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Effect of channelling on the concentration of bulk-phase intermediates as cytosolic proteins become more concentrated

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This paper shows that metabolic channelling can provide a mechanism for decreasing the concentration of metabolites in the cytoplasm when cytosolic proteins become more concentrated. A metabolic pathway in which two sequential enzymes can form a dynamic complex catalysing the direct transfer of an intermediate is compared with the analogous pathway lacking a channel (an 'ideal' pathway). In an ideal pathway a proportional increase in protein content does not result in a change in the steady-state concentration of the bulk-phase intermediate, whereas in a channelling pathway the bulk-phase intermediate either decreases or increases depending on the elemental rate constants within the enzyme mechanisms. When the concentrations of the enzymes are equal, the pool size decreases with increasing protein concentration if the elemental step depleting the bulk-phase intermediate exerts more control on its concentration than the step supplying the intermediate. Results are illustrated numerically, and a simplified dynamic channel is analysed in which the concentration of the enzyme–enzyme complex is negligible compared with the monomeric enzyme forms. For such a 'hit-and-run' channel it is shown that, when the product-releasing step of the enzyme located upstream is close to equilibrium, the pool size decreases as the concentrations of the enzymes increase in proportion, regardless of the rate, equilibrium constants and concentration ratios of the two sequential enzymes.

INTRODUCTION

The term metabolic channelling has been introduced to describe the mechanism whereby the reaction product of one enzyme is transferred to the next enzyme without mixing with the bulk-phase pool (for reviews see refs. [1,2]). Hand in hand with such a direct transfer, there usually exist reaction routes that allow some of the intermediate to enter the bulk phase [1–4]. Consequences of metabolic channelling on the concentrations of bulk-phase metabolites (pool sizes) have been the subject of intensive studies [2,5–8]. To discover whether channelling can specifically affect pool size, the effect of a parameter change enhancing the direct transfer flux in a channelled pathway can be compared with the effect of the same parameter change in the corresponding pathway lacking the channel. A pathway in which two enzymes form a complex (E_1XE_2) capable of channelling the intermediate (the dynamic channel [9–12]; Figure 1, top) and the same pathway without such complex-formation (Figure 1, bottom) can serve as a test system to examine this question [6–8].

In the present paper we apply a powerful method developed [13,14] within the framework of Metabolic Control Analysis ([15,16], see ref. [17] for a review) to examine how the concentration of the bulk-phase intermediate and the flux through a channel vary when the concentrations of the enzymes that constitute the channel are increased in proportion. The results suggest that, whereas the channelled flux increases with protein content, the bulk-phase pool can either increase or decrease depending on the elementary rate constants within both enzyme mechanisms. The paper shows that for some Gibbs energy profiles of the enzyme reactions, channelling of the intermediate provides a mechanism for decreasing bulk-phase pool size.

RESULTS

At very low enzyme concentrations when the fraction of the flux through a channel is almost zero, the two pathways in Figure 1 have almost the same flux and sustain the same concentration of the bulk-phase metabolite (X). A natural way of enhancing channelling is to increase the concentrations of the two directly interacting enzymes and hence the concentration of their complex (E_1XE_2). An equal relative change in both enzyme concentrations is a type of parameter adjustment that does not affect the pool of intermediate X in the pathway lacking the channel at all (Figure 1, bottom). However, these changes do affect the free metabolite pool in the dynamic channel of Figure 1 (top) [18,19].

To answer the question of how pool size is affected by such a proportional increase in enzyme concentrations, we shall rephrase it in terms of Metabolic Control Analysis: what does the sum of the enzyme control coefficients with respect to pool size amount to? The control coefficient (C) of an enzyme (i) over any steady-state flux (J) or pathway intermediate concentration (X) describes how J or X will change after a small change in enzyme concentration (e_i). A strict mathematical definition is given by [15,16]:

$$C_{e_i}^J = (dJ/J)/(de_i/e_i)_{s.s} = (d\ln|J|/d\ln e_i)_{s.s}$$

$$C_{e_i}^X = (d\ln X/d\ln e_i)_{s.s} \tag{1}$$

where subscript s.s signifies that the derivatives of the flux and the concentration are taken at the steady state of a pathway.

