Transient times in linear metabolic pathways under constant affinity constraints

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In the early seventies, Easterby began the analytical study of transition times for linear reaction schemes [Easterby (1973) Biochim. Biophys. Acta **293**, 552–558]. In this pioneer work and in subsequent papers, a state function (the transient time) was used to measure the period before the stationary state, for systems constrained to work under both constant and variable input flux, was reached. Despite the undoubted usefulness of this quantity to describe the time-dependent features of these kinds of systems, its application to the study of chemical reactions under other constraints is questionable. In the present work, a generalization of these magnitudes to linear metabolic pathways functioning under a constant-affinity constraint is carried out. It is proved that classical definitions of transient times do not reflect the actual properties of the transition to the steady state in systems evolving under this restriction. Alternatively, a more adequate framework for interpretation of the transient times for systems with both constant and variable input flux is suggested. Within this context, new definitions that reflect more accurately the transient characteristics of constant affinity systems are stated. Finally, the meaning of these transient times is discussed.

INTRODUCTION

The way in which a metabolic pathway responds to external changes is a fundamental problem in biology. In particular, the knowledge of the time needed for the pathway to reach the new state after a perturbation, which is commonly called the transition time, is of special interest (see, for instance, [1]). The transition time is a non-stationary magnitude that can strongly depend on the external constraints imposed on the system, unlike stationary properties that are independent of environmental conditions. The transitory behaviour of the system also depends on its state at the moment the perturbation takes place (i.e., the initial conditions), as well as on the final state achieved as a consequence of such perturbation (which could be similar to the initial state). Additionally, any model used to study the transition time must handle the problem of its evaluation. In fact, the measure of this transition time is a subject of important problems, both theoretical (mathematically the time needed to reach a steady state is infinite), and experimental (there is no way to determine the exact time at which the system enters the stationary state). So, any theory must find an operative definition of this magnitude.

Until now, most work has focused on the study of the transition time in linear enzymic chains [2–6]. The situations analysed are mostly the transition from rest, i.e., no intermediates are present in the system initially [2–4]. Moreover, although less extensively, transitions between any steady state have also been considered [3,5]. All of these models assumed that the system was constrained to function under either a constant input flux [2–4] or with a time-dependent input [5–7], generally as a consequence of keeping a constant concentration of substrate [6,7]. In both cases the output rate was considered to be proportional to the concentration of the final product, i.e., non-negative.

To obtain a measure of the transition time in systems forced to work under a constant input flux, Easterby [3] defined a magnitude, the so-called transition time, τ, as the quotient of the mass accumulated in the system, σ, and the flux passing through it, V, both taken at the steady state:

$$\tau = \frac{\sigma^{ss}}{V^{ss}} \tag{1}$$

It was proved that this definition corresponds to the transit time, i.e., the average time a molecule takes to cross the system [3].

Later, he showed that when a time-dependent input flux is considered, the transient time τ is given by [5]:

$$\tau = \frac{\sigma^{ss}}{V^{ss}} + \frac{\int_0^{V^{ss}} t\,\mathrm{d}V_{in}}{V^{ss}} \tag{2}$$

where the last term is associated with the variable input, V_{in}. However, σ^{ss}/V^{ss} is still related to the transit time of the system.

Despite the general acceptance of this theory, many authors state that τ hardly gives an estimate of the transition time, and that a more accurate calculation of this time needs new definitions. This criticism is based on the fact that the percentage of the steady state reached at time τ can vary from one system to another. This means that two systems with the same τ can differ appreciably in their transitory behaviour. For this reason, other magnitudes have been suggested to measure the way (and therefore the time) the system approaches the stationary state. The relationship between the transient time and the time at which the system approaches within 1 % of the steady state was studied by Easterby in 1973 [2]. Later, the time needed to reach 99 % of the steady state of a system variable was designated t_{99} [8]. In this context, it was demonstrated that, in long linear enzymic chains, τ gives an accurate evaluation of t_{99} [4]. In addition, Easterby used the quotient of t_{99} and τ as a way to obtain more information about the transition [3,4]. The reciprocal of this magnitude was referred to as passivity by Torres et al. [6].

