Biochem. J. (1986) 233, 871–875 (Printed in Great Britain)

The effect of feedback on pathway transient response

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The effect of variation of the rate of input of material on the transient behaviour of metabolic pathways is examined. This reveals the existence of three transient times which make up the overall pathway transient. Two of these have been described previously and represent the times required for the accumulation of the free intermediate pool and the pool of enzyme-bound intermediate. They are state functions and as such are independent of the way in which the steady state was reached. The third is attributable to the variation in the rate of input of material to the pathway. It is dependent on three further factors. These are (*a*) the time required for the initial enzyme to reach its own steady state, (*b*) substrate depletion and (*c*) feedback. The description of the transient is:

$$1/V_{ss} \cdot \int_0^{V_{ss}} t \cdot dV_0$$

where V_0 represents the rate of input and V_{ss} represents the steady-state flux. The transient time associated with the transition between steady-states is shown to be a simple function of the transients for the establishment of each steady state from rest and may be expressed as:

$$\tau = \tau_b - V_a/V_b \cdot \tau_a$$

where V_a and V_b refer to the fluxes in the two steady states and τ_a and τ_b represent the transient times for the establishment of each of the steady-states from rest. The total pathway transient may now be completely defined as:

$$\tau = 1/V_{ss} \cdot \int_0^{V_{ss}} t \cdot dV_0 + \Sigma[I]/V_{ss} + \Sigma[EI]/V_{ss}$$

where summation over all intermediates, I, is implied. The significance of this to the analysis of pathway behaviour is discussed with more general examples of pathway transient analysis.

INTRODUCTION

Any sequence of consecutive enzyme-catalysed reactions exhibits a transient or lag phase in product formation after its initiation. During this phase the intermediates of the sequence are accumulating to their steady-state concentrations. The nature of this transient has been extensively analysed in the case of sequences of coupled, irreversible enzyme-catalysed reactions (McClure, 1969; Hess & Wurster, 1970; Easterby, 1973; Storer & Cornish-Bowden, 1974; Kuchel & Roberts, 1974; Cleland, 1979; Garcia-Carmona *et al.*, 1981; Easterby, 1984) and also to a more limited extent in the case of reversible reactions (Heinrich & Rapoport, 1975; Easterby, 1981). The analysis of irreversible reactions is reminiscent of systems of radioactive transformation studied earlier (Bateman, 1910). It has also been shown that the idea of transcience may be extended to pathways and the transient may be simply described in terms of the steady-state flux and the concentrations of intermediates of the pathway (Easterby, 1981). An analogous approach has been applied to the study of the effects of protein–protein interaction on transient behaviour (Bartha & Keleti, 1979) and to the analysis of the residence times of compartmentalized systems (Hearon, 1981*a*, *b*). The description in my earlier paper (Easterby, 1981) is general and does not depend on the kinetic mechanism of the pathway or its constituent enzyme, but, like all other analyses, does assume a steady rate of input of material to the pathway. The transient so defined represents the time required for the accumulation of intermediates and is based on the principle of mass conservation within the pathway.

The analysis of transience in consecutive enzyme-catalysed reaction sequences falls into three main categories: (*a*) the analysis of coupled reactions, (*b*) the analysis of substrate channelling within multienzyme complexes and multifunctional proteins, and (*c*) the analysis of the temporal response of pathways. It was initiated by the study of assays using coupled enzyme systems (McClure, 1969; Hess & Wurster, 1970; Easterby, 1973). The analysis had two aims; firstly to minimize the lag in the formation of reaction product and accelerate the attainment of the steady state and secondly to optimize the concentrations of primary and auxiliary enzymes. This approach has been refined in order to improve the cost-effectiveness of coupled assay systems (Cleland, 1979; Garcia-Carmona *et al.*, 1981; Easterby, 1984) and has been the subject of extensive review (Rudolph *et al.*, 1979).