In an ideal pathway (as in Figure 1 for example), in line with the lack of effect of a proportional increase in the enzyme concentrations on the intermediate pool, the sum of the enzyme control coefficients over this pool equals zero (the summation theorem [15,16]). For channelled pathways, new summation theorems have been derived [13,20–22]. When the concentrations of the two enzymes in Figure 1 (top) are equal ($e_1 = e_2 = e$), the sum of the enzyme control coefficients with respect to the intermediate pool (X) is proportional to the sum of the elemental

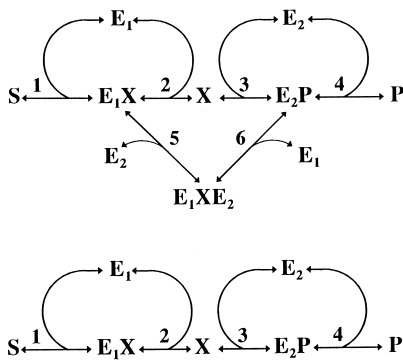‖ To whom correspondence should be addressed.

Figure 1   Representation of (top) Dynamic channel and (bottom) the analogous 'ideal' pathway lacking the channel

The concentrations of the initial substrate, S, and the end product, P, are constants. X is the intermediate in the bulk phase. The positive direction of the flux J is from S to P. (Top) The enzyme–enzyme complex E_1XE_2 is formed after binding the substrate S to E_1. The upper route represents the usual reaction pathway through the bulk-phase intermediate, catalysed by free enzymes, and the lower route represents the channelling. The numbering of the elemental steps is shown.

control coefficients of steps 2 and 3 (Figure 1, top) (see Appendix A):

$$\frac{\mathrm{d}\ln X}{\mathrm{d}\ln e} = C_{e_1}^X + C_{e_2}^X = \frac{J_{\mathrm{chan.}}}{(J - J_{\mathrm{chan.}})} \times \frac{(C_2^X + C_3^X)}{(1 + E_1XE_2/e)} \tag{2}$$

Here and below the concentrations of substances (e.g. X, E_1XE_2) are designated by the same symbols as the substances themselves. J is the total flux and $J_{\mathrm{chan.}}$ denotes the flux through the channel. $C_{e_1}^X$ and $C_{e_2}^X$ are the control coefficients of enzymes 1 and 2 with respect to X, as defined by eqn. (1), and C_2^X and C_3^X are the elemental control coefficients of steps 2 and 3 defined in terms of identical relative modulation of forward and reverse rate constants [13,14]:

$$C_i^X = (\mathrm{d}\ln X/\mathrm{d}\ln k_{+i})_{\mathrm{s.s.}}, \quad k_{-i}/k_{+i} = \text{constant} \tag{3}$$

Such a simultaneous modulation of the forward (k_{+i}) and reverse (k_{-i}) rate constants of elemental step i by the same factor does not violate microscopic reversibility [23].

In either pathway of Figure 1 the total flux will increase with the proportional increase in enzyme concentrations. For the channelled pathway the increase in total flux is accompanied by an increase in flux through the channel because more enzyme–enzyme complex is formed [13]. Eqn. (2) shows that whether the pool size increases, remains constant or decreases depends on whether the sum of the elemental control coefficients of steps 2 and 3 is greater than, equal to or smaller than zero. Step 2 supplies, whereas step 3 consumes, the bulk-phase intermediate X. Therefore, normally, C_2^X is positive and C_3^X is negative. As a consequence, the sign of their sum ($C_2^X + C_3^X$) depends on whether step 2 or step 3 is more limiting. Hence, whether the pool concentration X increases or decreases as cytosolic enzymes become more concentrated depends on the magnitudes of the kinetic constants within the enzyme mechanisms, as these ultimately determine the control coefficients.

When is the sum of the enzyme control coefficients over *X* negative or positive? A little bit of algebra

One of the fundamental concepts of Metabolic Control Analysis is that the control exerted by enzymes is determined by their
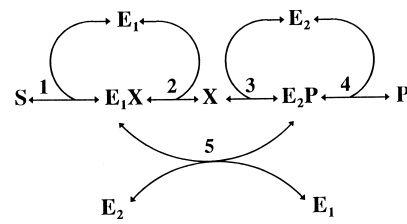

Figure 2   'Hit-and-run' channel

This case differs from the more general case of Figure 1 (top) in that the conversion of E_1X to E_2P is a single step as the steady-state concentration of the complex E_1XE_2 is assumed to be negligible.

