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How to determine the efficiency of intermediate transfer in an interacting enzyme system?

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A kinetic method, based upon measuring the transient time of coupled reactions, is proposed for the determination of the intermediate channel efficiency in a system of functionally interacting enzymes. The procedure rests upon a novel description in which the transient time is expressed as a function of channel efficiency and lifetime of the intermediate molecules. By this approach the reduction of transient time can be explained even if no changes in the kinetic parameters of the individual reactions occur. For determining channel efficiency, a linearized form has been evaluated and applied to the analysis of the kinetics of the aspartate aminotransferase-glutamate dehydrogenase coupled reaction, for which the data were taken from the literature [(1982) Eur. J. Biochem. 121, 511-517].

Enzyme interaction; Intermediate transfer efficiency; Channeling

1. INTRODUCTION

Channeling (i.e. direct transfer) of metabolites in enzyme clusters is often referred as a phenomenon probably playing an important role in metabolic economy and efficiency. The physiological advantages to an organized state capable of channeling over one unable to do so, are manifest in (i) segregation of competing pathways due to microcompartmentation of intermediates and in (ii) reduction of time required to reach the steady state (transient time) and enhancement in metabolite flux by providing high local metabolite concentration [2,3].

As a possible experimental indication of the channeling effect, reduction of transient time in a coupled reaction has long been accepted [1,4-8], however, no quantitative description of it has been presented when the kinetic parameters of the individual reactions were not altered due to the interaction.

The aim of this paper is to present a description of the transient time that takes into account the channeling effect, based on inherent parameters such as channel efficiency and intermediate lifetime. This approach makes it possible to provide a method for the quantitative determination of channel efficiency as well. Some of the properties of transient time came to light through this novel description, and the method is applied to the experimental data taken from [1], concerning the coupled reaction catalysed by aspartate aminotrasferase and glutamate dehydrogenase.

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2. THEORY

Let us assume, that enzymes E_1 and E_2 catalyze the consecutive irreversible conversion of S (substrate) to I (intermediate) and of I to P (product), according to the following scheme:

$$S \xrightarrow{E_1} I \xrightarrow{E_2} P$$

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In this enzyme system an intermediate molecule exists for a definite time, which includes the times required for release from E_1, diffusion (transit time) as well as for association to and conversion by E_2. Averaging the sum of these times for all molecules yields the characteristic lifetime of the whole population. In the case when the two enzymes form a heterologous complex, the intermediate population consists of (i) a fraction of the molecules which will be converted by the E_2 complexed to the same E_1 that generated them, due to the proximity effect of the active sites, and (ii) molecules consumed by an E_2 not complexed to the generating E_1. Each intermediate belongs to the latter if it is generated by a free E_1 or converted by a free E_2 or by a complex not identical with the generating one. The probability that an intermediate is converted within the generating complex is defined as channel efficiency (denoted α). Moreover, it seems obvious that the lifetime of the channeled (i.e. converted by the generating complex) intermediates ($\langle t \rangle'$) is shorter than that for the not-channeled ones ($\langle t \rangle$), since the average distance between active centres is markedly shorter within a complex than between separated enzyme molecules. In the theory we exploit the probabilistic meaning of the lifetime, i.e. that its reciprocal is a rate constant at which intermediates are converted. For sake of simplicity it is assumed that E_1 is saturated with S, hence generation of I proceeds at constant rate (v), and that the concentration of I is low enough to ensure that the reaction with E_2 is first order with respect to it. If the kinetic parameters of the individual enzymes are unaltered by the interaction, the concentrations of the channeled ($[I_i]$) and not channeled ($[I_e]$) intermediate molecules as a function of time can be obtained by solving the equations:

$$\frac{d[I_i]}{dt} = \frac{\alpha \cdot [C]}{[E_1]_t} \cdot v - \frac{[I_i]}{\langle t' \rangle} \quad (1)$$

$$\frac{d[I_e]}{dt} = \frac{[E_1]_f + (1-\alpha) \cdot [C]}{[E_1]_t} \cdot v - \frac{[I_e]}{\langle t \rangle} \quad (2)$$

where $[C]$, $[E_1]_f$ and $[E_1]_t$ represent the concentrations of the complexed, free and total E_1, respectively. It is worthy of note that $\langle t \rangle$ depends on enzyme concentration, since lifetime equals the reciprocal value of the rate constant of intermediate conversion ($1/k = K_{m,2}/k_{cat,2} \cdot [E_2]_t$, cf.[4]), while $\langle t' \rangle$ is concentration independent. It is also to be emphasized that division of the intermediate population into two parts can be accomplished as long as the velocities of the conversions of I_i and I_e are independent, namely when the concentration of intermediate is $\ll K_{m,2}$.

