Kinetic implications of metabolite channelling in β-oxidation

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Introduction

Direct transfer of metabolites between consecutive enzymes, or channelling, is a controversial topic, especially if the enzymes do not form a stable complex, i.e. 'dynamic' channelling [1–4]. The level of passion aroused by discussions of metabolite channelling reflects a belief, not always explicitly stated, that if it occurs then it must have major metabolic consequences [5]. Various authors have suggested that channelling can prevent accumulation of intermediates, which might strain the solvent capacity of the cell, or that it may prevent unwanted diversion of intermediates into competing pathways [5,6]. These two ideas are rather different from one another: if channelling occurred primarily to prevent accumulation of intermediates, one should find it in long unbranched pathways; if it occurred primarily to restrict competition, one should encounter it in highly branched pathways.

Srere [6] has estimated that ~80% of metabolites 'have just one use in the cell', in the sense that they do not occur at branch points, and suggests that this is a reason to expect channelling in unbranched pathways. Fatty acid metabolism offers what must surely be the longest unbranched pathways in metabolism: β-oxidation features 28 steps between palmitoyl-CoA and acetyl-CoA, with 27 intermediates, none of which has any other major role in metabolism; fatty acid synthesis provides a similar picture. The organization of the enzymes of fatty acid synthesis into a well defined multienzyme complex [7] supports the idea that preventing intermediates from accumulating may be an important function. Corresponding complexes in β-oxidation were unknown in mammalian systems until the recent identification of a three-enzyme complex located in the inner mitochondrial membrane [8], but even so, the involvement of channelling in β-oxidation has long been considered likely as an explanation for the low concentrations detected of the intermediates [9]. More sensitive techniques now allow many intermediates to be detected [10], but the concentrations are low enough to be still regarded as evidence for channelling [11].

The supposed effect of channelling on intermediate concentrations is however less clear than may appear from superficial study. Computer modelling studies [12] indicated that at a fixed net flux through a pathway, the free concentrations of the intermediates in the steady state were unaffected by the degree of channelling. This work was open to the objection [13] that it depended on the specific numerical values used in the simulation and that other values might have shown effects of channelling on the free concentrations. However, more recent analysis [14] using algebraic rather than numerical results has supported the earlier findings and has, incidentally, shown how some claimed counter-examples [13] arose from misinterpretation of the data.

The results of experimental studies of a multienzyme protein from yeast that carries carbamoylphosphate synthase (glutamine-hydrolysing) and aspartate carbamoyltransferase activities agree with this theoretical analysis: the free concentration of the common intermediate, carbamoyl phosphate, is independent of the degree of channelling [15].

It is now clear, therefore, that channelling of intermediates at constant net flux has no effect on their free concentrations in the steady state. Yet two questions remain unanswered, and these will be addressed in this article: what kinetic properties of the component enzymes in a highly repetitive pathway such as β-oxidation would allow the intermediate concentrations to remain very low even if channelling were not considered, and may channelling have large effects on metabolite concentrations during the period before a steady state is achieved?

Low intermediate concentrations without channelling

The simplest reasonable kinetic model of β-oxidation that one can propose is one in which the four kinds of reactions are catalysed by four enzymes – E_a (acyl-CoA dehydrogenase), E_b (enoyl-CoA hydratase), E_c (hydroxyacyl-CoA dehydrogenase) and E_d (thiolase) – according to reversible Michaelis–Menten kinetics with competition between all possible substrates and products for each enzyme. If the concentrations of the various co-substrates and co-products are treated as constants, they can be subsumed in the kinetic constants for the fatty acid intermediates. Such a model may seem almost hopelessly complicated to analyse, but with some plausible assumptions one can nonetheless arrive at useful conclusions. It is convenient to represent acyl-CoA, enoyl-CoA, 3-hydroxy-acyl-CoA and 3-oxoacyl-CoA with *n*

carbon atoms in the acyl group as A_n, B_n, C_n and D_n, respectively, and the corresponding concentrations as a_n, b_n, c_n and d_n, respectively. It is then straightforward (albeit complicated) to write down an expression for the rate of any reaction, and a corresponding expression for the steady-state concentration of any intermediate in terms of those of the flanking intermediates is produced by setting equal the rates of its formation and consumption. For example, the concentration of $CH_3(CH_2)_8CH=CHCOSCoA$, or B_{12}, may be written as:

$$b_{12} = \frac{k_{12a}e_a a_{12}/S_a + k_{-12b}e_b c_{12}/S_b}{k_{-12a}e_a/S_a + k_{12b}e_b/S_b}$$

where k_{12a} and k_{-12a} represent the forward and reverse specificity constants, respectively, for the reaction between A_{12} and B_{12} catalysed by E_a, k_{12b} and k_{-12b} are defined correspondingly, e_a and e_b are the concentrations of E_a and E_b, respectively, and S_a and S_b are saturation polynomials for E_a and E_b, respectively, that is:

$$S_a = 1 + \frac{a_{16}}{K_{14a}} + \frac{a_{14}}{K_{14a}} + \ldots + \frac{a_2}{K_{2a}} + \frac{b_{16}}{K_{-16b}} + \frac{b_{14}}{K_{-14b}} + \ldots + \frac{b_4}{K_{-4b}}$$

where each K represents a Michaelis constant, for a forward or reverse reaction according to whether its index is positive or negative, and similarly for S_b.