elasticities rather than by their relative distances from equilibrium [15–17]. The elasticities of enzyme reactions cannot be determined via the Gibbs energy differences of these reactions. They depend on (phenomenological) kinetic parameters such as Michaelis constants, allosteric constants, etc. However, at the level of the elemental steps within enzyme mechanisms, i.e. the level at which the dynamic channel is considered here, the relation between the elasticities (ϵ^i) of the steps and the Gibbs energy differences (ΔG_i) across steps is straightforward ([23]):

$$\epsilon_y^i = v_{+i}/v_i = 1/(1 - e^{\Delta G_i/RT}), \quad \epsilon_z^i = -v_{-i}/v_i = 1/(1 - e^{-\Delta G_i/RT}) \tag{4}$$

Here ϵ_y^i and ϵ_z^i are the elasticity coefficients of any step i of the dynamic channel with respect to its substrate (y) and product (z), v_{+i}, v_{-i} and v_i are the rates in the forward and reverse directions and the net rate of step i respectively, ΔG_i is the chemical potential of the product(s) minus the chemical potential of the substrate(s) of step i.

The elemental control coefficients (C_2^X and C_3^X) of steps 2 and 3 are related to their elasticities through the connectivity theorem (as applied at the level of the elemental steps [14,22,23]):

$$C_2^X \epsilon_X^2 + C_3^X \epsilon_X^3 = -1 \tag{5}$$

Using eqns. (4) and (5) one may conjecture that when step 2 is near equilibrium (ΔG_2 is close to zero) and step 3 is far from equilibrium ($-\Delta G_3 \gg RT$), the control exerted by step 3 is substantial and the control exerted by step 2 is small. From eqn. (2) one can conclude that, in such cases, the pool size decreases and the channel flux increases with an increase in enzyme concentrations.

To illustrate this conjecture we simplify the dynamic channel in Figure 1(a) to a 'hit-and-run' channel in which the concentration of the ternary complex, E_1XE_2, is negligible (see. ref. [8]). In such a simplified channel the conversion of E_1X to E_2P is a single step (step 5 in Figure 2). Similar thermodynamic restrictions apply to both models of a dynamic channel, i.e. $K_{\mathrm{eq.2}}K_{\mathrm{eq.3}} = K_{\mathrm{eq.5}}K_{\mathrm{eq.6}}$ for the channel in Figure 1 (top) and $K_{\mathrm{eq.2}}K_{\mathrm{eq.3}} = K_{\mathrm{eq.5}}$ for the 'hit-and-run' channel in Figure 2 ($K_{\mathrm{eq.}i}$ is the equilibrium constant of step i). These restrictions on the possible magnitudes of the rate constants reflect that, for any channel, the Gibbs energy change for each branch must be identical. Note that the 'hit-and-run' channel of Figure 2 can be considered as the limiting case of the dynamic channel of Figure 1 (top) when the rate constants k_5^- and k_6^+ are increased by the same factor (leaving the product $K_{\mathrm{eq.5}}K_{\mathrm{eq.6}}$ unaffected).

Let us consider the 'hit-and-run' channel (Figure 2) for the case when the activation barrier for step 2 is so low, and hence its rate constants are so high compared with other steps, that step 2 is near equilibrium, whereas steps 1, 3, 4 and 5 are further away from equilibrium. Under such conditions the ratio of the
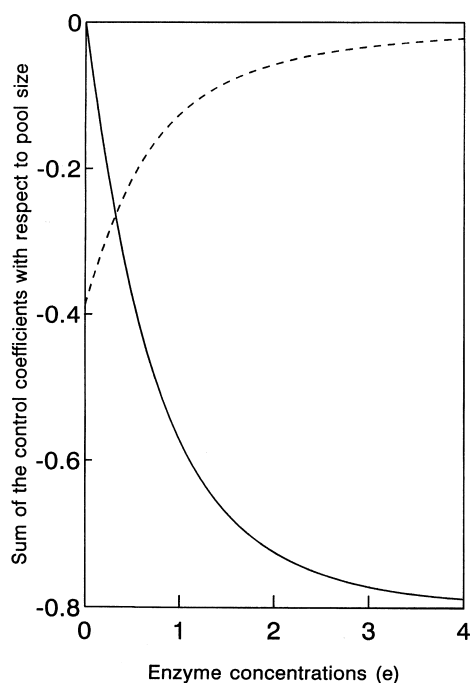
Figure 3   Variation with protein concentration of the extent to which the enzymes control the bulk-phase intermediate X

