Channelling can affect concentrations of metabolic intermediates at constant net flux: artefact or reality?

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We show that if a metabolic intermediate is directly transferred ('channelled') from an enzyme that catalyses its production to another that uses it as substrate, there is no change in its free concentration compared with a system with the same net flux in which there is no direct transfer. Thus the widespread idea that channelling provides a mechanism for decreasing metabolite concentrations at constant flux is false. Results from computer simulation that suggest otherwise [Mendes, P., Kell, D. B. & Westerhoff, H. V. (1992) Eur. J. Biochem. 204, 257–266] are artefacts either of variations in flux or of alterations in opposite directions of the activities of the relevant enzymes.

If two enzymes catalysing consecutive reactions in a metabolic pathway can form a loose complex with one another in solution, there is the possibility of direct transfer of a metabolite that acts as product of one and substrate of the other. This behaviour is often called 'channelling' and has been reported for numerous systems, though whether it actually occurs remains in dispute [1, 2], as may be judged from the commentaries by numerous authors that accompanied a recent review [3].

Part of the interest in channelling is due to a widespread belief that if it does occur it has major metabolic consequences, for example that it can avoid problems derived from the limited solvent capacity of the cytoplasm [3–5]. This idea comes from a belief that redistributing metabolic fluxes to replace fluxes through free pools of intermediates with fluxes through channels would decrease the free concentrations of the channelled intermediates, ultimately to negligible levels.

Computer modelling indicated, however, that such a redistribution of flux would have no effect on the free concentration of a channelled intermediate [6], a conclusion supported by theoretical studies [7]. Mendes et al. [5] subsequently presented results appearing to show that channelling could decrease intermediate concentrations as much as by a factor of 1000. These results were surprising, but they did not initially seem impossible because the disagreement with the earlier ones [6] could have been due to different numerical values assumed for some rate constants, and because we had no difficulty in reproducing them with a different computer program. If valid, they would be of great importance for metabolic regulation as they would imply strong evolutionary selection of channelling mechanisms even if the fundamental chemistry might appear unfavourable.

We now show by algebraic analysis that channelling cannot have any effect on metabolite concentrations under the reported conditions, and, to dissipate any residual doubts, we examine how the apparently contradictory results arose. The explanation is that, in this type of study, it is essential to take care to maintain the flux truly constant, to distinguish between zero changes in flux and changes that may be very small but are not negligible, and to pay attention to the flux and concentration control coefficients involved. A large change in activity of an enzyme that has a flux control coefficient close to zero but a large concentration control coefficient for the metabolite of interest may produce such a small flux change that it passes unnoticed even though accompanied by a large change in metabolite concentration. Such a combination of a large concentration control coefficient with a small flux control coefficient means also, of course, that the concentration in question is very small, so lowering it further may be physiologically insignificant.

We show that all of the apparent examples of effects of channelling on metabolite concentrations at constant flux are either artefacts of failure to maintain a truly constant net flux or classical cross-over effects of changing the catalytic activities of consecutive enzymes in opposite directions. No evidence remains that dynamic channelling in a pathway with no leak has any effect on the free concentration of the channelled intermediate at constant net flux, and algebraic analysis shows that no such effect is possible, regardless of the particular rate and equilibrium constants.

METHODS

All of the simulated results given in this paper were obtained on an IBM PC-compatible computer with the program MetaModel [8]. As a guard against false results derived from incorrect programming, many of the results were verified by hand calculation: although it is difficult to determine the steady state for a multi-enzyme model, it is easy to check whether a purported steady state does in fact lead to the claimed fluxes. As a further check, some output from MetaModel has been compared with results generated by a different program, SCAMP [9] and found to be identical. Mendes et al. [5] also reported that their program GEPASI was able to reproduce the results from MetaModel given earlier [6].
Fig. 1. Model for channelling in a pathway of four enzymes. The reactions catalyzed by $E_2$ and $E_3$ are shown as two elementary steps (steps 2a and 2b for $E_2$, steps 3a and 3b for $E_3$) to allow for the possibility that their common reactant $C$ may exist both as a free metabolite and as a bound complex $E_2CE_3$ in which it is channelled from $E_2$ to $E_3$. Apart from the values of some of the kinetic constants the model is the same as that in Fig. 1 of [5] and Scheme 3 of [6]. Variation of the proportion of flux channelled through the complex $E_2CE_3$ is achieved by varying the rate constants for steps 2c and 3c by a factor $p$, in most cases (but not all: see the text) consequentially varying the rate constants for steps 2b and 3a by a factor $q$ in order to maintain constant the net flux through the pathway.