The approach was later applied to multienzyme complexes and multifunctional proteins, where separation of individual enzyme activities was either impossible or undesirable in the context of the study of the integrated reaction sequence. Table 1 provides examples of the application of pathway-transient analysis in such systems. One of the earliest and most successful applications of this approach was that of Welch & Gaertner (1975) to the multifunctional *arom* enzyme conjugate of the fungus *Neurospora crassa*, catalysing

Table 1. Applications of the analysis of transient times

Application	Reference
(*a*) Coupled enzyme reactions	
Pyruvate kinase/lactate dehydrogenase	Hess & Wurster (1970)
Hexokinase/glucose 6-phosphate dehydrogenase	Easterby (1973)
Bifunctional enzyme aggregates	Matthiasson *et al.* (1974)
Sucrose phosphate synthetase assay	Harbron *et al.* (1980)
Multienzyme cellulase systems	Klesov & Grigorash (1981)
Arginase/ornithine carbamoyltransferase	Teasdale *et al.* (1982)
Sedoheptulose bisphosphatase assay	Woodrow & Walker (1982)
(*b*) Pathways and substrate channelling	
Tryptophan synthetase of *Neurospora crassa*	Matchett (1974)
Erythrocyte glycolysis	Rapoport *et al.* (1974)
arom complex of *N. crassa*	Welch & Gaertner (1975)
Aspartate aminotransferase/malate dehydrogenase	Bryce *et al.* (1976)
Cysteine synthetase complex of *Salmonella typhimurium*	Cook & Wedding (1977)
Chorismate mutase/prephenate dehydratase of *Escherichia coli*	Duggleby *et al.* (1978)
Aldolase/glyceraldehyde 3-phosphate dehydrogenase of rabbit muscle	Ovadi & Keleti (1978)
Dihydro-orotate synthesis by protein *pyr-3* complex of hamster cells	Christopherson & Jones (1980)
Pyrimidine biosynthesis on Syrian-hamster cells	Mally *et al.* (1980)
UMP synthesis in Ehrlich-ascites cells	Traut (1980)
Deoxyribonucleotide synthesis induced by bacteriophage T4	Chiu *et al.* (1982)
Aspartate aminotransferase/glutamate dehydrogenase	Salerno *et al.* (1982)

the biosynthesis of aromatic amino acids. This study revealed a reduction in the transient time within the multienzyme complex relative to that in free solution and provided evidence for substrate channelling of intermediates within the complex rather than their wasteful dispersal within the bulk medium. Moreover, Easterby (1973) had predicted the existence of 'time-limiting' enzymes which would govern the temporal response of a pathway as opposed to controlling its flux. These enzymes might determine the distribution of intermediates between competing, branched pathways. One such enzyme was identified by Welch & Gaertner (1976) in the *arom* complex, namely shikimate kinase, which determined the anabolic or catabolic fate of aromatic intermediates. Whereas shikimate kinase was 'time-limiting', other enzymes within the complex were subject to co-ordinated flux regulation. The idea of channelling of intermediates within complexes or compartments, or more generally between free cytosolic enzymes, as a means of improving efficiency has gained increasingly in importance and has been fully reviewed (Welch, 1977; Keleti, 1984). By comparison of the calculated transient time (based on the assumption of free diffusion of enzymes and intermediates) with the measured transient, one may decide whether intermediates are freely diffusing, channelled or compartmentalized.

The analysis of transience within complexes and pathways has suffered one major drawback; it has largely been based on equations derived to describe simpler coupled enzyme systems and has not approached the kinetic complexity found within the complex. Simpler models in which all enzymes are assumed to obey first-order kinetics in substrate have been applied. This problem has largely been overcome by the derivation of a general equation to describe the transient which makes no assumption of kinetic mechanism (Easterby, 1981). However, one major problem remains. All previous approaches have assumed a constant rate of input of material into the pathway, thus precluding any assessment of the effects of feedback on the transient time and hence channelling within the pathway.

The present report extends the idea of mass conservation to pathways in which the rate of input of source material is allowed to vary with time. This variation may be due to the transient behaviour of the initiating enzyme, the depletion of the substrate for the pathway or regulation of flux through the initial boundary of the system (e.g. feedback on the first enzyme of the pathway). The pathway is shown to possess three transient times, two due to the accumulation of intermediates and the third due to variation in input.

RESULTS

Derivation and significance of the feedback transient

Consider the following scheme in which a substrate, S, is input to a pathway at a rate V_0 and is converted through intermediates I and enzyme-bound intermediates EI to end product P:

$$S \rightarrow (I, EI) \rightarrow P$$

Mass conservation requires that the total matter input is accounted for either as intermediate, free or enzyme-bound, or as product. If the rate of input is allowed to vary with time then this conditions is met by the following equation:

$$\int V_0 \cdot dt = \Sigma [I] + \Sigma (EI) + [P] \quad (1)$$

where summation over all components is implied and the boundary condition at $t = 0$ is $[I] = 0$, $[EI] = 0$, $[P] = 0$, $V_0 = 0$. This may be written in the alternative form:

$$V_0 \cdot t - \int t \cdot dV_0 = \Sigma [I] + \Sigma [EI] + [P] \quad (2)$$

or more descriptively:

$$[P] = V_0 \cdot (t - 1/V_0 \cdot \int t \cdot dV_0 - \Sigma [I]/V_0 - \Sigma [EI]/V) \quad (3)$$

