The analysis of metabolite channelling in multienzyme complexes and multifunctional proteins

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Multienzyme complexes and multifunctional proteins may confer a kinetic advantage by channelling reaction intermediates between consecutive enzymes and reducing the transient time for the establishment of steady states. A general means for quantitatively assessing the contribution of channelling to the reduction of pool size and transient time is presented. Restrictions to the kinetic advantage are identified, and it is shown that no channelling advantage is obtained at high enzyme concentration or for enzymes which exhibit rapid-equilibrium kinetic behaviour.

INTRODUCTION

It has been recognized in the last few years that many pathways or consecutive enzyme sequences within the cell may be present in multienzyme complexes or multifunctional proteins (Reed & Cox, 1966; Yanofsky & Crawford, 1972; Welch & Gaertner, 1976; Nicholson *et al.*, 1987; Gontero *et al.*, 1988). Such complexes might confer a kinetic advantage to the cell by the direct transfer of intermediates between enzymes, without the need for release into the bulk phase of the cell and subsequent diffusion to the next enzyme of the sequence. In general, no flux advantage would result from this mechanism, except in the limited case where the direct transfer of substrate between two isolated or transiently interacting enzymes is being considered (Srivastara & Bernhard, 1986). In the more extended complex, the flux will generally be determined outside of any individual step in the pathway, and the kinetic advantage results from the reduction in the size of intermediate pools within the cell. In the case of direct transfer of metabolite between consecutive enzymes, there is no intermediate pool. In general, however, one may imagine that an intermediate pool exists within the complex, and the direct-transfer mechanism would be a limiting case in which this pool was reduced to zero. The internal pool can be of reduced size compared with the situation in which the enzymes and intermediates are freely diffusing, as the enzymes of the complex will see a higher, localized, concentration of the metabolite. Consequently, the transient time of the sequence is also reduced and hence the time it takes to approach a new steady state.

Despite the recognition that channelling may confer these advantages, there has been little attempt to make a quantitative kinetic analysis of the advantage in transient reduction. This is partly due to the perceived difficulty and complexity of such an analysis. Recently, Ovádi *et al.* (1989) have analysed the situation in a two-enzyme complex or in the case of two transiently interacting enzymes. However, their elegant analysis in terms of mean lifetimes of channelled and free intermediate is of limited general applicability as it assumes pseudo-first-order kinetic behaviour of the enzymes involved and is not easily extended to larger complexes and more complex kinetic behaviour.

The present paper describes an approach to channelling based on a method of transient-time analysis proposed

previously (Easterby, 1981, 1986). It does not require the solution of differential equations for the chosen model and identifies possible constraints on the kinetic advantage of channelling.

RESULTS AND DISCUSSION

Scheme 1 describes the minimum model considered compatible with channelling of intermediates within a multienzyme complex or multifunctional protein. According to the Scheme, an enzyme releases its product, the intermediate I , either into an internalized pool within the complex (I_c) or into free solution in the bulk medium (I_f). The channelling process is therefore regarded as 'leaky', with fraction α of the steady-state flux entering the internal pool and fraction $(1 - \alpha)$ being released into the bulk phase. α is termed the 'channelling efficiency' at this stage in the enzyme sequence (Ovádi *et al.*, 1989). The next enzyme in the sequence can receive intermediate either from the internal pool or from the external pool and convert it into the next intermediate of the pathway with rate constant k_{cat} . Release of product from the enzymes is considered irreversible in this model in order to reduce the mathematical complexity, but in principle reversibility could be included at these stages. No direct exchange has been included between the internal and external pools, as this, if significant, would effectively destroy the channelling advantage.

Scheme 1. Channelling of an intermediate within a multienzyme complex or multifunctional protein

I represents an intermediate in the pathway. The upper route represents the passage of channelled intermediate within the internalized pool. The lower route represents the path of unchannelled intermediate leaking into the bulk phase. I_c and I_f represents channelled and free-solution intermediate respectively. EI is the enzyme-intermediate complex, E being the next enzyme in the sequence for which I is substrate. Kinetic constants and coefficients are described in the text.

For the purpose of this analysis, the transient time has been taken as the intercept on the time axis of the steady-state asymptote to the curve of pathway product formation with time (Easterby, 1981). For a system of the sort shown in Scheme 1, the transient time is given by the ratio of pool sizes to flux (Easterby, 1981). Therefore simple analysis of the steady-state kinetic behaviour of the system, without solution of differential equations, allows comparison of the transient time for the channelled and non-channelled routes. Associated with each metabolite pool is a transient time which is the lifetime of the pool or the time required for the pool to turn over in the steady state. These transients are additive in determining the total transient response of the system (Easterby, 1981). Variation or fluctuation in the rate of input of material to the pathway, during the approach to the steady state, adds an additional term to the description of the pathway transient. This will alter the pathway transient, but will not alter the transient times associated with the intermediate pools or the kinetic advantage conferred by channelling (Easterby, 1986).