MODEL

Fig. 1 shows a model of channelling, the same as that used previously [6] except that the rate constants for steps 2 and 3 were previously assigned constant numerical values but are now treated as variables. It is also the same as the model shown in Fig. 1 of Mendes et al. [5] apart from the numbering of rate constants: all rate constants for $E_2$ have subscript 2, all rate constants for $E_3$ have subscript 3, and $v_r$ refers to $E_2$; this seems clearer than having subscripts 2, 3 and 7 refer to $E_2$, subscripts 4, 5 and 8 refer to $E_3$, and referring to the limiting rate of $E_3$ as $v_{max}$ of step 6. (To avoid any danger of confusion of the $a$, $b$ and $c$ used as subscripts with the metabolites $A$, $B$ and $C$, we shall use square brackets for concentrations rather than lower-case letters.) As previously [5, 6], the concentration of $A$ is assumed to have a fixed value of 10. All units are arbitrary (but consistent), though if one wishes one can take the concentrations to be in mM and the rates and fluxes to be in mM s$^{-1}$, as pointed out by Mendes et al. [5].

ALGEBRAIC ANALYSIS

General proof

The response of the net flux $J_{net}$ and the concentration $[C]$ to external parameters $p$ and $q$ that act on the rate constants of the channel and pool steps respectively may be written as follows:

$$\frac{d \ln J_{net}}{dp} = (C_{2b}^{C_2} + C_{3c}^{C_3}) d \ln p + (C_{2b}^{C_2} + C_{3c}^{C_3}) d \ln q \quad (1)$$

$$\frac{d \ln [C]}{dp} = (C_{2c}^{C_2} + C_{3c}^{C_3}) d \ln p + (C_{2c}^{C_2} + C_{3c}^{C_3}) d \ln q \quad (2)$$

where each $C_i$ represents a control coefficient [10] defined as follows:

$$C_i = \frac{\partial \ln y}{\partial \ln [E_i]} \quad (3)$$

y being an output variable of the system ($J_{net}$ or $[C]$ in the present example) and $[E_i]$ is the concentration of the $i$th enzyme. Eqs (1) and (2) follow from the combined response relationship [11], as all the relevant elasticities are unity (i.e. each rate constant affected by $p$ or $q$ is directly proportional to it). Varying $p$ and $q$ concomitantly to give no change in net flux, i.e. $d \ln J_{net} = 0$, defines a dependence of $q$ on $p$ such that

$$\frac{d \ln q}{d \ln p} = \frac{C_{2c}^{C_2} + C_{3c}^{C_3}}{C_{2b}^{C_2} + C_{3c}^{C_3}} \quad (4)$$

Hence Eqn (2) may be rewritten as follows

$$\frac{d \ln [C]}{d \ln p} = \frac{C_{2c}^{C_2} + C_{3c}^{C_3}}{C_{2b}^{C_2} + C_{3c}^{C_3}} - (C_{2c}^{C_2} + C_{3c}^{C_3})(C_{2b}^{C_2} + C_{3c}^{C_3})^{C_{2b}^{C_2} + C_{3c}^{C_3}} \quad (5)$$

However, Fell and Sauro [12] obtained a general relationship (their Eqn 6) for flux control coefficients in a branched pathway that may be expressed, for the present model and symbols, as follows:

$$\frac{C_{2b}^{C_2} + C_{3c}^{C_3}}{C_{2b}^{C_2} + C_{3c}^{C_3}} - (C_{2c}^{C_2} + C_{3c}^{C_3}) = 0 \quad (6)$$