Similar expressions may be written for all of the 27 intermediates in the pathway. If we suppose that a set of conditions exists such that each intermediate concentration is low, not merely on some unspecified scale but in relation to the Michaelis constants for the forward and reverse reactions in which it participates, then each of the four saturation polynomials becomes approximately equal to unity. The apparent complication that many different molecules compete for each active site and each polynomial has in consequence many terms then becomes an irrelevance. Even if this is not accurately true they may nonetheless be similar enough in magnitude to one another for them to be cancelled from the concentration expressions with little error; the expression given above for b_{12}, for example, simplifies to:

$$b_{12} = \frac{k_{12a}e_a a_{12} + k_{-12b}e_b c_{12}}{k_{-12a}e_a + k_{12b}e_b}$$

If we make the further reasonable assumption that all reactions have equilibria that sufficiently favour the forward reactions for all specificity constants for the reverse reactions to be negligible, then this further simplies to $b_{12} = k_{12a}e_a a_{12}/k_{12b}e_b$. All of the other expressions for intermediate concentrations simplify similarly. If all of the forward catalytic activities (taken as the products of enzyme concentrations and specificity constants) are similar in magnitude, then all of the intermediate concentrations will be similar in magnitude also, though not necessarily small. If, however, the first reaction in the pathway has a much lower catalytic activity than all of the others, which are approximately equal to one another, then all intermediate concentrations will be very small compared with the pool concentration of the initial reactant, and virtually all flux control will reside in the first step of the pathway.

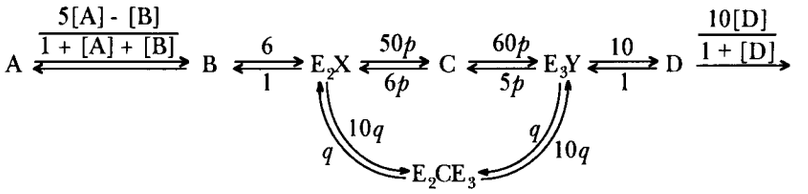
The assumption that all equilibrium constants are very favourable is not strictly true in β-oxidation. For example, the equilibrium constant between decanoyl-CoA and 3-hydroxydecanoyl-CoA is ~2.3 [16]. Although this complicates the quantitative analysis given above, it does not invalidate it qualitatively, because a highly active enzyme can always overcome an unfavourable equilibrium in the reaction that precedes it. In the metabolism of *trans*-ω-6-unsaturated fatty acids, for example, the corresponding equilibrium constant is much smaller, ~0.003 [17]. In such cases, it is likely to be much more difficult to achieve low concentrations of intermediates with free-diffusion kinetics.

It does not follow, of course, that this is the only set of assumptions that could produce this result, but that is unimportant. What matters is that a simple kinetic model, involving no implausible assumptions about the properties of any of the enzymes, is capable of predicting that all intermediate concentrations should be very small in the steady state and that all flux control should reside in the first step. There are thus no grounds for arguing that the low concentrations of intermediates by themselves require channelling or any other exotic hypothesis. There may nonetheless exist other reasons for postulating metabolite channelling. For example, although a mixture of enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase from different sources gave exactly the time course expected for a mixture of non-interacting enzymes [18], the complex having these two activities extracted from *Escherichia coli* did not and completely lacked the expected lag in the build-up of final product [16]. The second point to be examined, therefore, is whether or not channelling can account for the low concentrations of β-oxidation

Figure 1

Model of a dynamic channel

The by-pass via E_2CE_3 allows interconversion of the enzyme–substrate complexes E_2X and E_3Y without release into free solution of the channelled intermediate C. The proportion of the net flux from A passing through the channel was varied by varying the factors p and q in such a way as to maintain constant at 4.15 the steady-state flux from A.



intermediates, if not in the steady state [14] then at least during the approach to the steady state.