For the pathway of Figure 1(a) the dependences are shown of the sum ($C_{e_1}^X + C_{e_2}^X$) of the enzyme control coefficients (———) and of the sum ($C_2^X + C_3^X$) of the elemental control coefficients of steps 2 and 3 (– – – –) on the total concentration (e) of either enzyme (these equal concentrations, $e_1 = e_2 = e$, are shown in dimensionless units). The parameter values were (dimensionless units): $S = 10$, $P = 1$, $k_{+1} = 0.2$, $k_{-1} = 0.05$, $k_{+2} = 10$, $k_{-2} = 2$, $k_{+3} = 0.001$, $k_{-3} = 0.005$, $k_{+4} = 20$, $k_{-4} = 0.01$, $k_{+5} = 0.25$, $k_{-5} = 1$, $k_{+6} = 4$, $k_{-6} = 1$.

concentrations (thermodynamic activities) of the product and the substrates of step 2 approach the equilibrium constant:

$$(E_1 \times X)/E_1 X = K_{\mathrm{eq.2}} \tag{6}$$

The sum of the concentrations E_1 and $E_1 X$ equals the total concentration of the enzyme (e). As a consequence, the equilibrium ratio [eqn. (6)] fixes these concentrations as follows:

$$E_1 = e/(1 + X/K_{\mathrm{eq.2}}), \quad E_1 X = e(X/K_{\mathrm{eq.2}})/(1 + X/K_{\mathrm{eq.2}}) \tag{7}$$

Now we use eqn. (7) to express the total flux (J), which is equal to the steady-state rate of step 1 (v_1), in terms of X and e:

$$(v_1)_{\mathrm{s.s}} = J = e(k_{+1}s - k_{-1}X/K_{\mathrm{eq.2}})/(1 + X/K_{\mathrm{eq.2}}) \tag{8}$$

Differentiating eqn. (8) with respect to $\ln e$ and taking into account that, under conditions of equal concentrations of the enzymes, the resulting differential quotient should equal the sum of the enzyme control coefficients, one arrives at:

$$\frac{\mathrm{d}\ln J}{\mathrm{d}\ln e} = C_{e_1}^J + C_{e_2}^J = 1 - f(C_{e_1}^X + C_{e_2}^X) \quad \text{where}$$

$$f = (k_{+1}s + k_{-1}) \times E_1 \times E_1 X/Je \tag{9}$$

The factor f is positive provided that the flux J is positive (i.e. from S to P). Therefore eqn. (9) shows that the sum of the enzyme control coefficients over the pool concentration (X) is negative if, and only if, the sum of the enzyme control coefficients

over the total flux (J) is greater than 1. As has been shown for the general case of dynamic channelling [13,21,24], the latter is true for a wide range of rate constants. For the 'hit-and-run' channel (Figure 2) we shall now show that, if step 2 can be considered to be near equilibrium, the sum of the enzyme control coefficients over J exceeds 1. Expressing the sum of the enzyme control coefficients with respect to the flux J in terms of the elemental control coefficients, one obtains [13,25]:

$$C_{e_1}^J + C_{e_2}^J = 1 + C_5^J \tag{10}$$

C_5^J is the (elemental) control coefficient of channel step 5. In a 'hit-and-run' channel, sequestration of enzymes by the formation of the enzyme–enzyme complex is negligible (see refs. [26,27]). From this it is already intuitively clear that the total flux will always increase with the activity of the channel step, i.e. $C_5^J > 0$. One can also prove the latter inequality by applying the summation and connectivity theorems to the elemental control coefficients [14,22,23]. Taking into account that the control coefficient of near-equilibrium step 2 can be neglected in comparison with the control coefficients of the other steps and using the branch (summation) theorem [28], one can write:

$$C_3^J/C_5^J = (J - J_{\mathrm{chan.}})/J_{\mathrm{chan.}}$$

For thermodynamic reasons, the fluxes through the branches should flow in the same direction. As a consequence, C_5^J and C_3^J must have the same sign (i.e. both are positive or negative). Further, from the connectivity theorem with regard to $E_2 P$ it follows that C_4^J must have the same sign as C_5^J and C_3^J, and from the connectivity theorem with regard to X it follows that C_1^J also has the same sign. As the sum of all the (elemental) control coefficients over the flux J is equal to 1, each term must be positive. Hence C_5^J is greater than 0, and the sum in eqn. (10) is greater than 1. Therefore the sum of the enzyme control coefficients over the pool concentration (X) is negative under the conditions considered [see eqn. (9)]. Thus we have shown that, for some kinetic properties of the enzymes forming the channel, the pool concentration X decreases with an increase in protein concentrations and therefore with channelling (see also Appendix B).