Solution (with boundary conditions $[I_i] = [I_e] = 0$ at $t = 0$) results in:

$$[I_i] = \frac{\alpha \cdot [C]}{[E_1]_t} \cdot v \cdot \langle t' \rangle \cdot (1 - e^{-t/\langle t' \rangle}) \quad (3)$$

$$[I_e] = \frac{[E_1]_f + (1-\alpha) \cdot [C]}{[E_1]_t} \cdot v \cdot \langle t \rangle \cdot (1 - e^{-(t/\langle t \rangle)}) \quad (4)$$

The concentration of the intermediate (both $[I_i]$ and $[I_e]$) approaches a constant level as $t \to \infty$, consequently the system reaches a steady state characterized by

$$[I]_{ss} = \frac{v}{[E_1]_t} \cdot \{\alpha \cdot [C] \cdot \langle t' \rangle + \\ + ([E_1]_f + \{1-\alpha\} \cdot [C]) \cdot \langle t \rangle\} \quad (5)$$

Since $[P]_{t \to \infty} = v \cdot t - [I]_{ss}$, by extrapolating the linear part of the progress curve of product formation, its intercept on the time axis is the apparent transient time:

$$\tau_{app} = \frac{1}{[E_1]_t} \cdot \{\alpha \cdot [C] \cdot \langle t' \rangle + \\ + ([E_1]_f + \{1-\alpha\} \cdot [C]) \cdot \langle t \rangle\} \quad (6)$$

In limiting cases this expression reduces: (i) if no complexation occurs in the system ($[C] = 0$, $[E_1]_f = [E_1]_t$) then

$$\tau_{app} = \langle t \rangle \quad (6a)$$

τ_{app} in this case equals $K_{m,2}/k_{cat,2} \cdot [E_2]_t$ if $[E_2]_t \langle\langle K_{m,2}$ (cf. [4,8]); (ii) if the complexation of E_1 by E_2 is complete ($[E_1]_t = [C], [E_1]_f = 0$),

$$\tau_{app} = \alpha \cdot \langle t' \rangle + (1-\alpha) \cdot \langle t \rangle \quad (6b)$$

For the determination of α and $\langle t' \rangle$, eqn (6) is rearranged:

$$\frac{\tau_{app} \cdot [E_1]_t - \langle t \rangle \cdot [E_1]_f}{[C]} = \alpha \cdot \langle t' \rangle + (1-\alpha) \cdot \langle t \rangle \quad (7)$$

The left-hand side of eqn (7) (denoted τ_{trans}) is a linear function of $\langle t \rangle$, and can be calculated with the knowledge of the dissociation constant of the heterologous enzyme complex. Plotting τ_{trans} against $\langle t \rangle$ yields a straight line, the slope of which is $1-\alpha$, while the intercept on the ordinate is $\alpha \cdot \langle t' \rangle$.

By measuring τ_{app} at various enzyme concentrations ensuring different degrees of complexation, the channel efficiency and lifetime of the channeled intermediates can be experimentally determined. However, before showing an example of this linearization, it is worthwile examining the properties of τ_{app} on the basis of this novel expression.

In contrast to the equation for the transient time for a non-interacting system in which only the concentration of a second enzyme is involved [4], here the concentration of the first enzyme appears as well. This substantial difference is understandable, considering that the transient time in an interacting system must depend upon the degree of complexation, which is determined by the concentration of both enzymes, and, naturally, by the dissociation constant.

By computing τ_{app} vs $[E_2]_t$ at various values of channel efficiency (fig.1a), a remarkable feature can be recognized, especially in the concentration range ensuring almost complete complexation. That is, that the relative reduction of the transient time is much more pronounced when α is close to 1, than when it is around zero. Considering those parts of the curves for which $[E_2]_t$ is above 10^{-4} M, an increase of α from 0 to 0.5, as well as from 0.9 to 0.95, causes a reduction of the same extent (2-fold) in τ_{app}, as indicated by the Δ marks in fig.1a. However, it is to be emphasized that this phenomenon is observable when $\langle t' \rangle$ is negligibly small compared to $\langle t \rangle$, a condition usually realized with dilute aqueous solutions, and probably for in vivo systems as well. Considering that intermolecular distances between enzymes or enzyme complexes are much greater than the distance between active sites in a complex, one can assume that for these systems $\langle t' \rangle \ll \langle t \rangle$.