Combining Eqns (6) and (7) shows that

$$\frac{C_{2b}^{C_2} + C_{3c}^{C_3}}{C_{2b}^{C_2} + C_{3c}^{C_3}} = \frac{C_{2c}^{C_2} + C_{3c}^{C_3}}{C_{2b}^{C_2} + C_{3c}^{C_3}} \quad (8)$$

and applying Eqn (8) to Eqn (5) gives

$$\frac{d \ln [C]}{d \ln p} = 0 \quad (9)$$

Thus if $p$ and $q$ are varied simultaneously so as to leave the net flux unchanged there is no effect on $[C]$, i.e. varying the proportion of the total flux that passes through the channel cannot affect the free concentration of $C$.

As it stands, this argument is circular, because Eqns (6) and (7) were originally obtained [12-14] with the use of assumptions very similar to Eqn (9). However, Reder [15] and Cascante et al. [16, 17] have shown how the theorems of control analysis can be obtained without any such assumptions, as necessary consequences of the definition of a steady state. Specifically, our Eqns (6) and (7) follow from Eqns (25-29) of Cascante et al. [17].

As the general forms of Eqns (6) and (7) refer to any numbers of steps in the parallel pathways and to any intermediate in the system, the conclusion may be expressed more generally: regardless of how many metabolites are by-passed by a channel, and regardless of how many intermediate complexes there may be in the channel, concomitant alteration of the pool and channel rate constants so as to leave the net flux unchanged has no effect on the free concentration of any intermediate by-passed by the channel. Essentially the same arguments apply also to other models of channelling besides that of Fig. 1, such as ‘static’ channels, in which the complex $E_2E_3$ exists in the absence of bound metabolite, and multi-enzyme complexes in which the two activities represented here by $E_2$ and $E_3$ are properties of the same molecule. From the kinetic point of view these alternative models are more attractive than the ‘dynamic’ channel represented by Fig. 1, because they avoid the need for two macromolecules to encounter one another by free diffusion. However, they offer no escape from the conclusion that channelling at constant net flux has no effect on the free metabolite concentration.
Fig. 2. Model of an ideal channel. The model differs from that of Fig. 1 in that the conversion of E3X to E3Y is shown as a single step 23c, as the steady-state concentration of the ternary complex E3CE3 is assumed to be negligible.

It is tempting to argue that varying all four rate constants k+2b, k-2b, k+3a, and k-3a by the same factor q involves a loss of generality, but this is incorrect. If k-2b/k+2b and k+3a/k-3a were varied this would alter the thermodynamic relations between B, C and D unless compensating changes were made in k+2b/k-2b and k+3a/k-3a; as these latter changes would be outside the branched part of the pathway, one could not attribute any effects of the variation to the channelling as such. Alternatively, if one satisfied the thermodynamic relationships by keeping k+2b/k-2b and k+3a/k-3a constant but used different factors q2b and q3a in steps 2b and 3a, this would alter the relative activities of E2 and E3, a change well known to affect intermediate concentrations: again, therefore, one could not attribute any changes observed to the channelling as such. An example of this kind of improper variation is considered below in Table 2.

Simple proof for an ideal channel

The above proof is general, but it has the disadvantage of assuming certain results from metabolic control analysis [12–17], which is still regarded as controversial by some [18]. However, for an ideal channel, i.e. one in which the channel reactions are so efficient that the amount of material existing at any time as the ternary complex E3CE3 is negligible, one can prove the corresponding result using only universally accepted principles of kinetics.

