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Enzyme–enzyme interactions and metabolite channelling: alternative mechanisms and their evolutionary significance

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Metabolite channelling may result from different kinetic mechanisms in which enzyme–enzyme interactions occur, so that intermediates are not released into the bulk solution and cannot be used by enzymes outside the channel. From an evolutionary point of view, the emergence of such mechanisms may provide new functional possibilities for the system, which would result in a selective advantage. Hence, it would be useful to evaluate the objective advantages provided by the various options by considering different criteria for functional effectiveness. Following this strategy, the goal of this paper is to compare a model for a free-diffusion two-enzyme system with two different models with inclusion of enzyme–enzyme interactions. In addition, models with simultaneous free and interacting branches are also analysed, and their advantages or disadvantages are presented. Basic guidelines are suggested that help in predicting the occurrence of specific mechanisms in different circumstances, and provide theoretical evidence in support of the hypothesis that no single solution simultaneously optimizes all the possible desired properties of the system.

INTRODUCTION

The idea that one enzyme might act as an 'effector' of another enzyme, as proposed by Nichol et al. (1974), and that enzymes may organize into multienzyme complexes of several kinds *in vivo* has been supported by an increasing amount of experimental data (Keleti et al., 1977, 1989; Welch, 1977, 1985; Clegg, 1984; Friedrich, 1984; Welch and Clegg, 1986; Srere, 1987; Keleti and Ovádi, 1987; Ovádi, 1991). Metabolite-channelling mechanisms, in which there is direct transfer of products between active centres, appear to be a consequence of these enzyme–enzyme interactions within the cell [Ovádi (1991) and references therein]. The implications of this concept for understanding cell metabolism have stimulated the search for experimental confirmation of these mechanisms *in vivo* [Srere (1987), Cheung et al. (1989); see also references in Ovádi (1991) Table 2]. However, there is still controversy about the real occurrence and significance of these mechanisms [see the contributions after the review paper by Ovádi (1991) in the same issue of *J. Theor. Biol.*]. Although the channelling hypothesis is attractive, some authors point out the danger of using it to explain any unexpected observation (Shulman, 1991).

The advantages and disadvantages associated with metabolite channelling as a consequence of enzyme–enzyme interactions have been discussed from different perspectives. The main repercussions suggested are: (1) it protects chemically labile intermediates, (2) it prevents loss of metabolites by diffusion and (3) it segregates intermediates of competing chemical and enzymic reactions. Further, several effects of a channelling mechanism on the performance of the system have been suggested. In each case, there is contradictory evidence on whether the proposed property is present in a given example. Among others, the main advantages suggested include: (1) the maintenance of metabolite concentrations at low levels [see contradictory results in Mendes et al. (1992) and Cornish-Bowden (1991)], (2) the reduction of the transition time through the system [see discussion in Ovádi (1991), Meléndez-Hevia and Montero (1991) and Heinrich and Schuster (1991), and references therein; see also Friedrich (1985) for discussion of energy costs] and (3) provision of a co-ordinated response to a single regulatory signal (Savageau, 1972, 1991; Ovádi, 1991).

Mathematical models have been used to test the significance of the several kinetic mechanisms leading to metabolite channelling, and to evaluate expected advantages attached to this property. These studies were generally based on the assumption of a localized channel mechanism in parallel with a free-diffusion mechanism (Westerhoff et al., 1984; Cornish-Bowden, 1991; Sorribas and Savageau, 1989; Mendes et al., 1992). In these models, the channel was assumed to be a bypass of the free-diffusion reactions so that both alternatives compete, which results in the opening of two different routes from the initial substrate to the final product. According to the usual nomenclature (Friedrich, 1985; Keleti et al., 1989; Ovádi, 1991; Mendes et al., 1992), two classes for these two-way mechanisms have been recognized: (i) *dynamic channel*, in which the enzymes form a complex in the bypass branch only if one of them has already bound the common intermediate; (ii) *static channel*, in which an enzyme complex can exist in the bypass branch in the absence of the common intermediate.

Although it is doubtful whether this organization represents reality, the interpretation of the advantages of each kind of mechanism has been the subject of controversy [see, for instance, Cornish-Bowden (1991) and Mendes et al. (1992)].

If we consider the different kinetic possibilities for enzyme–enzyme interactions leading to metabolite channelling, the pattern found in a cell should provide an appropriate functionality for the pathway. Thus it would be useful to evaluate the objective advantages of the various options by using different criteria for functional effectiveness (Savageau, 1972, 1976; Irvine and Savageau, 1985a,b; Irvine, 1991). Following this strategy, the goal of the present paper is to compare a model for a free-diffusion two-enzyme system with two different models that include enzyme–enzyme interaction. In addition, models anal-

Abbreviations used: NIS, non-interactive system; IS, interactive system; MCS, multienzyme complex system.