In the steady state this may be written:

$$[P] = V_{ss}(t-\tau) \quad (4)$$

where:

$$\tau = 1/V_{ss} \cdot \int_0^{V_{ss}} t \cdot dV_0 + \Sigma [I]/V_{ss} + \Sigma [EI]/V_{ss} \quad (5)$$

and V_{ss} represents the steady-state flux.

The transient time can be seen to be composed of three components and may be written:

$$\tau = \tau_I + \tau_{EI} + \tau_F \quad (6)$$

where

$$\tau_I = \Sigma [I]/V_{ss}, \tau_{EI} = \Sigma [EI]/V_{ss}$$

and

$$\tau_F = 1/V_{ss} \cdot \int_0^{V_{ss}} t \cdot dV_0 \quad (7)$$

τ_I and τ_{EI} have been described previously and represent the time required to establish a steady state in the pool of intermediate (Easterby, 1981). They are functions of the steady state alone and are independent of the way in which it was arrived at. τ_F is a transient time associated with the fluctuation in the rate of input of material to the pathway. It is dependent on three factors: (*a*) the establishment of a steady state in the first enzyme of the pathway; (*b*) the depletion of substrate for the pathway; and (*c*) feedback on the first enzyme or boundary of the pathway.

Substrate depletion may generally be ignored in a context where steady states are being discussed, as any significant reduction in the substrate concentration sufficient to reduce the rate of input of material to the pathway would probably preclude the establishment of a steady state in product formation. In the absence of feedback, τ_F will be determined by the time required by the first enzyme of the sequence to reach a steady state in its own enzyme–substrate complexes. In fact it may be shown that, under these circumstances, τ_F defines the transient or characteristic time of the first enzyme. It has been shown generally that product formation by a single enzyme in the pre-steady-state time domain is described by the expression (Hijazi & Laidler, 1973*a*,*b*,*c*,*d*,*e*; Wong, 1975):

$$[P] = V_{ss} \cdot [t + \sum_i \beta_i \cdot \exp(-\lambda_i \cdot t) - \sum_i \beta_i] \quad (8)$$

Where $\Sigma\beta_i$ represents the transient or characteristic time of the individual enzyme. From this expression the transient, τ_F, may be derived as follows:

$$V_0 = V_{ss} \cdot [1 - \sum_i \cdot \beta_i \cdot \exp(-\lambda_i \cdot t)] \quad (9)$$

$$dV_0/dt = V_{ss} \cdot \sum_i \beta_i \cdot \lambda_i^2 \cdot \exp(-\lambda_i \cdot t) \quad (10)$$

$$\int_0^{V_{ss}} t \cdot dV_0 = \int_0^{\infty} t \cdot (dV_0/dt) \cdot dt \quad (11)$$

$$\int_0^{V_{ss}} t \cdot dV_0 = V_{ss} \cdot \int_0^{\infty} \sum_i \beta_i \cdot \lambda_i^2 \cdot t \cdot \exp(-\lambda_i \cdot t) \cdot dt \quad (12)$$

$$\int_0^{V_{ss}} t \cdot dV_0 = -V_{ss} \cdot [\sum_i \beta_i \cdot \lambda_i \cdot \exp(-\lambda_i \cdot t)(t + 1/\lambda_i)]_0^{\infty} \quad (13)$$

$$= V_{ss} \cdot \sum_i \beta_i \quad (14)$$

Hence τ_F is given by:

$$\tau_F = 1/V_{ss} \cdot \int_0^{V_{ss}} t \cdot dV_0 = \sum_i \beta_i \quad (15)$$

This is the characteristic or transient time for the initial enzyme of the pathway. This will normally be in the microsecond-to-millisecond range and as such will be negligible compared with the pathway transient, which will commonly be in the second-to-minute range. One may therefore conclude that τ_F, if significant, will be largely indicative of the effect of feedback on the pathway temporal response.

