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'Channelled' pathways can be more sensitive to specific regulatory signals

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In 'simple' metabolic pathways the response to an external signal is readily described in terms of the effect of the signal on its receptor enzyme and the control exerted by that enzyme. We show here that in the response of 'channelled' pathways to such a signal, additional terms appear that reflect the direct enzyme–enzyme interactions. They tend to enhance the responsiveness of the pathway. The normalized value of the response is called the signal transduction coefficient. We show that in channelled pathways these coefficients are usually larger than in corresponding non-channelled (simple) pathways.

Regulatory signal; Enzyme–enzyme interaction; Metabolic channelling; Control theory

1. INTRODUCTION

External effectors often play a role as signal molecules causing the system to modify its behaviour in order to meet altered environmental requirements. In order to understand the cell's regulatory structure it is important to realize how the response of the whole system is related to the 'local' response of affected reactions. In the present paper we show that the response to signals of 'channelled' pathways can differ drastically from the response of the corresponding non-channelled pathways.

The response of the flux (J) toward an external signal (effector) is quantified by the response coefficient, $R^J_{\sigma_i}$, defined as the log − log derivative of the steady-state flux (J) to the concentration (σ_i) of 'signal' molecules [1]:

$$R^J_{\sigma_i} = (\mathrm{d}J/J)/(\mathrm{d}\sigma_i/\sigma_i) = \mathrm{d}\ln|J|/\mathrm{d}\ln\sigma_i \qquad (1)$$

In simple pathways [2] a metabolic response to a signal is determined by the flux control coefficient ($C^J_{E_i}$), and by the elasticity coefficient ($\varepsilon^{v_i}_{\sigma_i}$) of the receptor ('target') enzyme (E_i) with respect to this signal [1]:

$$R^J_{\sigma_i} = \frac{\mathrm{d}\ln|J|}{\mathrm{d}\ln e_i} \cdot \frac{\partial \ln|v_i|}{\partial \ln\sigma_i} = C^J_{E_i} \cdot \varepsilon^{v_i}_{\sigma_i} \qquad (2)$$

Here e_i is the concentration of the receptor enzyme, v_i is the rate of the reaction catalyzed by e_i considered at constant concentrations of substrates and products. Eqn. 2 implies that regulatory molecules, σ_i, affect only one enzyme (E_i) in the pathway.

In metabolic pathways with enzyme–enzyme interactions Eqn. 2 is no longer valid. One of the reasons is lack of a one-to-one correspondence between the enzymes and the reactions, which is an obvious property of simple pathways [2]. In the present paper we derive a general expression for $R^J_{\sigma_i}$. We show how measuring the responses of a channelled pathway to external effectors (e.g. inhibitors) can enhance the insight into regulatory properties of the pathway (cf. [3]).

2. RESULTS AND DISCUSSION

In the companion paper [2] we have shown that pathways with enzyme–enzyme interactions may be treated in terms of control coefficients (C^J_{il}) with respect to the elemental processes (v_{il}). These processes correspond to transitions (cf. Figs. 1 and 2) between different enzyme subforms (states), or to sequences of such transitions that are not interrupted by branches:

$$C^J_{il} = (\mathrm{d}\ln|J|/\mathrm{d}\ln k_{il})/(\partial \ln v_{il}/\partial \ln k_{il}), \qquad (3)$$

In order not to change the equilibrium constant of the elemental process the condition k_{-il}/k_{il} = constant (k_{il} and k_{-il} are the forward and reverse rate constants of the process v_{il}) can be added [2]. Since definition 3 does not depend on the choice of the parameter if the latter affects only the rate v_{il} [4–6], this condition is not necessary. Although the numbering of the elemental processes may be arbitrary, here the designation implies the

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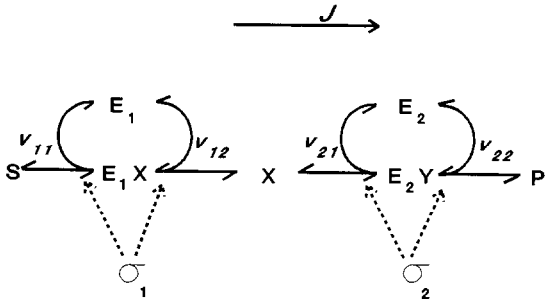

Fig. 1. 'Simple' pathway of two enzymes E_1 and E_2. The concentrations of the initial substrate, S, and the end product, P, are constants. X is the intermediate in the bulk phase. v_{11}, v_{12} and v_{21}, v_{22} are the rates of E_1- and E_2-dependent elemental processes, respectively. σ_1 and σ_2 are the signal molecules, specific to the receptor enzymes, E_1 and E_2, respectively. The positive direction of the flux J (from the substrate, S, to the product, P) is indicated.

subdivision of all the elemental processes in the network into the sets of E_i-dependent processes (v_{il}, $l = 1,2...$) corresponding to every of the pathway enzymes ($i = 1,2,...$). Note that some of the elemental processes depend both on E_i and E_j, if the enzymes E_i and E_j form a complex involved in catalytic transformations.