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ogous to a dynamic or static channel were analysed, and their advantages and disadvantages presented. We suggest some basic guidelines that may help in predicting specific mechanisms in different circumstances, and provide theoretical evidence that no single solution simultaneously optimizes all the possible desired properties of the system.

NUMERICAL PROCEDURES

Steady-state values were calculated by numerical integration of the set of differential equations with a Fortran77 program based on the Runge-Kutta fifth-order method (Franco and Canela, 1984a,b).

Normalized partial derivatives of dependent variables with regard to any independent variable (Logarithmic Gains) were calculated numerically as follows:

$$L(A_0,B_0) = \frac{A_{1.05}-A_{0.95}}{1.05B_0-0.95B_0} \times \frac{B_0}{A_0} \qquad (1)$$

A and B being respectively the dependent and independent variables. The subscript 0 refers to the steady-state value. $A_{1.05}$ refers to the value of A at $B = 1.05B_0$. $A_{0.95}$ refers to the value at $B = 0.95B_0$. A Logarithmic Gain refers to the same concept as a Control Coefficient. In this sense, the following correspondences should be noted:

$$L(V,E_i) \equiv C^J_{E_i} \quad L(X_i,E_i) \equiv C^{X_i}_{E_i}$$
$$L(V,A) \equiv R^J_A \quad L(\tau,A) \equiv C^\tau_A$$

The values of kinetic constants selected were chosen within the range 1×10^{-3}–1×10^{3}. For comparative purposes, the range of values used in this paper were selected to match those used recently in related literature (Cornish-Bowden and Cárdenas, 1993; Mendes et al., 1992). Alternative possibilities were tested using different values of kinetic constants inside this range. The results obtained were qualitatively similar to those reported in the Results section.

RESULTS

Alternative mechanism for metabolite channelling in a two-enzyme system

Channelling mechanisms may have evolved from free-diffusion systems through the formation of enzyme–enzyme complexes, with or without previous interaction with metabolites, by means of different alternatives. In a two-enzyme system catalysing consecutive reactions in the same metabolic pathway, the first step through a more complex organization corresponds to two independent catalysts working independently. The emergence of an interaction between the enzymes would open up the possibility of a direct transfer from active centre to active centre without the release of the first product into the bulk solution and leading to what is known as channelling. [We use the word channelling in a wide sense to indicate the fact that a product of a reaction is not released into the bulk solution before the next enzyme interacts with it. This includes direct transfer mechanisms and other alternatives. The reader is referred to the review by Ovádi (1991) and the contribution of Hervé (1991) for a complete discussion of these terms.] This could be considered a significant step in a more complex organization if it confers a functional advantage on the overall system. One can consider different kinetic possibilities leading to metabolite-channelling mechanisms. (1) The enzyme–enzyme interaction occurs when the first enzyme has bound to the substrate or (2) a complex between the enzymes has already been formed before the binding of the first substrate. This latter possibility can arise from a mutation involving an increase in enzyme–enzyme affinity. This new feature may provide an enhancement of the system's performance, or it could result in a drawback with respect to the former organization (Kell, 1991; Savageau, 1991).

The performance of the alternative patterns for metabolite channelling in a two-enzyme system can be studied using suitable kinetic models in which we can define specific conditions. This may provide specific predictions that may help in interpreting experimental data and in identifying potential systems in which to search for channelling mechanisms.

To explore the advantages of each kinetic mechanism, we first define a non-interactive system (NIS) (i.e. a free-diffusion mechanism with no interactions), which will be taken as a reference system to test the performance of kinetic mechanisms that include enzyme–enzyme interactions leading to metabolite channelling. For this NIS system, the reaction pattern is:

$$\begin{aligned} &A + E_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_1A \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E_1B \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E_1 + B \\ &B + E_2 \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E_2B \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} E_2C \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} E_2 + C \\ &C_{in} \overset{k_{13}}{\rightarrow} C_{out} \end{aligned} \qquad (2)$$

As the first mechanism accounting for metabolite channelling, we will consider the interactive system (IS), which is equivalent to the dynamic-channel branch (Cornish-Bowden, 1991; Mendes et al., 1992). For this mechanism, the reaction pattern is:

$$\begin{aligned} &A + E_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_1A \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E_1B \\ &E_1B + E_2 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E_1BE_2 \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E_2B + E_1 \\ &E_2B \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} E_2C \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} E_2 + C \\ &C_{in} \overset{k_{13}}{\rightarrow} C_{out} \end{aligned} \qquad (3)$$