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As stated above, the transition time must depend on the kind of constraint imposed on the system. Several examples described recently [9] show that some metabolic pathways work under a fixed concentration of both substrate and product. In these situations the chemical affinity is kept constant. Thus most of the studies on the regulation of pathways (using the metabolic control theory), as well as the optimization of kinetic parameters, assume that the system is functioning under a constant-affinity constraint (see, for instance, [9–12]). Nevertheless, so far, the study of transition times has been developed only for metabolic pathways in which the input flux is externally regulated, leaving the output of last intermediate to fit this constraint.

In this paper, the theory of transient times is extended to those systems working under a constant-affinity constraint. With this aim, it is convenient to reformulate the definitions given in systems evolving with an irreversible output. This new formulation allows the definition of the concept of transient time in a general framework, independently from the kind of constraint applied to the system. So, similar definitions to those stated previously can be established to measure the transient time associated with both the input and output rates, as well as with the overall transition, for systems under constant affinity. These definitions are valid for enzymic chains that yield monotonic input and output rates, i.e., the input velocity decreases (negative feedback) and the output velocity increases for all time. This requirement is held in all of the studies developed previously when assuming either pseudo-first-order [2] or Michaelis–Menten kinetics [3].

SYSTEMS UNDER CONSTANT INPUT FLUX

In 1973, Easterby [2] defined the transient time in chemical systems under a constant input flux, V_{in}. For the following reaction scheme:

$$S \xrightarrow{V_{in}} I_k,\ EI_k \xrightarrow{V_{out}} P \tag{3}$$

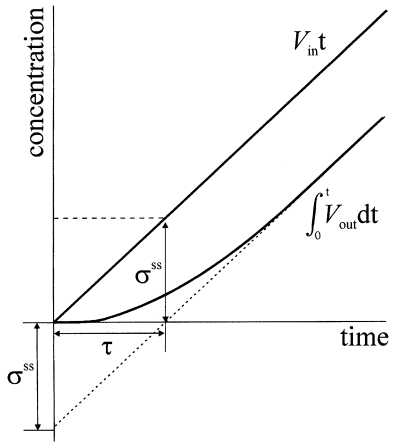

Figure 1 Progress curve of systems operating under a constant input flux restriction

The graph shows the mass that enters ($V_{in}t$) or leaves the system ($\int_0^t V_{out}\,dt$) versus time. The transient time, τ is defined as the time necessary for a mass equal to that accumulated in the steady state, σ^{ss} to enter the system. Graphically, it coincides with the point at which the asymptote to the output progress curve intersects the time axis.

the transient time τ was defined as:

$$\tau = \frac{\sum_{i=1}^{n} ([I_i]^{ss} + [EI_i]^{ss})}{V^{ss}} = \frac{\sigma^{ss}}{V^{ss}} \tag{4}$$

where $[I_i]$ and $[EI_i]$ are the concentrations of the free and enzyme-bound intermediates respectively. In this model the last step is considered irreversible yielding an output flux, V_{out}, proportional to the concentration of last intermediate, $[I_n]$. Figure (1) shows the geometrical meaning of this time. Strictly speaking, τ does not represent the time the system takes to attain the asymptotic regime, which from a deterministic point of view is infinite, but gives an idea of how long the transition is. In fact, to have a low τ is a necessary condition, but not sufficient, to carry out fast transitions. Therefore, despite being a magnitude defined from stationary values, it provides information about the temporal evolution of the system.

An alternative way of explaining the transient time τ can be deduced from Figure 1. As can be seen, τ is the time at which a mass equal to σ^{ss} has entered the system. Mathematically, this can be written as:

$$\int_0^{\tau} V_{in}\,dt = \sigma^{ss} \tag{5}$$

Since V_{in} is constant, this formula reduces to Easterby's expression, eqn (1). It is worth mentioning that, although the transient time is directly related to the stationary concentration of the intermediates, it gives the measure of the transition time associated with the output flux, but not of the temporal evolution of such intermediates. Nevertheless, as has been noted in the Introduction, this magnitude has also the meaning of the transit time, that is, the average time taken by a molecule to cross the system at steady state.