It is instructive to consider also an 'opposite' case in which step 3 (rather than step 2) is near equilibrium. When all the other steps (1, 2, 4 and 5) are further away from equilibrium, step 3 is not limiting and its control coefficients are close to 0. Similarly to the above, one can obtain the following relationship between the sum of the enzyme control coefficients with respect to the flux J and the corresponding sum with respect to the concentration X:

$$C_{e_1}^J + C_{e_2}^J = 1 + f^*(C_{e_1}^X + C_{e_2}^X) \quad \text{where}$$

$$f^* = (k_{+4} + k_{-4}P) \times E_2 \times E_2 P/Je \tag{11}$$

The factor f^* is positive for positive J, i.e. for positive values of the thermodynamic driving force. Therefore, in contrast with the previous case, the sum of the enzyme control coefficients over X is positive when the sum of the enzyme control coefficients over the pathway flux is greater than 1. Using the summation and connectivity theorems it is readily shown that, for this case also $C_5^J > 0$. Then from eqns. (10) and (11) it follows that the total control exerted by enzymes on the flux exceeds 1 and the control exerted on X is positive. Moreover, from the above proofs one can see that, for a 'hit-and-run' channel, a simplifying assumption, i.e. that the enzyme concentrations are equal (which was used for the more general case of dynamic channelling) can be relaxed provided that these concentrations increase at a constant ratio ($e_1/e_2 = $ constant).
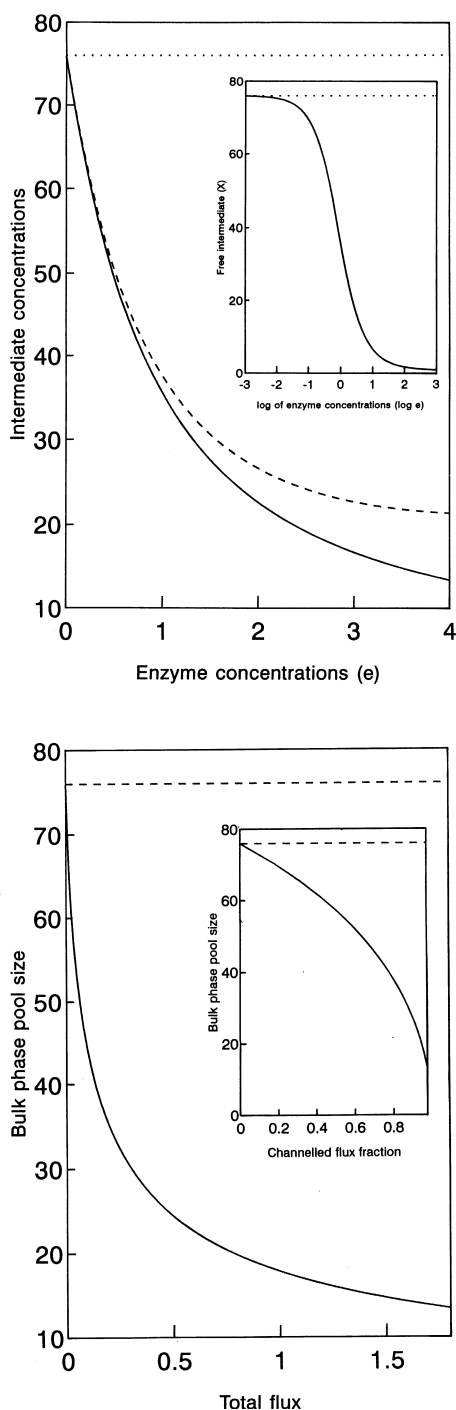
Figure 4   Comparison of the behaviour of the bulk-phase intermediate in the dynamic channel with that in the ideal pathway [depicted in Figures 1a (top) and 1b (bottom), respectively]

(Top) Dependence of the concentration of the intermediate (X) on the total concentration (e) of enzyme for either the dynamic channel (———) or the ideal pathway (••••), and the analogous dependence of the concentration of all substances in the bulk-phase for the dynamic channel (————). Inset: the concentration X for either pathway versus log e. (Bottom) The concentration of the bulk-phase intermediate (X) versus the total pathway flux (J) for the dynamic channel (———) and for the ideal pathway (————). Inset: the concentration X for either pathway versus the flux fraction through the channel ($J_{chan.}/J$). The values of the parameters are indicated in the legend to Figure 3.