An alternative model is the multienzyme complex system (MCS) which is equivalent to the static-channel branch (Mendes et al., 1992). For this mechanism, the reaction pattern is:

$$\begin{aligned} &E_1 + E_2 \underset{k_{15}}{\overset{k_{14}}{\rightleftharpoons}} E_1E_2 \\ &A + E_1E_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AE_1E_2 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} BE_1E_2 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E_1BE_2 \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E_1CE_2 \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} E_1E_2C \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} E_1E_2 + C \\ &C_{in} \overset{k_{13}}{\rightarrow} C_{out} \end{aligned} \qquad (4)$$

According to these mechanisms, a set of differential equations can be derived so that the performance of the different models can be investigated. (The corresponding equations are shown in the Appendix.) In each case, A is a source metabolite and C is the final product. The total concentration of intermediate B (σ) is defined as the sum of free and complexed forms.

Criteria for model comparison

Evaluation of system performance involves considering definite criteria related to the metabolic function of the alternative

systems. In analysing the kinetic mechanisms considered, the following criteria are examined.

Increment of the flux (V) through the system

It is believed that a direct consequence of the existence of a channelling mechanism is to increase the flux through the channelling reactions (Ovádi, 1991). To compare the three alternative schemes, we will decide upon, among other considerations, a common flux as a starting condition. This will constrain the possible values for the kinetic parameters and will provide common ground for comparison. This is required so that, once this characteristic is fixed, we can explore the remaining differences to validate the performance of each model when yielding the same flux. The effect of changes in pathway substrate [A in eqns. (2)–(4)] and total enzyme concentration (E_{iT}) on the flux through the system will be used to compare the alternative schemes.

Reduction in the concentration of the whole pool of intermediates (σ)

Metabolite channelling may provide a mechanism for reducing the concentration of free metabolites, which will result in a saving of solvent capacity in the cell (Savageau, 1974). As in the preceding case, evaluation of how a change in the substrate of the pathway or in the total concentration of enzyme affects the dependent concentrations will be considered a criterion of comparison.

Reduction in transition time (τ)

Transition time ($\tau = \sigma/V$) is defined as the mean time in which a molecule of substrate is converted into the end product (Easterby, 1973). It has been suggested that reduction in τ is a consequence of metabolite-channelling mechanisms. Easterby (1989, 1991) pointed out that, in these mechanisms, there is a trade-off between minimizing the pool sizes, transient and homoeostasis. In addition he stated (1991) that different strategies are appropriate to different cells. Evaluation of this property and the effect of a variation in the substrate or in the total concentration of enzymic forms on τ will help in discussing the relative performance of the alternative models.

Optimization of the dynamic response

For this, 95% of the time taken to move from an initial steady state to another steady state after a 50% increase in the source metabolite A (t_{95}) will be considered as a criterion for characterizing the performance of a given scheme. It should be noted that t_{95} quantifies the relaxation time of the system, in the sense of the time the system needs to attain a new steady state. Reduction of t_{95} will be an advantage because it is indicative of a quick response to a perturbation in A.

Conservation of properties at high enzyme concentration

Easterby (1989, 1991) has argued that certain enzymes are present at high concentrations inside the cell. He has shown what is meant by a high protein concentration in the context of channelling, and concluding that channelling would be of no advantage under these circumstances. He has also pointed out that others have observed high enzyme concentrations and apparent overcapacity *in vivo* and has suggested that these may be related to rapid system response rather than flux generation. Thus the properties deduced for the different enzymes at low concentrations may not be representative of the situation *in vivo*. We shall explore the performance of our models at different enzyme concentrations to simulate the conditions *in vivo*. In each case, we shall consider 10-, 100- and 1000-fold increments in the total concentration of the enzymes involved.

Comparison of alternative mechanisms for metabolite channelling

To make an appropriate comparison of the alternative mechanisms, we must establish a common basis for eliminating spurious differences that could affect the interpretation of the performance of the optional models. In evaluating the three schemes proposed, we have considered the following constraints to define the reference state: (1) same flux in all the systems; (2) same equilibrium constant for the transformation of A into C in all systems; (3) same equilibrium constant for the dissociation of A in all systems; (4) same equilibrium constant for the dissociation of C in all systems; and (5) same rate constants for the common steps. With these constraints, we have chosen a particular set of kinetic parameters for the three models (Table 1). With these, and according to the criteria defined above, we can perform the required simulations using eqns. (2)–(4) so that the performance of each alternative model can be discussed.