We can find the response of the flux to a signal specific to its receptor enzyme E_i via the control coefficients (C^J_{il}) of the E_i-dependent elemental processes (v_{il}) and their elasticities,

$$\varepsilon^{v_{il}}_{\sigma_i} = \partial \ln v_{il} / \partial \ln \sigma_i$$

with respect to the signal molecules present at concentration σ_i (the response theorem [7]):

$$R^J_{\sigma_i} = \sum_{\substack{\text{all} \\ E_i\text{-dependent} \\ \text{processes}}} C^J_{il} \cdot \varepsilon^{v_{il}}_{\sigma_i} \qquad (4)$$

Applying Eqn. 4 to the schemes of Figs. 1 and 2 one can see that for the channelled enzymes additional terms appear in Eqn. 4 in comparison to the unchannelled ones. For example, the flux responses to a signal (σ_1) affecting the enzyme E_1 in the simple (non-channelled) pathway (Fig. 1) and in the 'static' channel (Fig. 2A) are, respectively:

$$R^J_{\sigma_1} = C^J_{11} \cdot \varepsilon^{v_{11}}_{\sigma_1} + C^J_{12} \cdot \varepsilon^{v_{12}}_{\sigma_1} = C^J_{E_1} \cdot \varepsilon^{v_1}_{\sigma_1} \qquad (5)$$

and

$$R^J_{\sigma_1} = C^J_{11} \cdot \varepsilon^{v_{11}}_{\sigma_1} + C^J_{12} \cdot \varepsilon^{v_{12}}_{\sigma_1} + C^J_Q \cdot \varepsilon^{v_Q}_{\sigma_1} \qquad (6)$$

Obviously, the term $C^J_Q \cdot \varepsilon^{v_Q}_{\sigma_1}$ is absent in Eqn. 5 of the pathway of two unchannelled enzyme reactions. The analogous additional term $C^J_Q \cdot \varepsilon^{v_Q}_{\sigma_2}$ appears in the response $R^J_{\sigma_2}$ of the channelled flux to a signal σ_2 affecting the second enzyme E_2.

The flux responses for the 'dynamic' channel (Fig. 2B) are:

$$R^J_{\sigma_i} = C^J_{11} \cdot \varepsilon^{v_{11}}_{\sigma_i} + C^J_{12} \cdot \varepsilon^{v_{12}}_{\sigma_i} + C^J_{Q1} \cdot \varepsilon^{v_Q}_{\sigma_i} + C^J_{Q2} \cdot \varepsilon^{v_Q}_{\sigma_i} \qquad (7)$$

where $i = 1$ or 2 for signal molecules σ_1 or σ_2, respectively.

Eqns. 5–7 already suggest that the response of channelled pathways to signals differs from that of non-channelled ones. To compare the responses it is convenient to normalize them to the response of the receptor enzyme by itself. Accordingly, in the light of Eqns. 4–7 we define the 'signal transduction' coefficient of the enzyme E_i ($^RC^J_{E_i}$) as the ratio of the response of the whole pathway to the response of the 'isolated' enzyme:

$$^RC^J_{E_i} = R^J_{\sigma_i} / \varepsilon^{v_i}_{\sigma_i} = \sum_l C^J_{v_{il}} \cdot \varepsilon^{v_{il}}_{\sigma_i} / \varepsilon^{v_i}_{\sigma_i} \qquad (8)$$

In simple pathways the signal transduction coefficients coincide with the 'true' control coefficients (see