For this ideal channel the conversion of E3X to E3Y is a single step 23c, with rate constants k+3c and k-3c for the forward and reverse directions respectively (Fig. 2). There is only one concentration of B that allows any particular flux Jnet through E2 at any particular value of [A], and so [B] can be treated as a known quantity independent of any properties of E2 or E3. As B, E2 and E3X are the only reactants that participate in step 2a, and as [E2] + [E3X] is a constant, there is only one pair of values of [E2] and [E3X] that can support a flux of Jnet through step 2a, so they can also be treated as known quantities independent of the rate constants in steps 2b, 3a and 23c. The same argument applied to steps 3b and 4 shows that [E3] and [E3Y] are also known quantities independent of the rate constants in steps 2b, 3a and 23c. Thus the only concentration that can conceivably depend on the degree of channelling is [C], for which there are two steady-state expressions that must be identical:

\[
[C] = \frac{-J_{\text{pool}} + k_{+2a}[E2X]}{k_{-2a}[E2]} = \frac{J_{\text{pool}} + k_{-3a}[E3Y]}{k_{+3a}[E3]}.
\]

(10)

The need for these to be identical places restrictions on the possible values of the rate constants. If they are varied from some base values k'2a, etc. by a common factor q, Eqn (10) may be written as follows:

\[
\frac{[A] - [B]}{1 + [A] + [B]} \rightarrow \frac{k_{+2a}E2X}{k_{-2a}} \rightarrow \frac{k_{+3a}E2Y}{k_{-3a}} \rightarrow \frac{V_{\text{max}}[D]}{1 + [D]} \rightarrow B \rightarrow E2X \rightarrow E3X \rightarrow E3Y \rightarrow E3 \rightarrow C \rightarrow D.
\]

Table 1. Parameter values examined. The table lists particular sets of kinetic constants discussed in the text and elsewhere [5, 6] for the model shown in Fig. 1. The factor p was varied in order to vary the proportion of flux passing through the channel steps 2c and 3c. When the factor q is included (i.e. for Cases 1 and 2b) it was adjusted to give the same flux from A at each value of p.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value for</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_{+2a}</td>
<td>6</td>
</tr>
<tr>
<td>k_{-2a}</td>
<td>1</td>
</tr>
<tr>
<td>k_{+2b}</td>
<td>5q</td>
</tr>
<tr>
<td>k_{-2b}</td>
<td>6q</td>
</tr>
<tr>
<td>k_{+3a}</td>
<td>p</td>
</tr>
<tr>
<td>k_{-3a}</td>
<td>p</td>
</tr>
<tr>
<td>k_{+3c}</td>
<td>6q</td>
</tr>
<tr>
<td>k_{-3c}</td>
<td>q</td>
</tr>
<tr>
<td>k_{+3b}</td>
<td>1</td>
</tr>
<tr>
<td>k_{-3b}</td>
<td>6</td>
</tr>
<tr>
<td>V_{\text{max}}</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 3. Model without channelling. It is the same as that in Fig. 1 except that no possibility for channelling of C from E2 to E3 exists.

\[
[C] = \frac{-J_{\text{pool}} + qk_{+2a}[E2X]}{qk_{+3c}[E3]} = \frac{J_{\text{pool}} + qk_{+3c}[E3Y]}{qk_{+2a}[E2]}.
\]

(11)

The right-hand identity allows J_{\text{pool}}/q to be eliminated from the expression for [C], leaving only constants: [C] must therefore be independent of the degree of channelling.

RESULTS

Although the algebraic analysis could be considered to render computer simulation superfluous, this remains useful as a guard against logical errors in the algebra; it is also necessary to examine the previous numerical results in some detail to understand why apparent examples of effects of channelling [5] on metabolite concentrations were misleading.

