Transient-time analysis of substrate-channelling in interacting enzyme systems

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The kinetics of dynamically interacting enzyme systems is examined, in the light of increasing evidence attesting to the widespread occurrence of this mode of organization *in vivo*. The transient time, a key phenomenological parameter for the coupled reaction, is expressed as a function of the lifetime of the intermediate substrate. The relationships between the transient time and the pseudo-first-order rate constants for the coupled reaction by the complexed and uncomplexed enzyme species are indicative of the mechanism of intermediate transfer ('channelling'). In a dynamically interacting enzyme system these kinetic parameters are composite functions of those for the processes catalysed by the complex and by the separated enzymes. The mathematical paradigm can be extended to a linear sequence of N coupled reactions catalysed by dynamically (pair-wise) interacting enzymes.

INTRODUCTION

In contrast with the older widely accepted notion that macromolecular components are freely dissolved within the cytoplasm, more and more evidence indicates that enzyme proteins associate specifically with each other and/or with various membranous and skeletal elements of the cell (Keleti *et al.*, 1977; Welch, 1977a, 1985; Masters, 1981; Clegg, 1984; Friedrich, 1984; Srere, 1984, 1987; Damjanovich *et al.*, 1986; Welch & Clegg, 1987). Moreover, the intracellular organization of enzymes is not a time-invariant property but may change with the metabolic status of the cell (Wilson, 1978; Keleti & Welch, 1984; Kurganov, 1985). The physiological advantages of the organized state have been emphasized and reviewed extensively in the literature.

First indications of the structural orderliness of enzyme sequences came with the isolation and characterization of such stable multienzyme complexes as the fatty acid synthase and α -oxo acid dehydrogenase systems. Now we find that organization is evident in all of the major metabolic pathways (Srere, 1987; Welch & Clegg, 1987). However, the state of association is frequently seen to be loose and transient, highly dependent on environmental parameters and regulatory factors. On the basis of studies *in vitro* it seems that dynamic (pair-wise) enzyme interactions represent a common (perhaps the major) form of enzyme organization *in vivo* (Srivastava & Bernhard, 1986), as opposed to the isolatable ('static') enzyme clusters found on occasion. Such transient (but metabolically specific) enzyme complexes endow a metabolic pathway with the usual advantages of the organized state, while providing physiological variability and flexibility for metabolic flow (Friedrich, 1984; Keleti, 1984; Kurganov, 1985).

An oft-used analytical test for the functionality of a given multienzyme aggregate is the capability of 'channelling' intermediate substrate/product. In many documented cases the organized state leads to observation of this phenomenon (Welch, 1977a; Srivastava & Bernhard,

1986; Srere, 1987). Kinetically this can be assessed by observation of a decrease in the steady-state transient time, compared with the corresponding non-interacting coupled enzyme reaction (Hess & Wurster, 1970; Easterby, 1973, 1981, 1984; Welch, 1977b; Bartha & Keleti, 1979). Notionally, the transient time is that required for diffusion and accumulation of intermediates to concentrations necessary to sustain a given steady-state flux. This key temporal quantity has a well-known operational (and mathematical) meaning for the 'static' case, wherein the organizational state of the enzyme does not change.

Viewing the relative abundance of known multienzyme systems exhibiting dynamic organization, we extend the quantitative representation of the transient-time concept to include this regime. We give a general phenomenological description of the transient time, using standard kinetic parameters, which allows a mechanistic characterization of channelling in 'statically' as well as 'dynamically' interacting enzyme systems.

THEORY

The phenomenological form of the bulk-phase transient time

Let us consider the scheme shown in Scheme 1, involving enzymes E_1 and E_2 and metabolites S, I and P, which is often used in analysis of steady-state transient time (Easterby, 1973, 1984). For a system of two non-interacting enzymes the intermediate, I, exists in solution for a time, lasting from its generation (catalysed by E_1) until its enzymic conversion into P (catalysed by E_2). The

Scheme 1.

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lifetime, T , characteristic of the whole population of I molecules, can be obtained as the following average:

$$\langle T \rangle = \int_0^\infty t \cdot I(t) \cdot dT / \int_0^\infty I(t) \cdot dT \quad (1)$$

where $I(t)$ is the number density of I as a function of time, t . This lifetime includes the time required for release from E_1 , diffusional transit and binding to (and conversion by) E_2 .