Comparative steady-state values of the different models at the reference state

In the reference state considered, we compute the steady-state values corresponding to σ, τ and t_{95} (Table 2). Both the IS and the MCS models lead to a decrease in σ, τ and t_{95} when compared

Table 1 Kinetic constants considered

	NIS	IS	MCS
k_1	100	100	100
k_2	10	10	10
k_3	100	100	100
k_4	100	100	10
k_5	26.6	381 000	100
k_6	10	10	100
k_7	100	100	100
k_8	26.6	381 000	100
k_9	100	100	100
k_{10}	100	100	100
k_{11}	100	100	100
k_{12}	10	10	10
k_{13}	10	10	10
k_{14}	–	–	980 000
k_{15}	–	–	1
[A]	0.1	0.1	0.1
E_{1T}	1×10^{-4}	1×10^{-4}	1×10^{-4}
E_{2T}	1×10^{-4}	1×10^{-4}	1×10^{-4}

Table 2 Comparative steady-state values of the different models

The comparative values of flux (V), transition time (τ), total concentration of intermediates (σ) and t_{95} are shown for the different models considered for the reference state.

	NIS	IS	MCS
V	4.084×10^{-4}	4.084×10^{-4}	4.084×10^{-4}
τ	175	0.228	0.214
σ	7.136×10^{-2}	9.316×10^{-5}	8.740×10^{-5}
t_{95}	590	0.385	0.380

with the NIS model. The IS and MCS models behave in a similar way with respect to σ, τ and t_{95}. However, the MCS model is slightly superior in diminishing these parameters.

In consequence, the two channelling mechanisms can yield the same flux as the NIS model and maintain a low level of intermediates and a low t_{95} which, according to the criteria stated, may be considered advantageous for the system. Clearly, if the aim in optimizing a given metabolic pathway is to maintain a given flux with a low σ, τ and t_{95}, an IS or MCS mechanism may be adequate. To understand the possible advantages of each mechanism, complementary criteria involving the system response to variations in [E_{iT}], source metabolite and general behaviour at high enzyme concentrations should be considered. With these, we will be in a better position to discuss the possible evolutionary implications of the alternative mechanisms.

Steady-state characterization by means of Logarithmic Gains: normalized partial derivative of the system variable with regard to total concentration of enzyme in the reference state

Once we have established the comparison of the different models in the reference state, it is important to evaluate how they react to a small change in the total concentration of the enzymes. This can be quantified by means of the appropriate Logarithmic Gains (Table 3). With the kinetic constants considered in this example, E_{1T} has the greatest influence on the flux for the NIS model. The summation relationship holds because flux is linear with respect to the enzyme concentration. Both the IS and MCS models result in an increase in the influence of E_{2T} and a decrease in the influence of E_{1T}. The summation relationship does not hold in these conditions because the flux does not depend linearly on enzyme concentration (Sorribas and Savageau, 1989; Savageau and Sorribas, 1989). In each case, the IS and MCS models are superior to the NIS model in increasing the flux in response to a simultaneous increase in E_{1T} and E_{2T}, the IS model being the most sensitive to this change. Clearly, both channelling models result in a tendency to equate the effect of a change in any of the enzymes involved. In the case of the MCS model, the Logarithmic Gains of the flux with regard to any of the enzymes are the same. This means that a change in any of the enzymes produces the same effect. Further, a combined change in the enzymes is greater when the enzymes interact. We can postulate that channelling through an MCS mechanism leads to a device providing a coordinated response to any change in the enzymes. This property has been postulated to be a desirable feature for optimizing the design of feedback regulation of branchpoints in biosynthetic pathways (Savageau, 1972, 1976).

The effect of a percentage change in the total concentration of enzyme on the value of σ is shown in Table 3 (rows 3–6). The effect is similar to that observed in the flux. It should be noted that, in the IS and MCS models, a change in both E_{1T} and E_{2T} results in an increase in σ, making it impossible for the summation relationship to be equal to zero in these models. A positive value for these Logarithmic Gains does not detract from the fact that the IS and MCS models actually reduce the value of σ (see below).

To appreciate the differences between the alternative models, we turn to the effect of a change in the total concentration of enzyme on the transition time (Logarithmic Gains of transition time). This concept combines the effect on both flux and σ, so that we can appreciate a mean effect on the system. In Table 3 (rows 7–9), it is shown that both the IS and MCS models are less sensitive than the NIS model. This is especially true for the MCS model, in which the value of τ is almost insensitive to changes in enzyme concentration. These results suggest that channelling through either an IS or an MCS model may provide a mechanism for reducing τ and for making it insensitive to changes in enzyme concentration, which may be an advantage to the cell.