Transition between steady states

So far, only the establishment of steady states from rest has been considered. A more realistic model of the behaviour of a pathway *in vivo* is to consider the transition between steady states. This may be done in a manner similar to that described previously (Easterby, 1981). If at time $t = 0$ a transition begins from steady state (*a*) to steady state (*b*), then the following relationship holds:

$$\int_0^t V_0 \cdot dt = \Delta[P] + \Delta[I] + \Delta[EI] \quad (16)$$

Where:

$$\Delta[P] = [P]_{t=t} - [P]_{t=0} \quad (17)$$

$$\Delta[I] = [I]_{t=t} - [I]_{t=0} \quad (18)$$

$$\Delta[EI] = [EI]_{t=t} - [EI]_{t=0} \quad (19)$$

Similarly:

$$V_b \cdot t - \int_{V_a}^{V_b} t \cdot dV_0 = \Delta[P] + \Delta[I] + \Delta[EI] \quad 20)$$

$$[P] = V_b \cdot (t - \Delta[I]/V_b - \Delta[EI]/V_b - 1/V_b \cdot \int_{V_a}^{V_b} t \cdot dV_0) \quad (21)$$

$$[P] = V_b \cdot [t - (\tau_{Ib} - V_a/V_b \cdot \tau_{Ia}) - (\tau_{EIb} - V_a/V_b \cdot \tau_{EIa}) - (1/V_b \cdot \int_0^{V_b} t \cdot dV_0 - 1/V_b \cdot \int_0^{V_a} t \cdot dV_0)] \quad (22)$$

$$[P] = V_b \cdot [t - (\tau_{Ib} - V_a/V_b \cdot \tau_{Ia}) - (\tau_{EIb} - V_a/V_b \cdot \tau_{EIa}) - (\tau_{Fb} - V_a/V_b \cdot \tau_{Fa})] \quad (23)$$

$$[P] = V_b \cdot [t - (\tau_b - V_a/V_b \cdot \tau_a)] = V_b \cdot (t - \tau) \quad (24)$$

where:

$$\tau = \tau_b - V_a/V_b \cdot \tau_a \quad (25)$$

and:

$$\tau_a = \tau_{Ia} + \tau_{EIa} + \tau_{Fa}, \tau_b = \tau_{Ib} + \tau_{EIb} + \tau_{Fb} \quad (26)$$

and:

$$\tau_{Fa} = 1/V_a \cdot \int_0^{V_a} t \cdot dV_0, \tau_{Fb} = 1/V_b \cdot \int_0^{V_b} t \cdot dV_0 \quad (27)$$

where V_a and V_b represent the rates of input in states (*a*) and (*b*) respectively.

Eqn. (25) is quite general and represents the transition between any two steady states. The transient for the transition between steady states may be readily estimated from the transients for the establishment of each steady state from rest. An important consequence of this is that only a limited number of steady states need be considered and this will allow the calculation of the transient

Table 2. Example of the estimation of a feedback transient by the simulation of glycolysis in heart

Measured input parameter	Value
Hexokinase capacity (V_{Hk})	2.7_3 units/ml
Phosphofructokinase capacity (V_{Pfk})	40 units/ml
K_m of hexokinase for ATP (K_{ATP})	0.7 mM
K_i of hexokinase for ADP ($K_{i,ADP}$)	2 mM
K_i of hexokinase for glucose 6-phosphate ($K_{i,G6P}$)	20 μM
$[S]_{0.5}$ of phosphofructokinase for fructose 6-phosphate ($[F6P_{0.5}]$)	0.46 mM
Hill coefficient of phosphofructokinase for fructose 6-phosphate (n)	2.1_6
[ATP]	5.5 mM
[ADP]	2.3 mM
Equilibrium constant of phosphoglucose isomerase ($K_{eq.}$)	0.26
Rate of glycogenolysis (V_G)	0.33 mM/min

Steady-state parameter	Value	
	Measured	Result of simulation
Glycolytic rate (mM/min)	1.12	1.12
Hexokinase rate (mM/min)	0.79	0.79
[G6P] (μM)	340	340
[F6P] (μM)	90	90
τ_I (s)	23.2	23.1
τ_F (s)		-7.3_8
$\tau_{overall}$ (s)		15.7_2

responses of the pathway in passage between any pair of these states. It is not therefore necessary to analyse in detail all possible transitions.