The reciprocal value of the lifetime is the probability for an intermediate molecule to be converted into product within unit time. Hence the number density of I at any time is given by solution of the following equation (see Easterby, 1981):

$$dI/dt = v - [I]/\langle T \rangle \quad (2)$$

with the generation of I by enzyme E_1 proceeding at a constant rate, v . Since $I = 0$ at $t = 0$, one obtains:

$$I = v \cdot \langle T \rangle \cdot (1 - e^{-t/\langle T \rangle}) \quad (3)$$

and at steady state (i.e. $t \rightarrow \infty$):

$$I_{ss} = v \cdot \langle T \rangle \quad (4)$$

For a system of non-interacting enzymes, the average (bulk-phase) lifetime $\langle T \rangle$ is identifiable with the well-known steady-state transient time, τ (Hess & Wurster, 1970; Easterby, 1973, 1981, 1984; Heinrich & Rapoport, 1975).

The validity of the foregoing treatment rests on the non-interacting character of the bienzyme scheme, such that the same lifetime value $\langle T \rangle$ can be applied uniformly to the entire population of I molecules. However, a system of interacting enzymes does not meet this condition.

Now, let us consider the situation for a bienzyme complex (which in principle may be 'static' or 'dynamic'). We suppose that this complex is capable of 'channelling', partially or completely, the flux of substance I , according to the scheme shown in Scheme 2. Quantitatively, then, we stipulate that a fraction, α , of the intermediate molecules (I) produced by E_1 in the E_1E_2 complex is converted directly into P by E_2 within the same complex. Therefore we denote α as a transfer efficiency. The number density of such 'internalized' intermediates (or E_1E_2 'channelling' complexes) is termed I_i , and the associated lifetime is $\langle T_i \rangle$. The remaining ('externalized') fraction $(1 - \alpha)$ of I molecules, converted into P by enzyme E_2 not aggregated to the same E_1 that generated the given I , has a lifetime $\langle T_e \rangle$ and number density I_e . It is assumed that I_i and I_e are generated simultaneously and, as a first approximation in this initial model, that the unitary rate constants are all the same after rebinding as if no dissociation of I occurred. (Any effects of enzyme-enzyme interaction on the actual rebinding are included implicitly in our kinetic

analysis; see below under 'Relationship among macroscopic kinetic parameters in an interacting system'.) The lifetime $\langle T_e \rangle$ involves the time required for diffusion, which is roughly proportional to the square of the distance between the active sites of E_1 and E_2 (Welch, 1977a). Since the site-to-site distance within the bienzyme complex (for I_i) is shorter, as compared with that between E_1 and E_2 in different complexes (for I_e), we assume the condition $\langle T_i \rangle < \langle T_e \rangle$ for the first-order scheme considered herein (with $I_i + I_e = I_T \ll K_m^{E_2}$). This condition is consistent with experimental results on many known enzyme complexes [reviewed in Srere (1987), Welch (1977a,b) and Welch & Clegg (1987)]. (Although, some internal transfer mechanisms could be slow enough to invalidate this condition.)

Then, we arrive at the following equations for I_i and I_e :

$$dI_i/dt = \alpha \cdot v - I_i/\langle T_i \rangle \quad (5)$$

$$dI_e/dt = (1 - \alpha) \cdot v - I_e/\langle T_e \rangle \quad (6)$$

If at $t = 0$ $I_i = I_e = 0$, we have:

$$I_i = \alpha \cdot v \cdot \langle T_i \rangle \cdot (1 - e^{-t/\langle T_i \rangle}) \quad (7)$$

$$I_e = (1 - \alpha) \cdot v \cdot \langle T_e \rangle \cdot (1 - e^{-t/\langle T_e \rangle}) \quad (8)$$

The total of intermediates, I_T , is the sum of I_i and I_e :

$$I_T = v \cdot [\alpha \cdot \langle T_i \rangle \cdot (1 - e^{-t/\langle T_i \rangle}) + (1 - \alpha) \cdot \langle T_e \rangle \cdot (1 - e^{-t/\langle T_e \rangle})] \quad (9)$$

As $t \rightarrow \infty$, I_T tends to the steady-state value:

$$I_{T|t \rightarrow \infty} = I_{ss} = v \cdot [\alpha \cdot \langle T_i \rangle + (1 - \alpha) \cdot \langle T_e \rangle] \quad (10)$$

with product formation proceeding at constant velocity, such that:

$$P(t)|_{t \rightarrow \infty} = v \cdot t - I_{ss} \quad (11)$$

Extrapolating this linear form to the time axis yields the observed transient time:

$$\tau_{obs.} = \alpha \cdot \langle T_i \rangle + (1 - \alpha) \cdot \langle T_e \rangle \quad (12)$$

We have the following two extreme cases.