Another feature of τ_{app} is revealed by plotting it against enzyme concentration at various $\langle t' \rangle$ values, keeping α constant (fig.1b). Since $\langle t \rangle$ decreases with increasing $[E_2]_t$ while $\langle t' \rangle$, being an internal parameter of the complex is independent

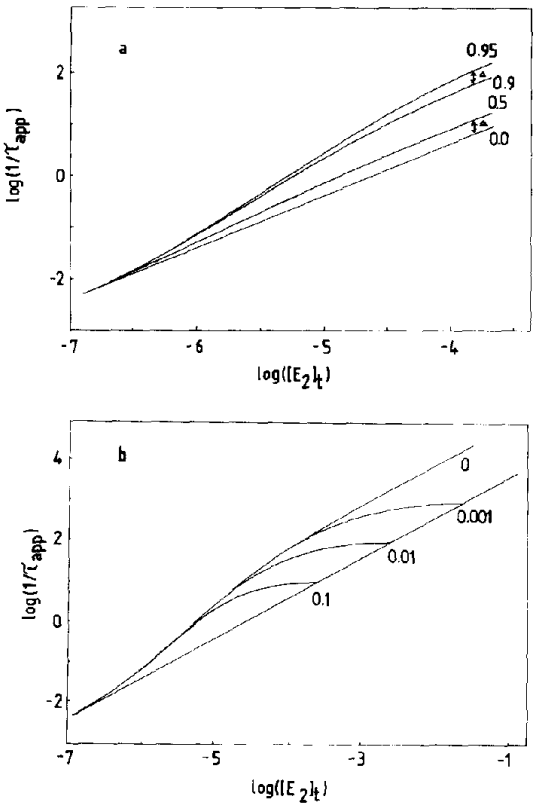

Fig.1. Theoretical curves of the dependence of the reciprocal transient time, $1/\tau_{app}$, on the concentration of E_2. Computations were performed according to eqn (6), using the following parameters: $[E_1]_t/[E_2]_t = 0.1$, $K_d = 1 \times 10^{-6}$ M, $K_{m,2} = 5 \times 10^{-4}$ M, $k_{cat} = 20$ s^{-1}. In (a) $\langle t' \rangle = 0$ and α is varied as indicated on the curves, and Δ indicates a 2-fold decrease, whereas in (b) α is kept constant (0.95), and $\langle t' \rangle$ is varied. A straight line is obtained when no interaction is assumed. All computations were performed with an Apple Macintosh computer.

of enzyme concentration, at extremely high concentrations $\langle t \rangle$ can approach or even reach $\langle t' \rangle$. This limiting concentration, where the transient time for the interacting and non-interacting systems are identical, depends on the numerical value of $\langle t' \rangle$ (fig.1b). However, such a high enzyme concentration may only exceptionally occur (e.g. glycosoma of *Trypanosoma brucei* [9]).

3. APPLICATION AND DISCUSSION

To demonstrate the applicability of the method described, transient time values for the aspartate

aminotransferase-glutamate dehydrogenase coupled reaction were taken from [1], where a deviation from linearity in plotting $1/\tau_{app}$ vs dehydrogenase concentration was observed, and conditions for the validity of our theory are fulfilled (neiter V_{max} of the transferase nor k_{cat} or K_m of the dehydrogenase is affected by the interaction, and the dehydrogenase reaction is first order with respect to the intermediate). In [1] the experimental points were fitted with a theoretical curve assuming perfect channeling of the intermediate 2-oxoglutarate. Since this model agrees with the special case of our new expression where $\alpha = 1$ and $\langle t' \rangle$ is negligibly small compared to $\langle t \rangle$ [In the calculations $\langle t' \rangle = 0$ was used, since solving eqns (1) and (2) in the limiting case of $\langle t' \rangle \rightarrow 0$ yields the same function obtained by giving the value of 0 to $\langle t' \rangle$ in eqn (6)], therefore the points can be fitted according to eqn (6), using the same dissociation constant ($K_d = 8.6 \times 10^{-6}$ M) of the enzyme complex as in [1] (fig.2). It is also seen, that with a considerably different dissociation constant ($K_d = 8.6 \times 10^{-7}$ M) the experimental points could not be fitted.