Table 3 Comparison of the logarithmic gains with respect to the enzymes (normalized partial derivative of the system variable with the total concentration of enzyme) on the different models

	NIS	IS	MCS
$L(V,E_{1T})$	0.9432	0.8692	0.5191
$L(V,E_{2T})$	0.0568	0.4676	0.5191
$\Sigma L(V,E_{iT})$	1	1.3368	1.0380
$L(\sigma,E_{1T})$	1.080	0.969	0.6831
$L(\sigma,E_{2T})$	−1.080	0.137	0.6831
$\Sigma L(\sigma,E_{iT})$	0	1.1063	1.3662
$L(\tau,E_{1T})$	0.148	0.098	-8.27×10^{-3}
$L(\tau,E_{2T})$	−1.148	−0.334	-8.27×10^{-3}
$\Sigma L(\tau,E_{iT})$	−1	−0.236	-1.65×10^{-2}

Table 4 Comparison of the Logarithmic Gains with respect to the concentration of substrate A (normalized partial derivative of the system variable with the concentration of substrate A) on the different models

	NIS	IS	MCS
$L(V,A)$	0.6173	0.5607	0.5191
$L(\sigma,A)$	0.5760	0.1567	0.4565
$L(\tau,A)$	0.0629	−0.3924	-2.181×10^{-4}

Steady-state characterization by means of Logarithmic Gains: normalized partial derivative of the system variable with regard to concentration of substrate A in the reference state

In Table 4 the normalized partial derivatives of system variables with regard to concentration of substrate A are shown. The behaviour of the three models is quite similar if we consider the increase in flux after a percentage increase in A. The differences arise when we consider the response of τ. Then the IS model significantly reduces τ, whereas the NIS and MCS models are insensitive to changes in A. So, if we consider the response to a change in A, the IS model is clearly superior; τ increases in the NIS model, which is clearly a disadvantage.

Changes in the steady-state values as a result of a large increase in the total concentration of enzyme

The performance of the alternative models at different enzyme concentrations can be simulated from eqns. (2)–(4). The results obtained for the reference state and 10-, 100- and 1000-fold increase in E_{1T} and E_{2T} are shown in Figures 1–4.

Figure 1 shows that the IS and the MCS models increase σ in parallel to an increase in enzyme, which is consistent with the corresponding Logarithmic Gains (Table 3). This increment is almost linear in log–log space between 1 and 100-fold increment in [E]. At [E] × 1000, the behaviour of these two models is similar to or even worse than that of the NIS model. Flux also increases as a function of [E] (Figure 2), although this increment is similar in all the models between a 1- and 100-fold increase in [E]. At [E] × 1000, the MCS model is not able to match the flux observed in the NIS or IS models. Finally, τ is maintained at low values by

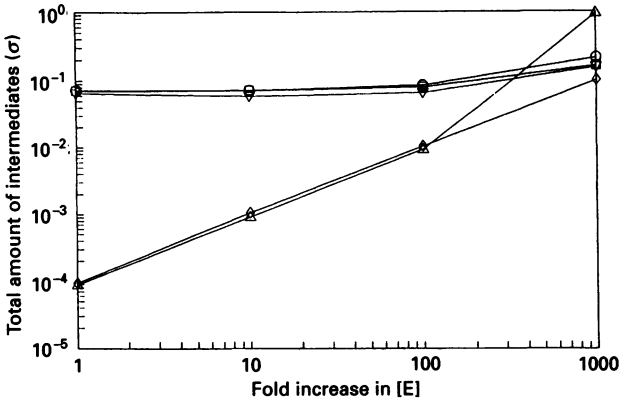

Figure 1 Effect of enzyme concentration on the total concentration of intermediates

The total concentration of intermediates (σ) is computed after an increment of 10-, 100- or 1000-fold in the total concentration of enzymes. This simulates the effect of compartmentation on the properties of the system. □, NIS; ◇, IS; △, MCS; ▽, NIS+IS; ○, NIS+MCS.

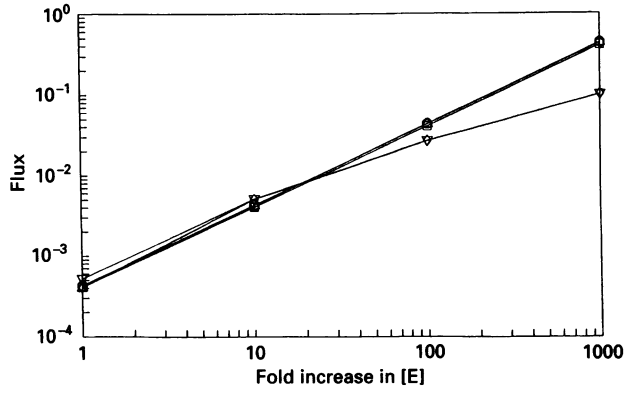

Figure 2 Effect of enzyme concentration on the flux through the system

The steady-state flux is computed after an increment of 10-, 100- or 1000-fold in the total concentration of enzymes. This simulates the effect of compartmentation on the properties of the system. □, NIS; ◇, IS; △, MCS; ▽; NIS+IS; ○, NIS+MCS.