SYSTEMS WITH VARIABLE INPUT FLUX

Let us consider now those linear chemical reactions working under a constant substrate concentration and irreversible output of the last intermediate. This situation can be schematically represented as:

$$S \overset{V_{in}}{\rightleftarrows} I_k,\ EI_k \xrightarrow{V_{out}} P \tag{6}$$

Notice that now the substrate S can be obtained from both the surroundings and the first intermediate I_1. The progress curve for this kind of system is as depicted in Figure (2). Contrary to the previous case, there exists a transition associated with both the input and the output velocities. Therefore, two contributions can be distinguished in the total mass accumulated in the steady state, σ^{ss}: one, σ^{ss}_{out}, due to the variable output, and another, $\sigma_{in}{}^{ss}$, consequence of the variable input, i.e., $\sigma^{ss} = \sigma^{ss}_{in} + \sigma^{ss}_{out}$. This theoretical distinction allows definition of two transient times, $\tau_{in} = \sigma^{ss}_{in}/V^{ss}$ and $\tau_{out} = \sigma^{ss}_{out}/V^{ss}$, being the sum equal to the transit time, i.e., $\tau_{in} + \tau_{out} = \sigma^{ss}/V^{ss}$. It has been proved that τ_{out} is given by the expression in eqn. (2), i.e., the transient time τ as defined by Easterby [5]. In addition, this author has shown that τ_{in} is associated with the feedback on the first enzyme of the pathway [5], and corresponds to:

$$\tau_{in} = -\frac{\int_0^{V^{ss}} t\,dV_{in}}{V^{ss}} \tag{7}$$

Although τ_{out} is a good measure of the transient time related to the output rate, Easterby and others suggest that, under particular conditions, this magnitude can also give a measure of

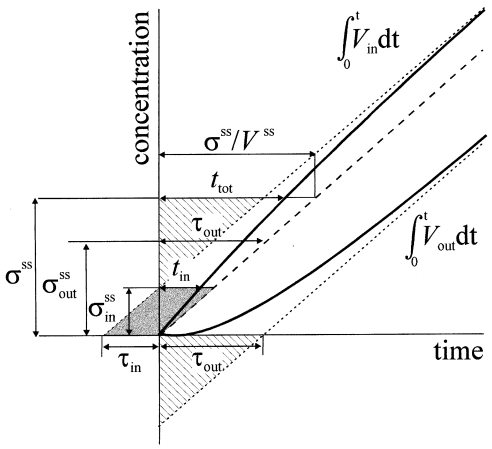

Figure 2 Progress curves of systems functioning under a constant concentration of the initial substrate and irreversible output of the last intermediate

Under these conditions, the total mass accumulated in the steady state, σ^{ss}, can be separated into two parts, σ^{ss}_{in} and σ^{ss}_{out}. The geometric meaning of the classical transient times, $\tau_{in} = \sigma^{ss}_{in}/V^{ss}$ and $\tau_{out} = \sigma^{ss}_{out}/V^{ss}$, is depicted. The new magnitude t_{in} is defined as the time needed for a mass equal to σ^{ss}_{in} to enter the system. In a similar way, t_{tot} is the time at which a mass equal to σ^{ss} enters the system. Notice that τ_{out} can be defined as the time required for a mass equal to σ^{ss}_{out} to enter, assuming an input rate equal to the steady state flux, and it coincides with the intersection point of the asymptote to the output curve and the time axis. Inequalities shown in eqns. (10), (13) and (14) can be easily proved. Since the hatched triangles are identical, it follows that $t_{tot} \geqslant \tau_{out}$. Similarly, from the shaded parallelogram, it is obvious that t_{in} is always a fraction of its shortest side, τ_{in}. Moreover, since $V_{in} \geqslant V^{ss}$ for all t, then $\sigma^{ss}/V^{ss} \geqslant t_{tot}$.