Linearization according to eqn (7) was also performed, using those points corresponding to

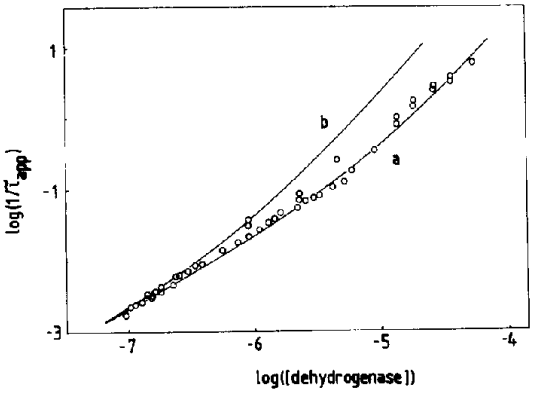

Fig.2. Relation between the reciprocal transient time, $1/\tau_{app}$, and the concentration of glutamate dehydrogenase. Data for the coupled reaction of aspartate aminotransferase and glutamate dehydrogenase are from the paper of Salerno et al. [1]. The theoretical curves are computed on the basis of eqn (6), with the following parameters: [transaminase]/[dehydrogenase] = 0.088, $K_{m,2} = 8 \times 10^{-4}$ M, $k_{cat} = 17\ s^{-1}$, $\alpha = 1$, $\langle t' \rangle = 0$, $K_d = 8.6 \times 10^{-6}$ M for (a), and the same but $K_d = 8.6 \times 10^{-7}$ M for (b).

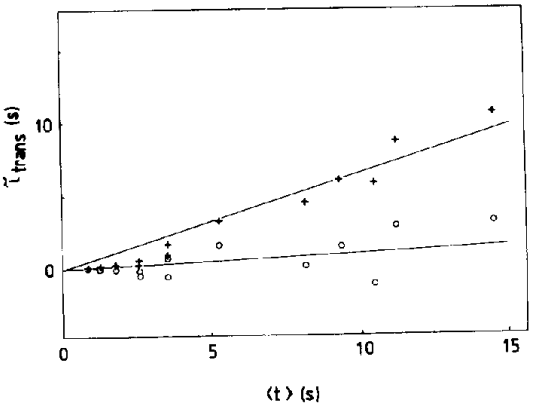

Fig.3. Linearization of the transient time for the aspartate aminotransferase-glutamate dehydrogenase coupled reaction. Data for glutamate dehydrogenase concentrations between 3×10^{-6} and 5×10^{-5} M are taken from fig.2. The transformation was performed as given by the left side of eqn (7) (τ_{trans}), with dissociation constants of 8.6×10^{-6} M (○) and 8.6×10^{-7} M (+). $\langle t \rangle$ was calculated as $K_{m,2}/V_{max}$ with $k_{cat,2} = 17\ s^{-1}$ and $K_{m,2} = 8 \times 10^{-4}$ M. The straight lines were obtained handdrawn, with the parameters $\alpha = 0.35$, $\alpha \cdot \langle t' \rangle = 0$ (upper line) and $\alpha = 0.9$, $\alpha \cdot \langle t' \rangle = 0$ (lower line).

glutamate dehydrogenase concentrations above 3×10^{-6} M (fig.3). Applying $K_d = 8.6 \times 10^{-6}$ M, points that could be fitted to a linear function of $\alpha = 0.9$ and $\langle t' \rangle = 0$ were obtained. This shows that the transformation introduced here yields a linear function in practice. By using the dissociation constant $K_d = 8.6 \times 10^{-7}$ M (fig.3), the points did not fit well to a linear function, indicating that the transformation yields points conforming to a straight line only if an appropriate dissociation constant is employed. Unfortunately no more data is available in the literature to further test the method. Nevertheless, this result, that a good fit could be achieved with a dissociation constant yielding α near to 1, may be regarded as limited experimental support of the idea developed in the following paragraph.

In section 2 it was rationalized that for dilute aqueous solutions, and probably even for in vivo systems, $\langle t' \rangle$ may be considerably shorter than $\langle t \rangle$. In this case, however, transient time is predominantly affected by channel efficiency and not by $\langle t' \rangle$. Since it was shown that reduction of transient time is most pronounced when α is near to 1, it means that $\alpha = 1$ endows a system with the

capability of reaching a new steady state faster than if α were considerably smaller than 1. In evolutionary terms it probably means that in order to reduce transient time, enzyme complexes have evolved to approach perfection in intermediate transfer (cf. [10]). Moreover, the flux rate of a pathway can be effectively modulated by changes in the complexation of the enzymes if α is near to 1.

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