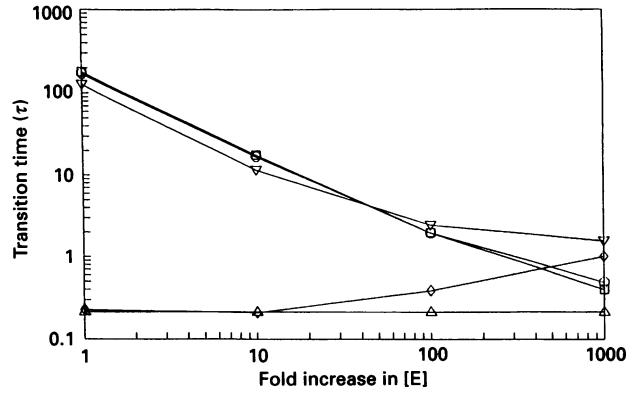

Figure 3 Effect of enzyme concentration on the transition time

The transition time (τ) is computed after an increment of 10-, 100- or 1000-fold in the total concentration of enzymes. This simulates the effect of compartmentation on the properties of the system. □, NIS; ◇, IS; △, MCS; ▽, NIS+IS; ○, NIS+MCS.

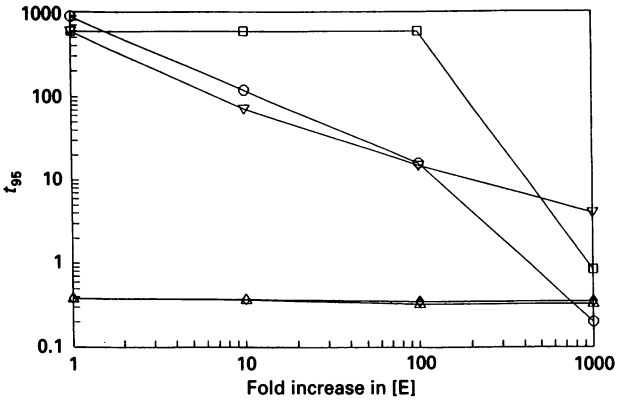

Figure 4 Effect of enzyme concentration on t_{95}

The t_{95} is computed after an increment of 10-, 100- or 1000-fold in the total concentration of enzymes. This simulates the effect of compartmentation on the properties of the system. □, NIS; ◇, IS; △, MCS; ▽, NIS+IS; ○, NIS+MCS.

Table 5 Comparative performance of mixed models at the reference steady state

We consider two mixed models: (1) NIS+IS; (2) NIS+MCS. In each case, we use the kinetic constants indicated in Table 1 (see the text for explanation).

	NIS+IS	NIS+MCS
V	5.717×10^{-4}	4.265×10^{-4}
σ	6.575×10^{-2}	7.280×10^{-2}
τ	127	170
t_{95}	590	880

the IS and MCS models, even for high enzyme concentrations. The increment in enzyme concentrations results in a decrease in τ for the NIS. At high enzyme concentration the value of τ is lower in the NIS model than in IS. According to the Logarithmic Gain (Table 3), the MCS model maintains the lowest τ irrespective of the enzyme concentration considered. The behaviour of t_{95} is shown in Figure 4. Between 1- and 100-fold increases in the enzyme concentration, t_{95} does not change, the MCS model being slightly better than the IS model with respect to this criterion. At the highest enzyme concentration considered, the NIS model approaches the behaviour of the IS and MCS models, and the MCS model maintains the lowest t_{95}.

These results agree with the observation previously made by Easterby (1989, 1991), to the effect that there is no channelling advantage at high enzyme concentration.

Performance of mixed models

As discussed in the Introduction, in considering the properties of channelling mechanisms, the models examined should include a mixed mechanism consisting of NIS and either IS or MCS. Table 5 and Figures 1–4 show the performance of mixed models defined following the parameters and conditions specified in Table 1. Clearly, the behaviour of the mixed models is worse than that of either the IS or the MCS models, and, in some cases, it approaches the behaviour of the NIS model. These results suggest that pure IS and MCS models may be more efficient and that, if an enzyme–enzyme interaction exists, evolution would

Table 6 Global comparison of the performance of the IS and MCS models

The plus sign means the model is superior with respect to this criterion (see the text for details, and Tables 3–5 for numerical results).