(i) $\alpha = 0$. No intermediate I molecules are converted into P within the confines of the bienzyme complex. The observed transient time equals $\langle T_e \rangle$. Consequently the system behaves as a non-interacting (homogeneous) solution of E_1 and E_2 .

(ii) $\alpha = 1$. Every nascent intermediate I molecule is converted into P within the E_1E_2 complex. Then the observed transient time is just $\langle T_i \rangle$. This circumstance is here denoted as 'perfect channelling'.

In general, α may be between 0 and 1, such that:

$$\langle T_i \rangle \leq \tau_{obs.} \leq \langle T_e \rangle \quad (13)$$

The decrease in transient time in interacting enzyme systems may be due either to the presence of physical barriers on the out-diffusion of the intermediate I or to the mere juxtaposition of sequential active sites. The physiological relevance thereof has been elaborated by many workers [reviewed in Friedrich (1984), Keleti (1984), Srere (1987), Welch (1977a,b) and Welch & Clegg (1987)]. Any alteration in the interacting system, relating either to the diffusional process or to the enzymic/kinetic parameters, will be manifest by changes of α , $\langle T_i \rangle$ and $\langle T_e \rangle$. {It should be noted that $\langle T_e \rangle$ decreases with increasing $[E_2]_T$, whereas $\langle T_i \rangle$ (being an intrinsic parameter for the enzyme complex) is independent of enzyme concentration. At extremely high concentration (which

Scheme 2.

occurs exceptionally) $\langle T_e \rangle$ may approach the value $\langle T_i \rangle$ (Tompá *et al.*, 1987). In the next section we discuss the analytical implications.

Relationship among macroscopic kinetic parameters in an interacting system

If we consider Scheme 1, for a non-interacting bulk-phase system, under conditions whereby the first reaction proceeds at constant velocity and the concentration of intermediate I is low enough for the second reaction to be first order, then the progress curve of product formation (with $\alpha = 0$) can be described as follows:

$$P(t) = v \cdot t - v \cdot \tau (1 - e^{-t/\tau}) \quad (14)$$

where v is the steady-state velocity of the overall reaction catalysed by the two-enzyme sequence (cf. eqn. 11). In non-interacting systems v is just the activity of free E_1 ($v = v_{E_1}$) and τ is inversely proportional to the concentration of E_2 (Hess & Wurster, 1970). If $[E_2] \ll K_m^{E_2}$ (Bartha & Keleti, 1979; Easterby, 1973, 1981), then:

$$\tau = K_m/V_{\max} = K_m/(k_{\text{cat}}[E_2]) = 1/k_r \quad (15)$$

where $V_{\max}$, K_m and k_{cat} all refer to E_2 . k_r is termed the 'kinetic power' (Keleti & Welch, 1984); for non-interacting systems it is determined in the coupled reaction simply as $1/\tau$. Here, k_r represents the pseudo first-order rate constant for the conversion $I \rightarrow P$ by E_2 .

If changes occur in the kinetic parameters of the reaction catalysed by E_2 and/or in the lifetime of the intermediate I, as the result of an interaction between enzymes E_1 and E_2 , this should be reflected in the two following inequalities: (i) the k_r values determined in the absence and in the presence of E_1 (adding I to the system in the absence of S), and (ii) the $1/\tau_{\text{obs}}$ value (determined in the coupled reaction catalysed by E_1 and E_2) and the k_r value (determined under pseudo-first-order conditions either in the absence or in the presence of E_1 , with the same enzyme concentration $[E_1]_T$ as in the coupled reaction). By determining the relationships among these parameters, one can delineate various possible mechanisms for molecular channelling in the enzyme aggregate. These possibilities, summarized in Table 1, are as follows.