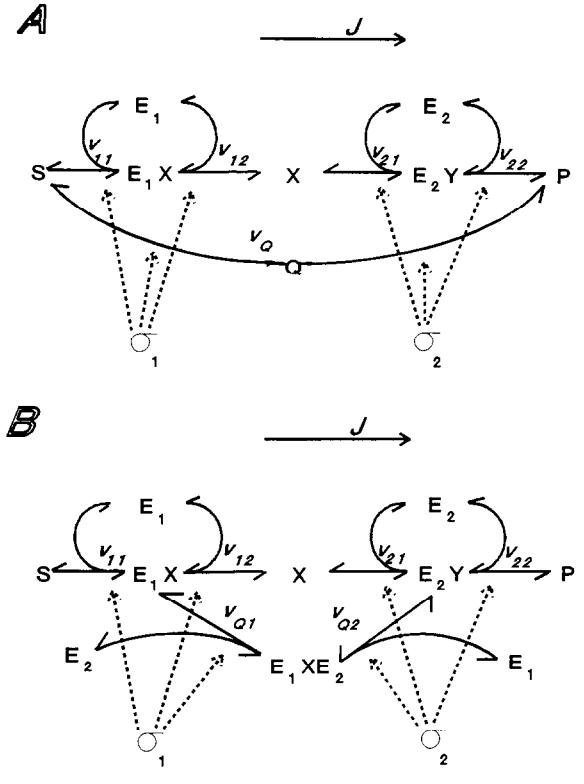

Fig. 2. 'Static' (A) and 'dynamic' (B) channels. The dynamic complex E_1XE_2 is formed only after binding X to E_1, while the static complex, Q, is formed independently of the presence of a common intermediate. In both systems the upper route represents the usual reaction pathway through the bulk phase intermediate, X, catalyzed by free enzymes, and the lower route represents the 'channelling'. The rates of E_1- and E_2-dependent processes are: (A) v_{11}, v_{12}, v_Q and v_{21}, v_{22}, v_Q, respectively; (B) v_{11}, v_{12}, v_{Q1}, v_{Q2} and v_{21}, v_{22}, v_{Q1}, v_{Q2}, respectively. σ_1 and σ_2 are the signal molecules, specific to the receptor enzymes, E_1 and E_2, respectively.

Eqn. 5 and [2]). However, in systems with enzyme–enzyme interactions (as well as in other 'non-simple' pathways [8]) the value of the signal transduction coefficients may depend on both the peculiarities of signal molecule action and the properties of the system.

Here we will consider the simple case when the elasticities ($\varepsilon^{v_{il}}_{\sigma_i}$) of all E_i-dependent elemental processes to the signal are equal to each other and to the elasticity of the reaction catalyzed by E_i in 'isolation' ($\varepsilon^{v_i}_{\sigma_i}$):

$$\varepsilon^{v_{il}}_{\sigma_i} = \varepsilon^{v_i}_{\sigma_i} \quad (9)$$

This case is obtained if: (i) the binding constants for σ_i are identical for all the subforms of the enzyme E_i, (ii) binding of σ_i transforms any E_i-subform into a more active (or inactive) state, and (iii) binding of σ_i does not change the ability of the enzyme E_i to form a complex with another enzyme. It follows from Eqns. 8 and 9 that in this case the expression for the signal transduction coefficient will be the following:

$${}^{R}C^{J}_{E_i} = \sum_{\substack{\text{all} \\ E_i\text{-dependent} \\ \text{processes}}} C^{J}_{il} \quad (10)$$

For signals satisfying Eqn. 9, the signal transduction coefficient coincides with the impact control coefficient which has been introduced in the parallel paper [2] in order to evaluate the total impact enzyme E_i has on the pathway flux via all E_i-dependent processes. This impact corresponds to the effect of a simultaneous and equal relative change in the elemental rate constants of all the processes in which any E_i-subform is involved.

Notably some irreversible or purely uncompetitive [9] inhibitors satisfy the condition in Eqn. 9. Therefore, using these inhibitors one can measure the impact control (signal transduction) coefficient. However, we should emphasize that in the general case the control coefficients determined by titrating a system with an inhibitor may depend (like the signal transduction coefficients) on the peculiarities of both the inhibitor and the system (see [3,10] for more complete consideration).

Let us compare the signal transduction coefficients for the simple and channelled pathways, Figs. 1 and 2, respectively. It follows from Eqns. 5–8 and 10 that for either enzyme the same additional term is present in the channelled pathway. It is equal to $C^J_Q = J_{chan}/J$ for the static channel (Fig. 2A) or $C^J_{Q1} + C^J_{Q2} = (J_{chan}/J) \cdot (1 - (C^J_{11} + C^J_{22}))$ for the dynamic channel (Fig. 2B). In case a significant fraction J_{chan}/J of the flux flows through the channel, the signal transduction coefficient of each of the enzymes E_1 and E_2 can be close to unity, while only one of them can have such a value in the pathway of two unchannelled enzyme reactions.