the overall transition time [5,6]. However, it must be pointed out that this is only strictly true in those situations in which the first enzyme is not limiting (i.e., when no feedback effect exists). In other words, when the time required for the input rate to reach the steady state is negligible with respect to that of the output velocity. In general τ_{in} does not yield useful information about this input transition (i.e., there can be systems with a high value of τ_{in} but with a very low transition time with respect to the input velocity), but it has been proposed that it provides information about the existence of feedback in the first enzyme of the sequence [5]. Nevertheless, σ^{ss}/V^{ss} still has the meaning of a transit time.

It follows that a careful evaluation of the transition time of a system constrained to work under a constant substrate concentration requires the definition of three new variables: one, t_{in}, associated with the variable input; a second one, t_{out}, that takes into account the variable output, and finally a third, which will be referred to as t_{tot}, that considers the global transition time, including the contribution of the two partial transient times (t_{in} and t_{out}). Fortunately, these definitions can be stated in a conceptually analogous way as shown in the previous section.

Let us define the global transient time, t_{tot}, as the time necessary for a mass equal to σ^{ss} to enter the system. Formally:

$$\int_0^{t_{tot}} V_{in}\,dt = \sigma^{ss} \tag{8}$$

Its geometrical meaning is shown in Figure (2). Moreover, from this Figure the next inequality is easily derived:

$$\frac{\sigma^{ss}}{V^{ss}} \geqslant t_{tot} \geqslant \tau_{out} \tag{9}$$

It can be shown that when a time equal to τ_{out} the input flux is already very close to its stationary value (which does not imply a low value of τ_{in}), t_{tot} can be approximated by τ_{out}. In this case, τ_{out} [or τ as defined in eqn. (2)] is a good evaluation of the overall transition time.

Similarly, t_{out} can be defined as the time that a mass equal to σ^{ss}_{out} needs to enter the system, assuming from the beginning an input flux equal to the stationary flux value, V^{ss}, i.e.:

$$\int_0^{t_{out}} V^{ss}\,dt = \sigma^{ss}_{out} \tag{10}$$

which clearly coincides with τ_{out} [τ in eqn. (2)].

Finally, to quantify the temporal evolution of the input velocity we introduce the new magnitude, t_{in}, as:

$$\int_0^{t_{in}} V_{in}\,dt = \sigma^{ss}_{in} \tag{11}$$

that represents the time at which a mass σ^{ss}_{in} has entered the system. From Figure (2), the following inequality can be derived straightforwardly:

$$t_{in} \leqslant \tau_{in} \tag{12}$$

In addition:

$$t_{tot} \geqslant t_{in} \tag{13}$$

Geometric considerations (Figure 2) allow us to show that, on the one hand, as t_{in} decreases, t_{tot} approaches t_{out} and, on the other hand, as t_{in} increases and tends towards τ_{in}, the value of t_{tot} approaches σ^{ss}/V^{ss}. Notice that, whereas the value of t_{out} depends only on steady-state variables (V^{ss} and σ^{ss}_{out}), both t_{in} and t_{tot} depend on the input flux dynamics.

These three times are not independent of each other. Indeed, from the expressions shown in eqn. (8), eqn. (10) and eqn. (11), and taking into account that $\sigma^{ss} = \sigma^{ss}_{in} + \sigma^{ss}_{out}$, the next equation can be deduced:

$$\int_{t_{in}}^{t_{tot}} V_{in}\,dt = V^{ss} t_{out} \tag{14}$$

from which t_{tot} can be interpreted as the time from t_{in} required to put into the system a mass equal to σ^{ss}_{out}. Besides, it is important to point out that the stationary magnitudes, τ_{in}, τ_{out} and σ^{ss}/V^{ss}, allow limits to the transient time [eqn. (9) and eqn. (12)] to be established. However, more exact measures can only be achieved from the new definitions stated above [eqns (8) and (11)].