In the present system it is necessary to write rate equations for each of the metabolite pools and set them equal to zero in the steady state. In this way the pool sizes are determined and the associated transient times are defined. In Scheme 1 the total transient time (τ) associated with intermediate I is given by:

$$\tau = ([I_c] + [I_f] + [EI])/J \quad (1)$$

where steady-state concentrations are implied and J represents the steady-state flux through the pathway. In this analysis intermediate concentrations are averaged over the total system volume. Therefore $[I_c]$ in eqn. (1) represents the mean concentration of channelled intermediate averaged over the total volume and not the local concentration within the enzyme complex. In the steady state, the pool sizes may be estimated by solution of the appropriate rate equations:

$$d[I_c]/dt = d[I_f]/dt = d[EI]/dt = 0 \quad (2)$$

The result of this analysis is:

$$\tau = 1/k_{cat.} + [\alpha \cdot k_{cat.}/k_c + (1-\alpha)k_{cat.}/k_f + k_{-c}/k_c + k_{-f}/k_f]/(V-J) \quad (3)$$

Eqn. (3) may be expressed in an alternative form:

$$\tau = \alpha \cdot \tau_c + (1-\alpha) \cdot \tau_f \quad (4)$$

where τ is the total transient associated with this stage in the sequence, and τ_c and τ_f represent the transient times associated with the wholly channelled (upper) and free solution (lower) routes of Scheme 1 respectively. τ_c is the value of τ obtained from eqn. (3) when α is unity; τ_f is the value of τ when α is zero. It should be noted that, even by the channelled route, intermediate will still be able to enter the free-solution pool by dissociation of the EI complex. This is necessary, as free-solution intermediate must be able to find its way back into the complex for further metabolism if it is not to accumulate. In the very restricted case where there is an alternative fate possible for the free-solution metabolite, re-entry into the complex may not be essential. Similarly, even by the free-solution route, intermediate can enter the internalized pool by dissociation of the EI complex.

The transient time for the free solution (τ_f) and channelled (τ_c) routes are given by:

$$\tau_f = 1/k_{cat.} + K_m/(V-J) + {}^cK_s/(V-J) \quad (5)$$

$$\tau_c = 1/k_{cat.} + {}^cK_m/(V-J) + K_s/(V-J) \quad (6)$$

where cK_s is the dissociation constant of intermediate from the EI complex into the channelled pool, K_s is the dissociation constant into the bulk phase and cK_m is the Michaelis constant of the channelled intermediate.

The definitions of the Michaelis and dissociation constants are as follows:

$$K_m = (k_{-f} + k_{cat.})/k_f \quad (7)$$

$${}^cK_m = (k_{-c} + k_{cat.})/k_c \quad (8)$$

$$K_s = k_{-f}/k_f \quad (9)$$

$${}^cK_m = k_{-c}/k_c \quad (10)$$

It can be seen that eqns. (5) and (6) each comprise three terms. The first is the lifetime or turnover time of the enzyme-substrate complex. The second is the time associated with the generation of the pool of intermediate either in free solution (eqn. 5) or within the complex (eqn. 6). The third term represents the time associated with the complex (eqn. 5) or in the bulk phase (eqn. 6) by simple dissociation of the enzyme-substrate complex on a branch to the main pathway flux.

The kinetic constant either superscripted or subscripted with c in eqns. (7)–(10) refer to processes involving channelled intermediate, those superscripted or subscripted with f, or without superscript or subscript, refer to processes involving intermediate in the bulk phase. The expression for the overall transient time, τ , derived from eqns. (3) or (4) may be expressed as follows:

$$\tau = 1/k_{cat.} + [K_m + {}^cK_s + \alpha({}^cK_m - {}^cK_s + K_s - K_m)]/(V-J) \quad (11)$$

This equation represents the transient associated with a single intermediate in the pathway. An analogous transient will be associated with each intermediate. The total pathway transient time, in the absence of variation in the rate of input to the pathway during the transient phase, will be the sum of the individual intermediate transients.

If any improvement in the transient response is due to the channelling process rather than to conformational and consequent kinetic changes in the enzymes on forming the complex, then eqn. (11) may be simplified. The advantage due to channelling will be the result of the higher local intermediate concentration within the complex and will result in an apparent increase in the 'on-constant' for the formation of enzyme-intermediate complex relative to the free-solution behaviour, without any change in the 'off-constant'. The increase in on-constant results from the greater probability of an encounter between enzyme active centre and intermediate within the complex and should therefore affect Michaelis and dissociation constants equally. The off-constant is unaffected, and therefore k_{-c} and k_{-f} are identical. The factor by which the on-constant, k_c , is increased in termed the 'channelling advantage' and given the symbol β . β is given by:

$$\beta = k_c/k_f = K_m/{}^cK_m = K_s/{}^cK_s \quad (12)$$

The transient time may now be written in terms of the

free-solution kinetic constants, the channelling efficiency, α , and the channelling advantage, β :

$$\tau = 1/k_{\text{cat.}} + [K_m + K_s/\beta + \alpha(K_m/\beta - K_s/\beta + K_s - K_m)]/(V - J) \quad (13)$$

Similarly the definitions of τ_f and τ_c become:

$$\tau_f = 1/k_{\text{cat.}} + K_m/(V - J) + (K_s/\beta)/(V - J) \quad (14)$$

$$\tau_c = 1/k_{\text{cat.}} + (K_m/\beta)/(V - J) + K_s/(V - J) \quad (15)$$

An alternative interpretation of the channelling advantage, β , can be given in terms of the volumes of the metabolite pools internal to, and external to, the complex. If the kinetic model is analysed in terms of local rather than average concentration of intermediate, then k_c and k_r can also be considered identical and β represents the ratio of internal to external metabolite concentration. This is equivalent to the ratio of system volume to internal pool volume.