Example of the application of the method to the measurement of the effects of feedback

This type of analysis should help to define the time scale on which pathways operate rather than the more common approach of defining their flux. As an example, an analysis of the glycolytic transient in erythrocytes (Rapoport *et al.*, 1974) demonstrated the importance of the reactions from hexokinase to phosphofructokinase to both the control and the transient response of the pathway. However, this analysis was unable to take full account of the effect of feedback inhibition of hexokinase by glucose 6-phosphate or the more general equations defining the intermediate-associated transients (Easterby, 1981). A similar study of the pathway in heart muscle has confirmed the dominance of these reactions in the pathway temporal response, but has additionally shown that feedback can reduce the pathway transient substantially and, for example, facilitate the transition of the pathway between normoxic and hypoxic states (G. Frodsham & J. S. Easterby, unpublished work). An example of the approach is given here.

In order to investigate the early part of the glycolytic pathway, the reactions from hexokinase to phosphofructokinase were computer-simulated. The following equations representing the rates of change of intermediate concentrations were integrated by a fourth-order Runge–Kutta procedure and the feedback-associated transient due to the inhibition of hexokinase by glucose 6-phosphate was evaluated by eqn. (30):

$$V_0 = \frac{V_{Hk}[ATP]}{K_{ATP}(1+[G6P]/K_{i,G6P}+[ADP]/K_{i,ADP})+[ATP]} + V_G \tag{28}$$

$$d[G6P+F6P]/dt = \frac{V_{Hk}[ATP]}{K_{ATP}(1+[G6P]/K_{i,G6P}+[ADP]/K_{i,ADP})+[ATP]} + V_G - \frac{V_{Pfk}[F6P]^n}{[F6P_{0.5}]^n+[F6P]^n} \tag{29}$$

$$\int_0^{V_{ss}} t\cdot dV_0 = \frac{1}{1+K_{eq.}}\int_0^{\infty} t\cdot(dV_0/d[G6P])\cdot(d[G6P+F6P]/dt)\cdot dt \tag{30}$$

The meanings of the symbols and the numerical values assigned to them are given in Table 2. The first term in the description of V_0 in eqn. (28) allows for glucose 6-phosphate and ADP inhibition of heart hexokinase (E. E. Farmer & J. S. Easterby, unpublished work). n represents the best estimate of the Hill coefficient of heart phosphofructokinase measured *in vitro* under '*in vivo*' conditions of pH, ionic environment and nucleotide

concentration as determined by ^{31}P n.m.r. V_G represents the measured rate of glycogenolysis in the perfused heart. For the purposes of simulation, phosphoglucose isomerase was assumed to be at equilibrium. This was justified on the basis of measured glucose 6-phosphate and fructose 6-phosphate concentrations in the perfused heart, and the assumption was made to speed to integration process and avoid unstable solutions. However, the result was not materially affected if no assumption of equilibrium was made. $K_{eq.}$ represents the equilibrium constant. In the analysis it was assumed that the individual enzymes maintained their steady states. This assumption relies on the fact that the characteristic times of the enzymes are much less than the transient times of the pathway. However, even if this were not the case, the estimates of transient times would be unaffected (Easterby, 1981).

It can be seen from the data in Table 2 that the effect of feedback on hexokinase is to reduce substantially the overall transient time of this section of the pathway. About a 30% reduction in transient time was attributable to feedback and, owing to the self-regulating nature of the feedback, this percentage was relatively insensitive to change in the kinetic parameters of the enzymes. For the purposes of simulation a value of 2.7_3 units/ml was used for the intracellular hexokinase capacity. The measured value was actually 12 units/ml, but at this level it was impossible to achieve a sufficiently low glycolytic flux. This suggests that only a small proportion of the hexokinase capacity is expressed *in vivo*. It is relevant to note that the major part of the hexokinase in heart is bound to the mitochondrion, and only a small proportion is cytosolic. It is therefore tempting to speculate that the enzyme is compartmentalized in the cell.

DISCUSSION

It has been shown that any sequence of consecutive enzyme-catalysed reactions will exhibit a transient or lag time, which is the simple sum of three components. Two of these are associated with the accumulation of intermediates within the sequence and are functions of state. The third is associated with variation in the rate of input of material to the sequence. In the case of a metabolic pathway the third term will generally be associated with feedback on the first enzyme or boundary of the pathway. In the case of transition between steady states, the description of the transient is still applicable, but each transient must be modified to allow for the flux already established in the initial steady state. The feedback transient is actually a consequence of the acceleration or deceleration of the pathway and, in the case of negative feedback, will help to establish the pathway in its steady state more rapidly, thereby improving its responsiveness to altering metabolic requirements.

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Received 3 June 1985/23 September 1985; accepted 10 October 1985