SYSTEMS UNDER A CONSTANT AFFINITY CONSTRAINT

As stated in the Introduction, some metabolic pathways have evolved under both the substrate S and the product P concentrations constant in time. For linear enzymic chains this means that both reactions connecting these external metabolites with the internal intermediates must be considered reversible. Schematically:

$$S \overset{V_{in}}{\rightleftarrows} I_k,\ EI_k \overset{V_{out}}{\rightleftarrows} P \tag{15}$$

Notice that by maintaining a constant concentration of both substrate and product the total affinity of the reaction is fixed. It is well known that the affinity of a linear reaction is proportional to $\log\left(\frac{qS}{P}\right)$ (q being the equilibrium constant). Thus if $q[S] > [P]$

the reaction progresses towards product formation. In all of the Figures a positive affinity is assumed, although the definitions proposed here are equally valid for reactions functioning in the opposite direction. As before, the study focuses on transitions from rest, i.e., from an empty system to a final stationary state with a net flux V^{ss}. It is notable that the situation presented in the previous section corresponds to the limit case [P] = 0, i.e., infinite affinity.

It is easy to demonstrate that, in the general hypothesis stated in the Introduction, the progress curve for this type of system has the qualitative shape shown in Figure (3). As can be seen, whereas the curve associated with the input mass is similar to that shown in Figure (2), now, as a result of keeping the product concentration constant with time, the curve of the output mass presents a minimum. This minimum is reached when the sign of the output velocity changes (from negative to positive), i.e., when $V_{out} = 0$. This time is defined as t_0. Because of this negative output rate, until $t = t_0$, the system is filled from both sides of the reaction chain.

Similar arguments to those presented in the previous section show that neither σ^{ss}/V^{ss} nor τ_{in} offers accurate information about the transition time. In addition, because of the dynamic difference in the curve of the output mass, τ_{out} cannot be used to measure the transition time associated with the output rate. In fact, there are situations in which at a time much less than τ_{out} the system has already reached the steady state. However, σ^{ss}/V^{ss} still defines the time that a molecule takes to cross the system. Therefore, as discussed in the previous section, new definitions to evaluate the transition time, both partial and global, are necessary.

Let us define an overall transient time, t_{tot}, as the time required for a mass equal to that accumulated in the steady state, σ^{ss}, to enter the system. As stated above, while $t < t_0$ both V_{in} and V_{out} contribute to fill the system. From t_0, mass accumulates only due to the time-dependent input, V_{in}. Thus:

$$\int_0^{t_0} (V_{in} - V_{out})\mathrm{d}t + \int_{t_0}^{t_{tot}} V_{in}\,\mathrm{d}t = \sigma^{ss} \tag{16}$$

This expression can be rewritten in a different form:

$$\int_0^{t_{tot}} V_{in}\,\mathrm{d}t = \sigma^{ss} + \int_0^{t_0} V_{out}\,\mathrm{d}t \tag{17}$$

showing that t_{tot} can also be interpreted as the time required for a mass equal to $\sigma^{ss} + \int_0^{t_0} V_{out}\,\mathrm{d}t$ (integral that is always non-positive) to enter the system, taking into account only the input rate V_{in} (see Figure 3).

As for systems with a constant concentration of substrate and irreversible output, we can define t_{in} as the time in which a mass, σ_{in}^{ss}, enters the system from the substrate S. This magnitude is given by the expression in eqn. (11) and its meaning is shown graphically in Figure (3). Obviously, inequality expressed in eqn. (12) remains. In addition, it is not difficult to prove that eqn. (13) also holds.