The model in Fig. 1 was studied earlier [6] with the parameter values shown as Case 1 in Table 1: p was varied so as to vary the proportion of the net flux passing through E3CE3 from less than 1% to more than 99%, and q was adjusted at each p value to maintain the net flux from A constant (at a value of 0.4576). The smallest concentration obtained for free C was 9.9972 and the largest was 10.0065, with the other values distributed haphazardly between these extremes (Table 4 of [6]). Mendes et al. [5] criticized this study on several grounds, arguing that a less constrained choice of conditions would allow channelling to have significant effects on the concentration of the channelled intermediate. They described various conditions in which they found significant (and in some cases large) changes in metabolite concentrations. We now show why none of them alters the general conclusion that channelling does not affect metabolite concentrations at constant net flux, all of the changes
Table 2. Variation of rate constants in a model where no channeling is possible. The table shows results obtained with the model of Fig. 2, with \( k_{-1a} = 6, k_{-2a} = 1, k_{-b} = 6, k_{-c} = 6 q_{ba}, k_{-d} = 6 q_{sa}, k_{-e} = 6 q_{ea}, k_{-f} = 6 q_{fa}, k_{-g} = 6 q_{ga}, k_{-h} = 1, k_{-i} = 1, k_{-j} = 1, V_{-4} = 10^3 \). The rate constants for steps 2b and 3a were varied by varying \( q_{ba} \) and \( q_{sa} \) in such a way as to keep the flux through the pathway constant. As no channeling is possible in the model, channeling cannot explain the variation in the free concentration of \( C \).

<table>
<thead>
<tr>
<th>( q_{ba} )</th>
<th>( q_{sa} )</th>
<th>( [C] )</th>
<th>Flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.6788</td>
<td>2.4669</td>
<td>0.6957</td>
</tr>
<tr>
<td>1</td>
<td>0.3181</td>
<td>3.1034</td>
<td>0.6957</td>
</tr>
<tr>
<td>2</td>
<td>0.2513</td>
<td>3.4216</td>
<td>0.6957</td>
</tr>
<tr>
<td>5</td>
<td>0.2233</td>
<td>3.6119</td>
<td>0.6957</td>
</tr>
<tr>
<td>10</td>
<td>0.2152</td>
<td>3.3661</td>
<td>0.6957</td>
</tr>
</tbody>
</table>

Table 3. Control coefficients in an example where channeling was reported to have a significant effect. Control coefficients for net flux and the concentration of \( C \) (defined as in Eqn 1) are shown for the model of Fig. 1 with the parameter values shown as Case 2a of Table 1 and \( p = 0.01 \).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control coefficient for net flux</th>
<th>( [C] )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_1 )</td>
<td>0.9994</td>
<td>11.03</td>
</tr>
<tr>
<td>( E_2 )</td>
<td>0.00041</td>
<td>0.4781</td>
</tr>
<tr>
<td>( E_3 )</td>
<td>-0.00013</td>
<td>-1.0074</td>
</tr>
<tr>
<td>( E_2 + E_3 )</td>
<td>0.00028</td>
<td>-0.5293</td>
</tr>
<tr>
<td>( E_4 )</td>
<td>0.00013</td>
<td>-10.2432</td>
</tr>
</tbody>
</table>

that they attributed to channeling being due either to varying the relative activities of the enzymes involved in the channel (the cross-over effect) or to variations in the net flux.

Variation of rate constants in different proportions

Mendes et al. [5] argued that varying the rate constants for steps 2b and 3a in constant proportion was too restrictive. They accordingly examined seven of the nine possible combinations of the values 0.05, 0.5 and 5 and for the rate constants \( k_{-b} \) and \( k_{-3a} \) adjusting \( k_{-2b} \) and \( k_{-3a} \) so as to keep constant the ratios \( k_{-b}/k_{-2b} = k_{-3a}/k_{-3a} = 5/6 \). They set \( V_{-4} \) to \( 10^3 \), and for each combination they adjusted \( p \) to give the same value of 0.6957 for the net flux through the pathway. They found that the free concentration of \( C \) varied in the range 3.200–3.488, describing these effects as ‘rather modest’. Modest or not, they are not effects of channeling, as one can readily demonstrate by examining the model shown in Fig. 3, in which no possibility for channeling exists. Table 2 shows some results obtained with this model by introducing factors \( q_{ba} \) and \( q_{sa} \) as multipliers of the rate constants for steps 2b and 3a, and varying them in such a way as to maintain the net flux constant at 0.6957. Notice that perturbations of the rate constants by a factor of 2 produce much larger variations in the free concentration of \( C \) in this simulation than those obtained before [5] with perturbations by a factor of 10 or even 100.