	Model	
	IS	MCS
Reference state		
Minimize σ		+
Minimize τ		+
Minimize t_{95}		+
Co-ordinate % change in total [E]		
Maximize $\Sigma L(V,E_{iT})$	+	
Minimize $\Sigma L(\sigma,E_{iT})$	+	
Reduce τ	+	
Make τ insensitive		+
% change in A		
Augment flux	+	
Maintain low σ	+	
Reduce τ	+	
Make τ insensitive		+
Behaviour at high [E]		
High flux		+
Low σ	+	
Low τ		+
Low t_{95}		+

tend to select a pure model instead of selecting a mixed alternative. Previous data seem to confirm these tentative conclusions (Clegg and Jackson, 1989).

DISCUSSION

The performance of a given metabolic design cannot be evaluated by a single criterion. Usually, what is recognized as functional effectiveness for a metabolic pathway can be dissected into different features that, globally considered, yield a measure of the efficiency of a given design [see Irvine and Savageau (1985a,b), Savageau (1972, 1976), Savageau and Sands (1991) for examples].

In the present paper, we have selected a set of different criteria for evaluating the advantages provided by two different mechanisms accounting for the phenomenon known as channelling. The results obtained are summarized in Table 6. Neither of the models involving enzyme–enzyme interactions is superior to the other if we consider the different criteria as a whole. Clearly, the IS model is better able to reduce the effect that a percentage change in $[E_{iT}]$ or in the pathway substrate causes on the flux and on σ. However, the MCS model is superior according to the other criteria considered. As a main feature, this model is more effective in reducing τ and t_{95}.

These observations suggest that both mechanisms may be related to different strategies of optimization. An IS mechanism may be preferred if it is critical for the system to control σ and to maximize the effect of small changes in $[E_{iT}]$ on the flux without losing control of σ. In contrast, the MCS mechanism may be preferred if the questions related to a quick response (t_{95}) and τ are critical for the performance of the system. However, it must be pointed out that, although it may be possible to obtain similar behaviour from the IS and MCS models by an appropriate selection of rate constants, as has been indicated by Cornish-Bowden and Cárdenas (1993), from the kinetic point of view, the MCS model is more attractive than the IS model because it avoids the need for two macromolecules to encounter one another by free diffusion. The results obtained with the mixed models show that maintaining a combined mechanism with an NIS component competing with either an IS or MCS model results in a lack of performance with respect to the corresponding IS or MCS models. Mixed models appear to be less effective than pure IS and MCS models, and evolution would tend not to favour these kinds of design. In particular, the results obtained by Cornish-Bowden and Cárdenas (1993) agree with those obtained in the present paper with the mixed model NIS + IS. According to Tables 2 and 5, the σ values in mixed models are as high as in the non-channelled NIS model. The introduction of a channelled branch in parallel to the free-diffusion mechanism is not enough by itself to reduce free-solution pool sizes at constant total flux. However, in perfect channel models (IS or MCS), pool sizes are comparatively low (see Table 2). In addition, when the channel branch carries mainly the flux, the mixed model becomes close to a perfect channel (IS or MCS) and the pool sizes are considerably reduced, in accordance with the results of Mendes et al. (1992).

The results presented in the present paper suggest further developments in analysing the phenomenon of channelling. First, it is clear that no single channelling mechanism is optimum under every circumstance (Meléndez-Hevia and Montero, 1991), and that, as pointed out by Kell (1991), some of the assumed advantages may turn out to be a handicap under certain conditions. Second, our results show that there may be evolutionary pressure to select specific channelling mechanisms from among alternative possibilities. If this is true, it suggests the need to search for IS or MCS mechanisms in accordance with the expected performance of the system.

Besides this interpretation, it remains to be determined to what extent channelling mechanisms are present in the cell. Giersch (1991) and Shulman (1991) indicated that the fact that a given mechanism provides an advantage does not imply that the mechanism exists. Moreover, Shulman (1991) argues that it would be better to leave some results unexplained rather than to label them as explained by channelling and thereby to take the emphasis away. Although we have shown that these mechanisms can provide clear advantages for better functionality, this does not mean that a system must evolve to become either an IS or MCS system. Many metabolites are common to several pathways, either as substrates or as regulatory signals, and hence they must have freedom to diffuse in order to accomplish their metabolic roles (Meléndez-Hevia and Montero, 1991). In these cases, a channelling mechanism would limit the performance of the system. There are other considerations to be taken into account in reaching a better understanding of this problem. Savageau (1991) indicated that optimization of a process does not mean that the system that includes this process would be optimized. In this sense, metabolite channelling has still not been analysed from a systematic point of view. Therefore it would be useful to evaluate the theoretical advantages of these mechanisms.

Finally, our results also show that the system performance depends on the total concentration of the enzyme. Further, the data shown in Figures 1–4 indicate that an NIS can match the performance of a channelling mechanism if the enzyme concentration is high enough. Although this result should be explored in more detail, it opens up an interesting question on the meaning and performance of the channelling mechanisms in these conditions.