Contrary to what happens in systems with irreversible output [inequality, eqn. (9)], now τ_{out} is not a lower limit of t_{tot} (see Figure 4). To recover this meaning, and to simultaneously measure the time taken for the output rate to reach the stationary regime for systems under this kind of constraint, we define the time t_{out} as follows: as can be observed in Figure (3), if the origin of coordinates is placed at the point $(t_0, \int_0^{t_0} V_{out}\,\mathrm{d}t)$, the rest of the progress curve is similar to the corresponding graph for systems with irreversible output. So, t_{out} can be defined as the time at

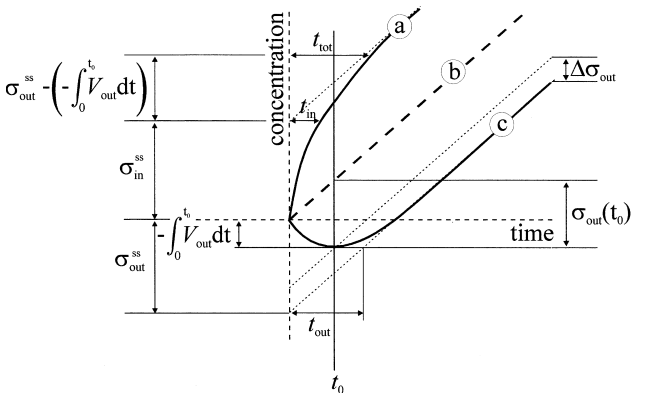

Figure 3 Progress curves of systems evolving under a constant affinity restriction

(**a**) is the evolution curve for the input rate, $\int_0^t V_{in}\,\mathrm{d}t$. The straight line (**b**) represents $V^{ss} \cdot t$, while (**c**) corresponds to the evolution curve for the output velocity. Unlike the output curve shown in Figure (2), the graph (**c**) presents a minimum at a time t_0. However, if the origin of the coordinates is displaced to the point $(t_0, \int_0^{t_0} V_{out}\,\mathrm{d}t)$, the shape of this curve is similar to that shown in Figure (2). Again, the total mass accumulated in the steady state, σ^{ss}, can be separated into two parts, σ_{in}^{ss} and σ_{out}^{ss}. In its turn, two contributions can be distinguished in σ_{out}^{ss}: one, $\sigma_{out}(t_0)$, which is the accumulated mass at time t_0, due to both the variable input, V_{in}, and the variable output, V_{out} (notice that the matter that enters from V_{out} is equivalent to $-\int_0^{t_0}\mathrm{d}t$). The other contribution to σ_{out}^{ss} is $\Delta\sigma_{out}$, as a consequence of the positive output flux from t_0. With regard to this decomposition, the three times defined analytically in the text can be interpreted graphically: t_{out} is the time at which a mass $\Delta\sigma_{out}$ has entered from t_0, assuming an input flux value equal to the steady-state flux. Obviously, definitions for t_{in} and t_{tot} are equal to those for systems with irreversible output.

which a mass $\Delta\sigma_{out}$ enters the system from t_0, assuming an input-flux equal to the net flux at the steady state, i.e.:

$$\int_{t_0}^{t_{out}} V^{ss}\mathrm{d}t = \Delta\sigma_{out} \tag{18}$$

where, as can be seen in Figure (3), $\Delta\sigma_{out}$ is the difference between the mass accumulated at the steady state as a result of the time-dependent output flux, σ_{out}^{ss}, and the value of this mass at time t_0, $\sigma_{out}(t_0)$. Solving the integral, the expression in eqn. (18) is simplified to:

$$t_{out} = t_0 + \frac{\Delta\sigma_{out}}{V^{ss}} \tag{19}$$

The term $\Delta\sigma_{out}/V^{ss}$ is conceptually equivalent to the previously defined transient time in systems with a constant input flux, τ, when the reaction product is measured from t_0.

A simple geometric consideration (Figure 4) shows that:

$$\frac{\sigma^{ss}}{V^{ss}} \geqslant t_{tot} \geqslant t_{out} \tag{20}$$

t_{out} recovering the meaning of the lower limit of the global transient time, t_{tot}, that it had in those systems with an irreversible output.