This result is not surprising, of course, as it has been understood for many years that increasing the activity of an enzyme in a linear pathway tends to increase the concentrations of metabolites downstream from it, and to decrease those of metabolites upstream from it [19, 20]. It shows, however, that one cannot invoke channeling as the explanation of the behaviour seen in Table 1 of [5]: far from being the cause of the variations in \( [C] \), the channel tended to damp the cross-over effect of changing the relative activities of the two enzymes.

Equilibrium constant for interconversion of \( E_2X \) and \( E_1Y \)

Mendes et al. [5] argued that assuming an equilibrium constant of unity for the interconversion \( E_2X \) and \( E_1Y \) resulted in ‘rather strange’ values of some of the rate constants. Accordingly they varied the flux through the channel by varying \( p \) with the set of parameter values listed in Table 1 as Case 2a. They observed no change in net flux, although they took no precautions to ensure that it remained constant, but the free concentration of \( C \) varied by a factor of about 3 as the proportion of channelled flux increased from negligible to 100%; they commented that ‘importantly, these effects of the channel were observed under conditions of constant flux’. However, the net flux cannot have been exactly constant, because the catalytic activity for conversion of \( B \) to \( D \) increased by more than an order of magnitude: we repeated the same simulation, and found a change in net flux from 0.908771 to 0.908839 as \( p \) varied from 10^{-2} to 10^{4} (channel flux changing from 0.3% to 99.98% of the total). This 0.0075% increase in net flux may seem negligible, as indeed Mendes et al. took it to be, but it accounts for the whole of the variation in \( [C] \): when the simulation was repeated with the parameters shown as Case 2b of Table 1, adjusting \( q \) at each value of \( p \) to give a net flux of 0.908800 ± 0.0000001, \( [C] \) was constant at a value of 0.02037.

It may appear surprising that such a small variation in net flux could account for a threefold variation in metabolite concentration, but it becomes less surprising when one realizes that in the conditions studied \( E_1 \), the first enzyme of the pathway, had almost all of the flux control, so that to alter the net flux even by as little as 0.0075% required a large increase in activity of the channel enzymes, with a significant effect on the concentration of \( C \), their common metabolite. This is illustrated by Table 3, which shows the control coefficients for each enzyme for net flux and for the concentration of \( C \) at the starting point of the simulation, i.e. for \( p = 0.01 \). Note that the combined effect of \( E_2 \) and \( E_3 \) on \( [C] \) is about 2000 times greater than their effect on the net flux.

One could reasonably argue that the very small change in flux ignored in the paper of Mendes et al. [5] was physiologically insignificant, but it would not be reasonable to argue simultaneously that the decrease in \( [C] \) from a very small value (appropriate for the substrate of an enzyme with a negligible flux-control coefficient) had physiological importance.

Although we are primarily concerned in this paper with the effects of channeling if it does occur, rather than with the controversy about whether it occurs at all, it is worth noting that a value of \( p = 10^{4} \) with the parameter values of Case 2a means that the on rate constant for binding of \( E_2 \) to \( E_2X \) is \( 10^{4} \) whereas the corresponding rate constant for binding of \( E_2 \) to \( C \) is 1000, i.e. the model supposes that diffusion of a macromolecule to \( E_2 \) is \( 10^{4} \) times faster than diffusion of a metabolite. This is not very plausible in general [21], especially if one recognizes, as discussed elsewhere [22, 23], that the high concentration of macromolecules in the cell...
milieu hardly affects the translational motion of small molecules, whereas it greatly hinders that of other macro-molecules [24, 25]. 'Static' channels and multi-enzyme complexes avoid this difficulty, as they assume that the enzymes are already associated before the channelled intermediate is produced; thus they do not involve any diffusion of macro-molecules during the catalytic process.