We conclude that, when the protein concentrations in the 'hit-and-run' channel (Figure 2) increase proportionally (at any e_1/e_2 ratio), the concentration of the bulk-phase intermediate

will increase if step 3 is near equilibrium and decrease if step 2 is near equilibrium. Most importantly, this conclusion does not depend on the rate constants of steps that are not near equilibrium. Therefore it does not depend on which enzyme exerts more control on the flux (or which reaction, $S \rightarrow X$ or $X \rightarrow P$, is closer to thermodynamic equilibrium).

Numerical illustrations

For the dynamic channel of Figure 1 (top), Figure 3 shows the sums of the control coefficients of the enzymes (solid line) and of steps 2 and 3 (dashed line) with respect to the pool concentration X for the case in which step 3 is more limiting. One can see that the sum of the enzyme control coefficients approaches zero at very low enzyme concentrations. In line with eqn. (2), it assumes negative values if, and only if, the sum of the elemental control coefficients of steps 2 and 3 does the same. These negative values imply that the concentration of the intermediate (X) decreases with increasing total enzyme concentration. For the same case, Figure 4 compares the behaviour of the bulk-phase intermediate (X) for the two pathways of Figure 1. In the channelled pathway the free intermediate concentration decreased with increasing enzyme concentration, whereas in the same pathway lacking the channel it remained constant (Figure 4, top). Moreover, the concentration of all species in the bulk phase including the enzymes also decreased (Figure 4, top), possibly enhancing the solvent capacity of the cytoplasm. The inset to Figure 4, top shows that, with a further increase in enzyme concentration, the free intermediate concentration (X), which starts above 70 (μM), decreases to less than 1.

The simulations show that, as long as the activation barriers of steps flowing to the branch points (steps 1 and 3 of Figure 1, top) are significantly higher than the activation barrier of any step (2 and 4) flowing away from these points, changes in particular values of the equilibrium constants of the enzymes have little if any effect on pool-size behaviour when enzymes become more concentrated.

The present demonstration was not for constant total flux. However, in all cases the total flux can be maintained constant by simultaneous equal modulation of all rate constants in addition to the modulation of total protein concentration. Indeed, such a modulation leaves all pathway concentrations unchanged and affects only pathway fluxes [13,29]. Consequently our results are also relevant for the constant-flux condition. Figure 4 (bottom) confirms this by plotting the bulk-phase pool size versus the total flux through either pathway. The inset to Figure 4 (bottom) shows how pool size depends on the flux fraction flowing through the channel.

For the dynamic channel of Figure 1 (top) the case of equal concentrations of the two enzymes was examined. Using eqn. (A3) one can show that the condition in which pool size (X) falls with a proportional increase in enzyme concentration remains valid if the concentration of the second enzyme exceeds the concentration of the first. Although X decreases with an increase in e_2 only, it continues to decrease when both e_1 and e_2 increase in proportion.

DISCUSSION

The suggestion that channelling can provide a mechanism for decreasing the concentration of bulk-phase intermediates has caused a flurry of discussions [2,6–8]. To elucidate the topic, the present paper applies a new method of Metabolic Control Analysis. This method descends to the level of the elemental

processes within the enzyme mechanism, then returns to the enzyme reactions (for a recent review see ref. [30]). The results show that channelling may cause either a decrease or an increase in pool size when compared with an analogous pathway lacking a channel. The behaviour of the bulk-phase intermediate when the enzymes become more concentrated strongly depends on the absolute values of the rate constants (i.e. on the activation barriers) of elemental steps located upstream and downstream of the branch points rather than on the standard Gibbs energy differences across those steps. As mentioned above, simultaneous equal relative changes in all the (elemental) rate constants do not alter any of the concentrations, whereas they do affect the pathway flux. Therefore our results imply that the channelling pathway and the same pathway without the channel may have the same flux (although the enzyme concentrations in the pathways differ), but pool size and even the concentrations of all the species is smaller in the case of channelling. Specifically, when the elemental step supplying the bulk-phase intermediate is sufficiently fast and the concentration of a dynamic enzyme–enzyme complex is much smaller than that of monomeric forms, the concentration of the bulk-phase intermediate decreases as proteins become more concentrated.

Constraints on the possible kinetic advantage of channelling have been identified by Easterby [5]. If the two enzymes of a channel are embedded within a larger pathway, an increase in their concentrations may not result in an appreciable increase in the pathway flux. Under these conditions and at high concentration of enzyme 2, there was little if any reduction in total pool size in the channelled compared with the non-channelled case, as both the free and channelled pools are minimized [5].