In addition, when at time t_{in} the velocity V_{in} is close enough to V^{ss}, t_{tot} tends toward t_{out}, but now, with reference to Figure (4), it can be stated that:

$$t_{out} \leqslant \tau_{out} \tag{21}$$

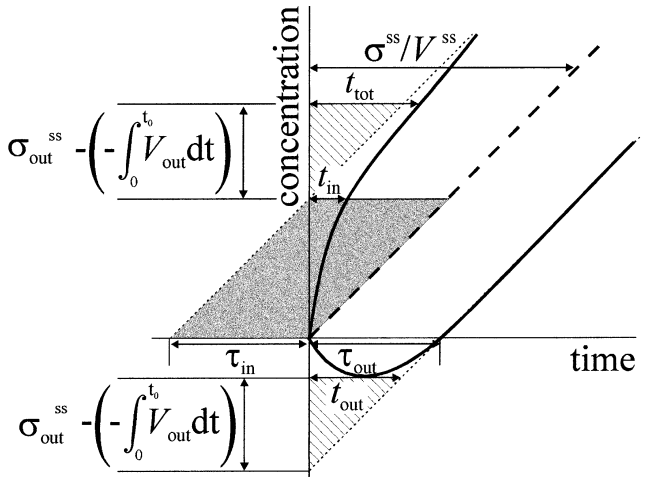

Figure 4 Geometric proof of inequalities in eqns. (13), (14) and (21) for systems under a constant affinity constraint

Since the hatched triangles are equal, then $t_{tot} \geqslant t_{out}$. From the shadowed triangles it can be seen that t_{in} is always a fraction of τ_{in}, i.e., $t_{in} \leqslant \tau_{in}$. The inequality $\sigma^{ss}/V^{ss} \geqslant t_{tot}$ can be easily shown taking into account both (i) $V_{in} \geqslant V^{ss}$ for all t, and (ii) unlike the definition of t_{tot}, the evaluation of σ^{ss}/V^{ss} also takes into consideration the mass entering the system until t_0. Finally, notice that $t_{out} \leqslant \tau_{out}$ for this kind of system.

Finally, it is easy to see that the dependence among the three times defined above, t_{in}, t_{out} and t_{tot}, is still given by the expression in eqn. (14).

DISCUSSION

Recent publications have pointed out that metabolic pathways are forced to work under different external constraints [12]. It seems that two main boundary constraints exist in Nature: constant input flux and constant affinity, i.e., keeping both substrate and product concentrations fixed in time. Although the stationary features of a pathway are independent of the kind of restriction imposed on the system, time-dependent magnitudes are clearly dependent upon it. An important characteristic of metabolic transformations is the time they need to return to the stationary state after perturbation, the so-called transition time. Hitherto, most of the works have focused mainly on measuring the transition time in linear reaction chains working under both constant input flux [2–4], and constant concentration of substrate with irreversible output [5–7].

In this article we have extended this theory to analyse linear reaction chains working under a constant affinity constraint. The study has focused on transitions from rest, i.e., assuming that initially the system is empty. To obtain an estimate of the rapidity with which the system reaches the stationary state we have defined general transient times that are valid regardless of the kind of constraint under which the system is functioning. Thus when a constant input flux is imposed on the system, these definitions correspond to the magnitudes established previously [2–4]. However, we have proved that this correspondence is lost when dealing with systems evolving under constant affinity.

Let us begin by discussing the particular case of infinite affinity, that is, when the substrate concentration is kept constant and the last intermediate product leaves the system by an irreversible reaction. It has been argued that an accurate evaluation of the global transient time can be obtained from the transition time associated with the output flux, i.e., measuring τ_{out} [τ as defined in eqn. (2)] [5]. This author assumes implicitly that the transient time related to the input rate (which changes with time until the stationary state is reached) is negligible. However, consideration of this magnitude can become essential in at least two situations. First, in those systems in which the transition of the input velocity is so slow that its contribution to the global transition time is significant. Secondly, when the main goal of the reaction chain is to remove a specific substance from the milieu (here referred to as the substrate).