It is important to distinguish in a simulation of this type between zero channelling and negligible channelling, i.e. between zero \( p \) and infinitesimal \( p \). In the former case the concentration of \( C_{E_p C_p} \) is zero by definition, whereas in the latter it is a limit that may be much greater than zero. This probably accounts for an anomaly in Fig. 4 of Mendes et al. [5] that they noted but did not explain, a discontinuity close to zero channelling in the plot of concentration of \( C \) against channelled flux.

Other supposed examples of effects of channelling

We have also examined other points made by Mendes et al. [5], such as their views that it was unrealistic to assume that \( E_p \) and \( E_p \) bind \( C \) with similar rate constants, and that one should consider the effects of assuming very large values of \( V_{+} \). However, the additional apparent effects of dynamic channelling on metabolite concentration that they found, such as those in their Fig. 3 and Table 3, do not require separate discussion here, as they are explained in the same way as in the last example: they were due to variations in net flux (as much as by a factor of 2), and when the simulations were repeated with the net flux genuinely constant the effects were not present.

DISCUSSION

We have proved that channelling of an intermediate cannot affects its free concentration at constant net flux, regardless of whether the enzymes forming the channel associate in a dynamic or a static complex, or even if they form part of the same molecule. This result is general and does not depend on computer simulation; for the simplest case of a highly efficient channel with negligible accumulation of ternary complex, it can be proved without recourse to the theory of metabolic control analysis. Our conclusion is concordant with that of Heinrich and Schuster [7], who used a more complex argument to show that redistribution of a constant net flux by channelling cannot alter transient times (which we may loosely take to be the times required for a system to switch from one steady state to another). Their view about both transient times and pool concentrations is essentially the same as ours, that all effects that have been ascribed to channelling are due to changes in other parameters that could arise equally easily without channelling, and are thus nothing to do with channelling.

In criticizing the generalization that ‘channelling has no effect on the free concentration of a channelled intermediate in a pathway’ [6], Mendes et al. [5] ascribed to channelling effects that are fully explained by changes in rate constants (their Table 3, see also Table 2 of the present paper) or by changes in flux through the pathway (their Figs 3, 4 and 5). Provided that one takes the generalization to apply to the context in which it was made, i.e. to pathways in which the net flux is constant and there is no possibility of ‘leaking’ of the free intermediate out of the system, no valid counter-examples have been reported, and the algebraic analysis shows that no such examples can exist.

In mechanisms where the free concentration of intermediate is already low in the unchannelled state, either because of a favourable equilibrium constant in the reaction that removes it, or because of a favourable equilibrium constant followed by a highly active reaction to remove the product, the low concentration of metabolite was reported [5] to be made even lower by channelling it from one enzyme to the next. We have shown that these results were artefactual, but even if they were not they would offer no comfort to investigators seeking a biological role for channelling. For channelling to have a useful moderating role on metabolite concentrations it must be able to affect concentrations that are high because of unfavourable equilibrium constants or because of low activity in later reactions in the pathway. Alternatively, if channelling is a mechanism for scavenging scarce intermediates it must be capable of acting in conditions where the enzymes concerned have significant flux control, but there is no evidence that it can do this either.

There are at least two pitfalls to be avoided if metabolic simulation is to yield reliable information about the behaviour of metabolic systems. First, it is important to remember the cross-over effect [17], whereby inhibition of any step in a pathway normally causes upstream metabolite concentrations to increase and downstream concentrations to decrease, whereas activation has similar effects in the opposite direction. A simulation intended to reveal effects of channelling should therefore be designed to exclude classical cross-over effects. Far from causing changes in metabolite concentrations, channelling can buffer changes induced by opposite changes in the activities of consecutive enzymes.

The second point is that careful attention must be paid to the control coefficients of the enzymes considered. If an enzyme has a very small flux control coefficient its activity can be increased substantially without any apparent effect on the flux unless the computations are done with very high precision; the same change in catalytic activity may nonetheless produce easily observable effects on concentrations, because the concentration control coefficients of an enzyme can be orders of magnitude greater than its flux control coefficient.

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REFERENCES