In conclusion, it is clear that kinetic mechanisms incorporating enzyme–enzyme interactions can provide specific advantages over non-interactive mechanisms. This can be considered as an advantage leading to an increase in the performance of a metabolic pathway, which may be an evolutionary advantage. If this is so, it would be expected that the different channelling

mechanisms would be selected on the basis of the performance expected for the system. Further experimental data are needed to confirm this prediction and to understand the relevance of this kind of organization *in vivo*.

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APPENDIX

Kinetic equations for the alternative mechanisms

According to eqns. (2)–(4) of the main paper, the kinetic equations for the three mechanisms considered in this paper are:
Non-interactive system [eqn. (2)]:

$$
\begin{aligned}
E_1 &= E_{1T} - E_1A - E_1B \\
E_2 &= E_{2T} - E_2B - E_2C \\
\\
V_1 &= k_1(A)(E_1) - k_2(E_1A) \\
V_2 &= k_3(E_1A) - k_4(E_1B) \\
V_3 &= k_5(E_1B) - k_6(B)(E_1) \\
V_4 &= k_7(B)(E_2) - k_8(E_2B) \\
V_5 &= k_9(E_2B) - k_{10}(E_2C) \\
V_6 &= k_{11}(E_2C) - k_{12}(E_2)(C) \\
V_7 &= k_{13}(C)
\end{aligned}
\qquad
\begin{aligned}
\frac{d(E_1A)}{dt} &= V_1 - V_2 \\
\frac{d(E_1B)}{dt} &= V_2 - V_3 \\
\frac{d(B)}{dt} &= V_3 - V_4 \\
\frac{d(E_2B)}{dt} &= V_4 - V_5 \\
\frac{d(E_2C)}{dt} &= V_5 - V_6 \\
\frac{d(C)}{dt} &= V_6 - V_7
\end{aligned}
\qquad \text{(A1)}
$$

Interactive system [eqn. (3)]:

$$E_1 = E_{1T} - E_1A - E_1B - E_1BE_2$$
$$E_2 = E_{2T} - E_1BE_2 - E_2B - E_2C$$

$$V_1 = k_1(A)(E_1) - k_2(E_1A)$$
$$V_2 = k_3(E_1A) - k_4(E_1B)$$
$$V_3 = k_5(E_2)(E_1B) - k_6(E_1BE_2)$$
$$V_4 = k_7(E_1BE_2) - k_8(E_1)(E_2B)$$
$$V_5 = k_9(E_2B) - k_{10}(E_2C)$$
$$V_6 = k_{11}(E_2C) - k_{12}(E_2)(C)$$
$$V_7 = k_{13}(C)$$

$$\frac{d(E_1A)}{dt} = V_1 - V_2$$
$$\frac{d(E_1B)}{dt} = V_2 - V_3$$
$$\frac{d(E_1BE_2)}{dt} = V_3 - V_4$$
$$\frac{d(E_2B)}{dt} = V_4 - V_5$$
$$\frac{d(E_2C)}{dt} = V_5 - V_6$$
$$\frac{d(C)}{dt} = V_6 - V_7$$

(A2)

Multienzyme complex system [eqn. (4)]:

$$\frac{E_1E_2}{(E_1)(E_2)} = \frac{k_{13}}{k_{14}}$$

$$E_1 = E_{1T} - E_1E_2 - AE_1E_2 - BE_1E_2 - E_1BE_2 - E_1CE_2 - E_1E_2C$$
$$E_2 = E_{2T} - E_1E_2 - AE_1E_2 - BE_1E_2 - E_1BE_2 - E_1CE_2 - E_1E_2C$$

$$V_1 = k_1(A)(E_1E_2) - k_2(AE_1E_2)$$
$$V_2 = k_3(AE_1E_2) - k_4(BE_1E_2)$$
$$V_3 = k_5(BE_1E_2) - k_6(E_1BE_2)$$
$$V_4 = k_7(E_1BE_2) - k_8(E_1CE_2)$$
$$V_5 = k_9(E_1CE_2) - k_{10}(E_1E_2C)$$
$$V_6 = k_{11}(E_1E_2C) - k_{12}(E_1E_2)(C)$$
$$V_7 = k_{13}(C)$$

$$\frac{d(AE_1E_2)}{dt} = V_1 - V_2$$
$$\frac{d(BE_1E_2)}{dt} = V_2 - V_3$$
$$\frac{d(E_1BE_2)}{dt} = V_3 - V_4$$
$$\frac{d(E_1CE_2)}{dt} = V_4 - V_5$$
$$\frac{d(E_1E_2C)}{dt} = V_5 - V_6$$
$$\frac{d(C)}{dt} = V_6 - V_7$$

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