At high enzyme concentrations any reduction in free intermediate (X) might be offset by an increase in bound X [5]. Whether this takes place depends on the particular values of the elemental rate constants within a channel and on the range of protein concentrations. Figure 4 (top) shows that, for some kinetic constants, total (free + bound) X decreased with increasing enzyme concentration, in an appreciable range of the latter.

Effects of high protein concentrations on metabolic channelling considered in this paper are of importance for extrapolation of information obtained *in vitro* to the situation *in vivo*. High concentrations of enzymes are commonly found *in vivo* where they can greatly exceed metabolite concentrations [31]. For these conditions channelling can provide a mechanism for decreasing pool size. Here the phenomenon of macromolecular crowding [32–34] may play a key role. An increase in total protein concentration reduces the effective solvent volume. Macromolecular crowding then increases the apparent effective concentrations of enzymes (resulting from an increase in their thermodynamic activities) much more than those of low-molecular-mass metabolites, in some cases to dramatic extents [32–34]. Under conditions of macromolecular crowding therefore a relatively small increase in the concentrations of two particular enzymes forming a channel may result in quite a significant decrease in pool sizes of small intermediate molecules. For organisms that are subject to transient changes in cytoplasmic water content, because of changes in the environment, this may be important.

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APPENDIX A

We shall apply to the channelled pathway of Figure 1(a) the approach developed for an arbitrary 'non-ideal' pathway [1–3]. Let, in the initial steady state of the dynamic channel, the concentrations of E_1-containing species, i.e. E_1, E_1X and E_1XE_2, be increased by a factor λ. Simultaneously one decreases the forward and reverse rate constants (k_{+i} and k_{-i}) of the elemental steps 1, 2, 5 and 6 (in which E_1-containing species participate) by the same factor (λ). Then the pathway attains a new steady state, in which all the rates (e.g. the flux J) and the concentration of the bulk-phase intermediate (X) retain their initial (unperturbed) values. However, some parameters, i.e. the rate constants (k_{+i}, k_{-i}; $i = 1, 2, 5, 6$) and the total enzyme concentrations (e_1 and e_2),

differ between the original and the new steady state. The new values of these parameters are related to the original values through:

$$k_{+i}(\lambda) = k_{+i}/\lambda, \quad k_{-i}(\lambda) = k_{-i}/\lambda, \quad i = 1, 2, 5, 6$$

$$e_1(\lambda) = E_1(\lambda) + E_1 X(\lambda) + E_1 X E_2(\lambda) = \lambda e_1 \tag{A1}$$

$$e_2(\lambda) = E_2 + E_2 P + E_1 X E_2(\lambda) = e_2 + (\lambda - 1) E_1 X E_2$$

The concentrations of different enzyme forms (e.g. E_1, $E_1 X$, $E_1 X E_2$) are denoted by the same symbols as the forms themselves.

Taking into account that the steady-state concentration (X) is a unique function of the parameters (k_{+i}, k_{-i}, e_i) and using eqn. (A1) and the definitions [eqns. (1) and (3)] of the main text, one may write:

$$\mathrm{dln}\, X / \mathrm{dln}\, \lambda = C_{e_1}^X + (C_{e_2}^X E_1 X E_2 / e_2) - (C_1^X + C_2^X + C_5^X + C_6^X) = 0 \tag{A2}$$

On an analogous perturbation of all the E_2-containing species and rate constants (k_{+i}, k_{-i}, $i = 3, 4, 5, 6$), one arrives at an equation system relating the enzyme control coefficients to the control coefficients of the elemental steps:

$$\begin{aligned} C_{e_1}^X + (C_{e_2}^X E_1 X E_2 / e_2) &= (C_1^X + C_2^X + C_5^X + C_6^X) \\ (C_{e_1}^X E_1 X E_2 / e_1) + C_{e_2}^X &= (C_3^X + C_4^X + C_5^X + C_6^X) \end{aligned} \tag{A3}$$

