could also be negative (activation of the first enzyme) or zero [$V_{in}(t)$ constant] as considered above. In any cases, if our interest is to know about the transition from one steady state to another, the useful term is δ. However, β is the interesting term if we want to know about the interactions acting on the first enzyme that are responsible for the changes in the duration of the transition (reversibility, product inhibition, feedback loops etc.).

Having reached this point, two important observations should be made. One refers to the distinction between the Relaxation Time, t_{99}, and the Transition Time, τ_δ, in order to assay the duration of transitions. The absolute time scale of transitions is strictly described by the Relaxation Time, t_{99}, or any other fraction of the time needed to reach the actual final steady state. However, the Transition Time, τ_δ, does not give us exact indication of the duration of the transition but gives only a relative measure referred to the final steady state. In fact its actual meaning as the ratio of the total metabolite concentration, σ, over the flux through the system at steady state, V_{ss}, is the mean time that a molecule takes to be converted through the system at steady state. The second observation refers to the differences among the two terms referred to above, τ_δ and t_{99}, and the qualitative characterization of the dynamics of the system along the transition from one steady state to another, which we describe by means of the Output Passivity, ρ. This analysis illustrates the differences between the response time t_{99} and the Output Passivity, ρ, showing how this last parameter gives us some information on the shape of the transient curve.

These different aspects are well illustrated in Fig. 4(*b*). In this Figure, in spite of the fact that $(\tau_\delta)_1$ is less than $(\tau_\delta)_2$, the longer transition is transition 1 because its Relaxation Time $(t_{99})_1$ is greater than $(t_{99})_2$. The sole comparison of τ_δ values could lead to wrong conclusions about the speed of reaching the steady state. On the other hand comparison of the passivity response should be done by comparing the values of ρ. In Fig. 4(*a*) the two transitions evolve towards the same steady state flux, V_{ss}. Transition 1 has a lower value of δ but the t_{99} value is greater than in transition 2: $\delta_1 < \delta_2$ and $(t_{99})_1 > (t_{99})_2$. Accordingly $(\tau_\delta)_1$ is less than $(\tau_\delta)_2$ and ρ_1 will be less than ρ_2. These results inform us that the response of system 2 during the transition has more passivity to the movement than system 1: the output flux of system 2 remains far away from the final steady-state flux longer than the first one. Therefore, with regard to the passivity of the transition, system 2 shows greater resistance to reaching the steady state than system 1, in spite of the fact that the duration of transition 1 is greater than the duration of transition 2.

The results shown in the Results section illustrate this new aspect of the metabolic regulation, which is the passivity of the transition between steady states. Until now some attention has been focused on the absolute time scale of transition processes. The importance of the temporal length of the transition between steady states is obvious. A given system can change from one state to another fast or slowly in absolute terms (i.e. low or high values of t_{99}), but in any case the path along this transition can be (see Fig. 4*a*) either low (ρ near unity) or high (ρ near zero). This aspect could be of crucial importance in the fitness of the cellular metabolism of species or tissues.

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