Case 1. As a consequence of enzyme interaction, changes in kinetic parameters may result just from alterations in the conformations of the enzymes, without producing channelling of the intermediate I. In this case the steady-state velocity of the coupled reaction, v , may differ from the velocity of the reaction catalysed by E_1 . Moreover, the τ_{obs} value determined in the coupled reaction would not be equal to the reciprocal of the pseudo-first-order rate constant, $1/k_r$, of the individual reaction catalysed by E_2 . However, the rate constant $k_{r2}^{(1)}$ for the conversion $I \rightarrow P$ by E_2 in the presence of E_1 should equal $1/\tau_{\text{obs}}$ (Table 1, line 2). In this case the kinetic parameters determining τ_{obs} (see eqn. 15) change, as a result of interaction between the two enzymes.

Case 2. The intermediate I produced by free E_1 is bound with the same probability to free E_2 as to the E_1E_2 complex. The conversion $I \rightarrow P$ by E_2 proceeds with the same pseudo-first-order rate constant, both in the absence and in the presence of E_1 . Some of the nascent I molecules produced by complexed E_1 are channelled between E_1 and E_2 , owing to the juxtaposition of sequential active sites, resulting in the relationship between τ_{obs} and k_r shown in Table 1, line 3. In addition,

Table 1. Relationships of kinetic parameters determined in the coupled and uncoupled reactions

v_{E_1} is the velocity of the individual reaction catalysed by E_1 ; v is the steady-state velocity of the coupled reaction catalysed by E_1 and E_2 ; $1/\tau_{\text{obs}}$ is the reciprocal of the time intercept of the linear part of the [P]-versus-time plot; k_{r2} is the pseudo-first-order rate constant of the individual reaction catalysed by E_2 ; $k_{r2}^{(1)}$ is the same as k_{r2} but determined in the presence of E_1 .

Type of interaction	Parameters referring to the actions of enzymes E_1 and E_2	
	E_1	E_2
1. Non-interacting	$v_{E_1} = v$	$1/\tau_{\text{obs}} = k_{r2}^{(1)} = k_{r2}$
2. Interaction inducing conformational changes	$v_{E_1} \geq v$	$1/\tau_{\text{obs}} = k_{r2}^{(1)} \geq k_{r2}$
3. 'Leaky channel'	$v_{E_1} = v$	$1/\tau_{\text{obs}} > k_{r2}^{(1)} \leq k_{r2}$
4. 'Perfect channel'	$v_{E_1} = v$	$1/\tau_{\text{obs}} \rightarrow \infty$ $k_{r2}^{(1)} \rightarrow 0$

the steady-state velocity, v , for the coupled reaction equals the velocity v_{E_1} determined in the individual reaction. This phenomenon may be designated as a 'leaky channel' (whereby $0 < \alpha < 1$). If escape of I molecules from the complex is impeded to some degree, a decrease of τ_{obs} and $k_{r2}^{(1)}$ with respect to k_{r2} is observable. This relationship among kinetic parameters, determined in the coupled and in the individual reactions, is indicative of the efficiency of the channel.

Case 3. This is the extreme case, whereby the intermediate I is quantitatively converted into P by E_2 before leaving the complex (i.e. $\alpha = 1$). This phenomenon we denoted above as a 'perfect channel'; its kinetic characteristics are found in Table 1, line 4. Of course, any intermediary mechanism between 'leaky' and 'perfect' channels may exist; such can be characterized by the relationship among the parameters $1/\tau_{\text{obs}}$, k_{r2} and $k_{r2}^{(1)}$.

The foregoing considerations are not generally valid if changes in the intrinsic value of K_m and/or k_{cat} arise as a result of interaction of the two enzymes (Table 1, line 2). In this case $k_{r2}^{(1)}$ may be even greater than k_{r2} and, consequently, the change in τ_{obs} as defined in eqn. (15) should be considered.

Dynamically interacting enzyme systems

In the most general case one must allow for the possibility that the interacting enzymes do not exist as a physical aggregate all the time (i.e. the complex is dissociable). Therefore the observed kinetic parameters would be weighted sums of parameters for the interacting and the non-interacting enzyme species:

$$k_{\text{obs}} = (k_{\text{free}}[E]_{\text{free}} + k_{\text{complexed}}[E]_{\text{complexed}})/[E]_T \quad (16)$$

$$1/\tau_{\text{obs}} = ([E]_{\text{free}}/\tau + [E]_{\text{complexed}}/\tau')/[E]_T \quad (17)$$

where τ' and τ denote the transient time for the complexed and free states respectively. An important consequence of this weighting comes from the condition that $k_{r2} = k_{r2}^{(1)} = 1/\tau_{\text{obs}}$ for the non-interacting regime (Table 1, line 1). That is, the relationship among parameters for the complexed portion of enzymes E_1 and E_2 will determine relationships among the observed parameters. Thus for any state of interaction the relationships in Table 1, lines 2–4, will hold.