For the case where the total concentrations of the two enzymes are equal ($e_1 = e_2 = e$), one may sum the two equations (A3) to obtain (see ref. [4]):

$$C_{e_1}^X + C_{e_2}^X = (C_5^X + C_6^X)/(1 + E_1 X E_2 / e) \tag{A4}$$

Here we assumed that the sum of the control coefficients with respect to X over all the elemental steps equals 0 [5]:

$$C_1^X + C_2^X + C_3^X + C_4^X + C_5^X + C_6^X = 0 \tag{A5}$$

The relative contribution of the channelled and unchannelled steps to the control of X is quantified by the branch theorems [6]:

$$(C_2^X + C_6^X)/J_{\mathrm{chan.}} = (C_2^X + C_3^X)/(J - J_{\mathrm{chan.}}) \tag{A6}$$

where $J_{\mathrm{chan.}}$ denotes the flux through the channel. Using eqns. (A4) and (A6) one arrives at eqn. (2) of the main text. Taking into account eqn. (A5), one can obtain an expression equivalent to eqn. (2), but now expressing the sum of the enzyme control coefficients in terms of the elemental control coefficients of steps 1 and 4:

$$C_{e_1}^X + C_{e_2}^X = -(J_{\mathrm{chan.}}/J)(C_1^X + C_4^X)/(1 + E_1 X E_2 / e) \tag{A7}$$

A comparison of eqns. (2) and (A7) helps us to understand the conclusion of the main text about the distribution of the control between the steps that result in a decrease in pool size concomitant with a proportional increase in enzyme concentration.

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APPENDIX B

This Appendix gives direct proof that, in a 'hit-and-run' channel, the pool size can decrease when enzyme concentrations increase proportionally. In order to avoid algebra that is too complex, the case is considered when steps 2 and 4 are near equilibrium and steps 1, 3 and 5 are not at equilibrium (hence steps 2 and 4 are not limiting).

Quasi-equilibrium conditions for steps 2 and 4 determine the concentrations of the enzyme intermediates as follows:

$$\begin{aligned} E_1 &= e_1/(1 + X/K_{\mathrm{eq.2}}), \quad E_1 X = e_1(X/K_{\mathrm{eq.2}})/(1 + X/K_{\mathrm{eq.2}}), \\ E_2 &= e_2/(1 + P/K_{\mathrm{eq.4}}), \quad E_2 P = e_2(P/K_{\mathrm{eq.4}})/(1 + P/K_{\mathrm{eq.4}}) \end{aligned} \tag{B1}$$

Here e_1 and e_1 are the enzyme concentrations, X and P are the concentrations of the bulk-phase intermediate and the (ultimate) product respectively and $K_{\mathrm{eq.2}}$ and $K_{\mathrm{eq.4}}$ are the equilibrium constants of steps 2 and 4. Using eqn. (B1) one obtains the following expressions for the rates of steps 1, 3 and 5:

$$\begin{aligned} v_1 &= e_1(k_{+1}s - k_{-1}X/K_{\mathrm{eq.2}})/(1 + X/K_{\mathrm{eq.2}}) \\ v_3 &= e_2(k_{+3}X - k_{-3}P/K_{\mathrm{eq.4}})/(1 + P/K_{\mathrm{eq.4}}) \\ v_5 &= e_1 e_2(k_{+5}X/K_{\mathrm{eq.2}} - k_{-5}P/K_{\mathrm{eq.4}})/[(1 + X/K_{\mathrm{eq.2}})(1 + P/K_{\mathrm{eq.4}})] \end{aligned} \tag{B2}$$

After the substitution of eqn. (B2) into the steady-state

relationships for the elemental rates:

$$v_1 = v_3 + v_5 \tag{B3}$$

the concentration of the bulk-phase intermediate (X) is uniquely determined as a function of enzyme concentrations and rate constants. When the enzyme concentrations e_1 and e_1 increase proportionally with one another, the ratio ($e_2/e_1 = \rho$) is clamped, i.e.:

$$e_1 = e \text{ and } e_2 = \rho e \tag{B4}$$

Substituting eqn. (B4) into eqns. (B2) and (B3) and differentiating eqn. (B3) with respect to e, one obtains:

$$\frac{\mathrm{d}X}{\mathrm{d}e} = \rho v_5/[e(1 + P/K_{\mathrm{eq.4}})(\partial v_1/\partial X - \partial v_3/\partial X - \partial v_5/\partial X)] \tag{B5}$$

Using eqn. (B2) one can see that the partial derivative ($\partial v_1/\partial X$) of rate v_1 with respect to X is negative, whereas such derivatives of rates v_3 and v_5 ($\partial v_3/\partial X$ and $\partial v_5/\partial X$) are positive. Then, from eqn. (B5) it follows that $\mathrm{d}X/\mathrm{d}e$ is negative (for positive flux through the channel, i.e. for positive values of the thermodynamic driving force).