In realistic cases the intermediates may be subject to leakage. In such a case the input flux (J_{in}, the substrate consumption) differs from the output flux (J_{out}, synthesis of the product), the difference equaling the sum of the leaks of bulk phase (X) and channelled (E_1XE_2) intermediates. Fig. 3 shows how the signals can control the output flux at various degrees of channelling in the case when channelled and unchannelled leaks are proportional to the corresponding channelled and unchannelled fluxes. We can see that the signal transduction coefficient of either enzyme increases in parallel with the fraction of the flux that runs through the channel.

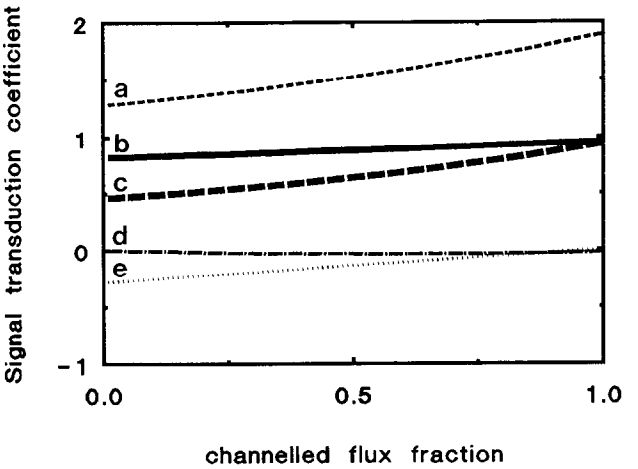

Fig. 3. Channelling enhances response to a signal. Signal transduction coefficients for enzymes 1 (b) and 2 (c) of Fig. 2B and their sum (a) were calculated as functions of the fraction of the total flux running through the channel. Leakage of bulk phase (X) and of channelled (E_1XE_2) intermediates was added to the scheme shown in Fig. 2B. (d) and (e) are the control coefficients of the 'channelled' and 'unchannelled' leaks, respectively. Total concentrations of enzymes 1 and 2 were set to 1. Elemental rate constants were equal to: $k_{11} = 10$, $k_{-11} = 0.1$, $k_{12} = 10 \cdot \alpha$, $k_{-12} = \alpha$, $k_{21} = \alpha$, $k_{-21} = \alpha$, $k_{22} = 10$, $k_{-22} = 0.01$, $k_{Q1} = 100 \cdot \beta$, $k_{Q1} = \beta$, $k_{Q2} = 0.0045 \cdot \beta$, $k_{-Q2} = 0.045 \cdot \beta$, $k_l = 0.05 \cdot \alpha$, $k_{lc} = 2.316 \cdot 10^{-3} \cdot \beta$. α and β were chosen such that the total output flux (J_{out}, to product P) and the total leak flux remained constant (at 0.3869 and $0.323 \cdot J_{out}$, respectively). The channelled flux fraction was defined as the flux from E_1XE_2 to E_2Y divided by J_{out} (in the case considered it also equals the channelled flux before the leak reaction divided by J_{in}). The signal transduction and control coefficients were calculated by increasing elemental rate constants (k_{11}, k_{-11}, k_{12}, k_{-12}, k_{Q1}, k_{-Q1}, k_{Q2}, k_{-Q2}, k_{lc} for enzyme E_1, and k_{21}, k_{-21}, k_{22}, k_{-22}, k_{Q1}, k_{-Q1}, k_{Q2}, k_{-Q2}, k_{lc} for enzyme E_2, k_l and k_{lc} for leaks) by 0.01%.

In a simple case only the bulk phase intermediate is subjected to leakage. Then, the signal transduction coefficient of either enzyme includes an additional term which is equal to J_{chan}/J_{out} for the static channel or $(J_{chan}/J_{out}) \cdot (1 - C^J_{22}) - (J_{chan}/J_{in}) \cdot C^J_{11}$ for the dynamic channel. Under the condition that most of the bulk phase flux ends up in the leak, and the binding of S and P to E_1 and E_2, respectively, is a near-equilibrium step, the signal transduction coefficient of each of the enzymes E_1 and E_2 should be close to unity, even if the channelled flux is much smaller than total bulk phase flux but comparable to the output flux. We conclude that channelling tends to increase the response of a pathway to signals.

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