Although τ_{in} is related to the mass accumulated in the steady state due to the input flux excess, σ_{in}^{ss}, it has been shown that it does not give information about the transition time associated with the input rate. Nevertheless, it is easy to see that τ_{in} is an upper limit of a new magnitude designated t_{in} (i.e., the time required for a mass equal to σ_{in}^{ss} to enter the system). In fact, most of the situations tested numerically (results not shown) present a t_{in} much lower than τ_{in}. As a direct consequence, having a low τ_{in} is sufficient (but not necessary) to bring about a low t_{in}. However, an important difference between these times is that, whereas, for the evaluation of τ_{in} one only needs to know the value of stationary variables (particularly, the flux and concentration of intermediates) together with the asymptotic behaviour of the output mass, in order to compute t_{in} an explicit knowledge of the temporal evolution of the input mass is required.

Even knowing both partial transient times, τ_{out} and t_{in}, a measure of the time at which the reaction rates (input and output) attain the stationary state is still necessary. To evaluate this global transition time of the pathway, we have defined a transient time, t_{tot} (see eqn. 14). This magnitude defines the time at which a mass equal to that accumulated in the steady state (shared among the different intermediates) enters the system. This variable gives useful information not only about the output process, but also about the temporal evolution of the input rate. Only in those situations in which at a time τ_{out} the input rate approaches its steady-state value, can t_{tot} be approximated by τ_{out}. Obviously, this only occurs if $t_{in} < \tau_{out}$ (although this condition is not sufficient to assure this approximation). In these cases, useful information about the transition can be obtained without knowing the temporal evolution of the input mass. It is noticeable that, even in these cases, σ^{ss}/V^{ss} loses its significance of global transient time. Nonetheless, it still has two meanings. On the one hand, it represents the average time of the transit of a molecule through the reaction chain. On the other hand, σ^{ss}/V^{ss} is the upper limit of t_{tot} (in fact, t_{tot} tends to σ^{ss}/V^{ss} when t_{in} approaches τ_{in}).

As a general conclusion, to compare the transition times of two systems constrained to work at a fixed substrate concentration and irreversible output, it must be noticed that, whereas τ_{out} could give an adequate evaluation of the transient time under particular conditions, neither σ^{ss}/V^{ss} nor τ_{in} can be used to compute the temporal characteristics of the transition. Then, new definitions such as those stated in this paper are required.

The major difference between the evolution curves of those systems working under the restriction discussed above and those constrained to function under constant affinity is the existence of a minimum in the graphics of the output mass in the last case (Figure 3). Because initially the system is empty, a negative local affinity appears in the last reaction and the mass enters from the product. Contrary to the previous cases, τ_{out} does not give an accurate measure of the output transient time. In fact, it is not difficult to find situations in which at time τ_{out} the output rate is already infinitesimally close to its steady-state value, and lacks the meaning of lower limit that it had under infinite affinity. To get a better measure of the output transition a new magnitude

has been defined, t_{out}. Its meaning is easily understood as follows. Two contributions can be distinguished in the accumulated mass due to the variable output, σ_{out}^{ss}: one, arising from the input mass entered from the product P, and another, after changing the direction of the output velocity, as a consequence of the delay in reaching the steady output flux. The first period ends at the time the input mass from P reaches its minimum, i.e., $t = t_0$. The second one can be estimated by a similar transient time to τ_{out} after translating the origin of coordinates to the point $(t_0, \int_0^{t_0} V_{out}\,dt)$. So, for this kind of system, t_{out} is defined as t_0 plus the quotient between the mass accumulated from t_0, $\Delta\sigma_{out}$, and the stationary flux, V^{ss}.

Obviously, since the qualitative behaviour of the input rate is similar to those systems under infinite affinity, both t_{in} and t_{tot} remain as valid magnitudes to be used under a constant affinity constraint. Again, for a system in which t_{in} is lower than t_{out}, then t_{tot} approaches t_{out}. In these cases, useful information about the transition can be obtained without knowing the input curve from S.

In the light of the definitions stated in this work, the necessity of reviewing several magnitudes previously proposed to measure other aspects of the transition must be pointed out. This is the case of the evaluation of the transition time between steady states (different from the rest) for those systems evolving under a constant affinity constraint. Similarly, the concept of passivity must be re-defined for this kind of system [6]. The establishment of these new definitions, as well as their functional implications, are the subject of current research.

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