If the equilibrium dissociation constant, K_c , for the enzyme interaction $E_1 + E_2 \rightleftharpoons E_1E_2$ is known, we can write eqn. (17) as follows:

$$1/\tau_{\text{obs.}} = (1/\tau' - 1/\tau) \{ ([E_1]_T + [E_2]_T + K_c - \sqrt{([E_1]_T + [E_2]_T + K_c)^2 - 4[E_1]_T[E_2]_T}) / 2[E_2]_T \} + 1/\tau \quad (18)$$

This relation represents the generalized form of the familiar transient time for a two-enzyme system (Easterby, 1973). Under the constraint of the (irreversible) linear-kinetic approximation employed herein (and frequently used for analysis *in vitro*), eqn. (18) is completely interpolative. In the extreme case of a non-interacting enzyme system in bulk solution, we have $K_c = \infty$ and $\tau_{\text{obs.}} = \tau$, where τ is given by eqn. (15), whereas at the other extreme we have a 'static' bienzyme complex, with $K_c = 0$ and $\tau_{\text{obs.}} = \tau'$. The form of τ' would depend on the nature of the internal transfer of substance I in the intact complex I (and on the perfection of channelling; see eqn. 12).

Formally, the representation in eqn. (17) might be extended to a sequence of N dynamically (pair-wise) interacting enzymes (i.e. E_i with E_{i+1} , E_{i+1} with E_{i+2} etc.). If the enzymes in the sequence catalyse (quasi-)irreversible reactions rate-limited by an initial governing step, then the observed transient time $\tau_{\text{obs.}}$ may be calculated by a generalization of eqn. (17). From the detailed mathematical expressions of Easterby (1981), it is seen that the overall transient for a multienzyme sequence is the sum of the individual transient times for the intermediary steps. If the first enzyme is saturated and all others follow pseudo-first-order kinetics (i.e. all the $V_{\text{max.}}$ values in the system are greater than the driving velocity of the first reaction), then the transient time of each individual enzyme reaction in the sequence corresponds to that in eqn. (15) (Easterby, 1973, 1981). Accordingly, one arrives at the following expression:

$$1/\tau_{\text{obs.}} = \sum_{i=1}^{N-1} \{ [E_{i+1}]_{\text{free}} (1/\tau_i) + [C_i] (1/\tau'_i) \} / ([E_{i+1}]_{\text{free}} + [C_i]) \quad (19)$$

where C_i represents the complex between E_i and E_{i+1} , and τ_i and τ'_i are the transient times of the coupled reaction for E_i - E_{i+1} enzyme pairs when no complex exists or when catalysed by the complex C_i respectively.

Expressions such as those in eqns. (18) and (19) extend the usefulness of the transient time to the analysis of microcompartmentation in dynamic aggregates, allowing a characterization in terms of accessible experimental factors [e.g. K_m , $k_{\text{cat.}}$, enzyme concentration, enzyme-enzyme equilibrium dissociation constants (K_c)]. The applicability to actual enzyme systems has already met with experimental success (Keleti, 1984; Orosz & Ovádi, 1986; Ovádi, 1986).

We have yet to explore the consequences of relaxing the assumptions of thermodynamic irreversibility and first-order kinetics (cf. Hearon, 1981a,b). Explicit allowance must also be made for the possibility that substrate/product affinity and catalytic turnover may be functions of the state of enzyme association (cf. Nichol *et al.*, 1974).

Heretofore, most discussions of transient time in

organized multienzyme systems have been couched in rather simplistic empirical analysis, whereby an observation of a decrease in transient time is employed as a gross qualitative indication of 'channelling' in a given enzyme aggregate. Increasing awareness of the widespread occurrence of enzyme organization in cell metabolism, in conjunction with realization of the variform nature of the organizational state, demands that we pursue transient-time analysis with more exactitude. The transient time can be a useful kinetic window into both the mechanism of enzyme interaction and the degree of channelling of intermediate substrates. In the present treatment we have made an initial attempt to elaborate such usefulness for a simple bienzyme system under (pseudo-)first-order reaction conditions.

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