For a significant difference to exist between the transient times for the free-solution and channelled routes and for channelling to represent a kinetic advantage, τ_c must be much less than τ_f . From eqns. (5), (6), (14) and (15) the following inequalities must hold:

$$^cK_m + K_s \ll K_m + ^cK_s \quad (16)$$

$$K_s - ^cK_s \ll K_m - ^cK_m \quad (17)$$

$$K_s[1 - (1/\beta)] \ll K_m[1 - (1/\beta)] \quad (18)$$

Hence: $K_s \ll K_m \quad (19)$

$$k_{-f}/k_r \ll (k_{-f} + k_{\text{cat.}})/k_r \quad (20)$$

Hence: $k_{\text{cat.}} \gg k_{-f} \quad (21)$

This means that K_m must be greater than the dissociation constant of the enzyme-substrate complex and implies that enzymes exhibiting rapid-equilibrium kinetic mechanisms are unlikely to provide a kinetic advantage through channelling. This condition may also be expressed as the need for $k_{\text{cat.}}$ to be much greater than the off-constant of the enzyme-substrate complex. This means that the intermediate will be removed along the enzyme sequence before it can dissociate from the enzyme-substrate complex into free solution. Eqns. (5) and (6) indicate a further constraint on kinetic advantage due to channelling. If the enzyme concentration is increased at constant flux, V increases and the transients associated with free solution and channelled pools decrease (i.e. the second and third terms of the equations decrease). Both τ_f and τ_c then reach the same limiting value, namely $1/k_{\text{cat.}}$, the lifetime of the enzyme-substrate complex (Easterby, 1981). Thus at high protein concentration the free and channelled pools are minimized, and no kinetic advantage is obtained from channelling. Examination of eqns. (5) and (6) reveals that by high enzyme concentration we mean enzyme concentrations comparable with K_m or K_s , whichever is the greater. Eqn. (19) suggests that this will generally mean that enzyme concentration must be comparable with, or greater than, K_m .

To summarize, there is no kinetic advantage or reduction of transient time due to the channelling process if either of the following conditions obtain: (a) The enzyme has a rapid-equilibrium kinetic mechanism. This restriction only obtains for channelling efficiencies less than

unity, where exchange between free-solution intermediate and EI complex is necessary; (b) the enzyme concentration is high (i.e.: $[E_{\text{total}}] \geq K_m$).

Eqns. (11) and (13) also permit the determination of α and β . Eqn. (11) may be expressed as:

$$\alpha = [(\tau - 1/k_{\text{cat.}})(V - J) - ^cK_s - K_m] / (^cK_m - ^cK_s + K_s - K_m) \quad (22)$$

The alternative form using eqn. (13) is:

$$\alpha = [(\tau - 1/k_{\text{cat.}})(V - J) - (K_s/\beta) - K_m] / [K_m/\beta - (K_s/\beta) + K_s - K_m] \quad (23)$$

In practice β is likely to be large and therefore cK_s and cK_m are likely to be very small. Eqns. (22) and (23) then reduce to:

$$\alpha = [(\tau - 1/k_{\text{cat.}})(V - J) - K_m] / (K_s - K_m) \quad (24)$$

This is a more convenient form, as cK_s and cK_m are likely to be difficult to determine. It is equivalent to saying that β tends to infinity, and direct transfer of intermediate between enzyme active centres occurs. This has been the starting point for most previous analyses. Under these conditions the pool of channelled intermediate vanishes. Eqn. (24) can be further simplified if K_m is greater than K_s . The expression becomes:

$$\alpha = 1 - (\tau - 1/k_{\text{cat.}})(V - J)/K_m \quad (25)$$

Here there is no dissociation of the enzyme-substrate complex into the free solution pool, and the transient time for the channelled intermediate is simply the lifetime of the enzyme-substrate complex. All quantities in eqns. (24) and (25) refer to free-solution behaviour of the enzyme and should be more readily measurable. They represent the simplest starting point for the analysis of channelling efficiency.

An alternative use of the equations might be to estimate the channelling advantage, β . This may be done by using eqn. (23), providing an independent method is available for the determination of α . One such method would be to measure the isotopic dilution of exogenously added labelled intermediate (Lue & Kaplan, 1970; Matchett, 1974; Christopherson & Jones, 1980; Christopherson *et al.*, 1981).

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