Model of a dynamic channel

A model of a dynamic channel is shown in Figure 1. It is structurally the same as that studied previously [12,14], but the kinetic equations have been modified from the original version [12] to give all steps equilibrium constants that favour the forward reactions, i.e. they have been modified in the direction considered by Mendes et al. [13] to make the model more realistic and to favour the possibility of effects of channelling on the concentration of C. The behaviour of this model was studied using the program MetaModel [19] in a modified form that uses the fourth-order Runge–Kutta method with adjustable step size [20] to simulate the approach to the steady state.

Effect of channelling on the approach to the steady state

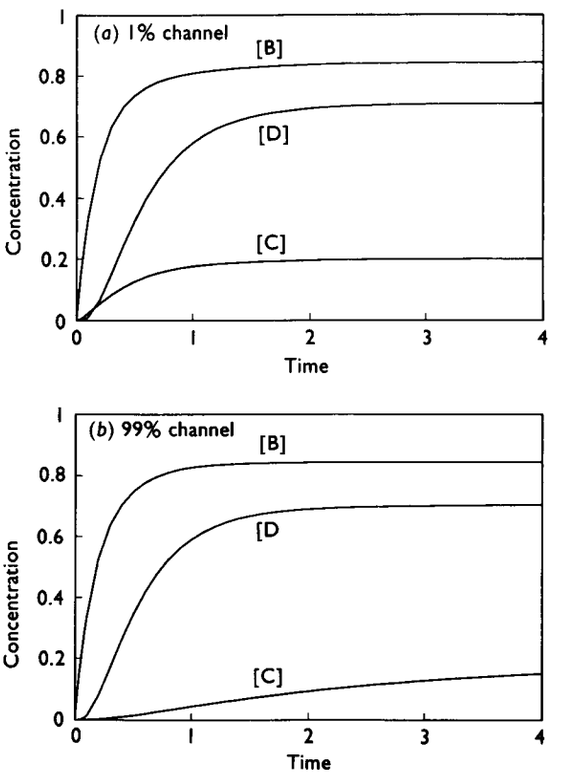
Figure 2 shows the approach to steady state of the concentrations of the three intermediates B, C and D in the model of Figure 1 under conditions where 1% (or 99%) of the steady-state flux passed through the channel. Variation in the channelled proportion has no perceptible effect on the curves for the non-channelled intermediates B and D; this was also true of the pre-steady-state fluxes from A to B, from B to D and from D, which are not shown in Figure 2. Only the concentration of the channelled intermediate C has a time course that varies with the degree of channelling, approaching its steady-state value more slowly as the degree of channelling increases.

If 99% of the flux passes through the channel, the concentration of the channelled intermediate is far from its steady-state value at times when the rest of the pathway is in a state indistinguishable from the steady state. Before concluding that this provides a role for channelling, however, one should note that going from 1 to 99% channelling produces only a five fold increase in the half time for [C] to approach its steady state.

Figure 2

Approach of metabolic intermediate concentrations to their steady-state values

The model of Figure 1 was simulated with (*a*) $p = 1.175$, $q = 0.1193$, for which 1% of the steady-state flux passes through the channel, and (*b*) $p = 0.0118$, $q = 11.5$, for which the net steady-state flux is unchanged at 4.15 but now 99% passes through the channel.



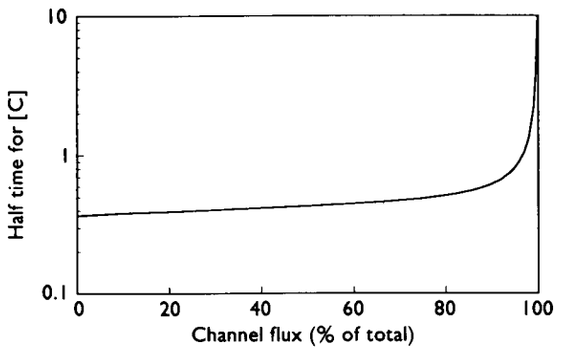
As the smallness of this effect is perhaps rather surprising, it was studied in more detail by examining the dependence of the half time for [C] on the channelling proportion over the range 0–99.8% (Figure 3). Once the channel becomes highly efficient, the half time does show the steep dependence on the channel efficiency that one might have expected. But the channel has to be highly efficient before this becomes evident: > 95% of the flux must pass through the channel before the half time for [C] is doubled.

It follows, then, that for channelling to be seen as a mechanism for preventing build up of free intermediates in the pre-steady-state period, one must postulate either that virtually all the flux passes through the channel (because a 90% channel is only trivially different from no channel at all), or that the pathway is in operation for such a short time that even the net flux does not reach its steady state. The latter condition seems most unlikely to be met in β-oxidation, and the former would likewise be very unlikely in the absence of clear evidence for static organization of all the enzymes involved. The recent discovery of a three-enzyme complex [8] is thus a useful step, but it is insufficient: unless all of the enzymes constitute a static complex, a channelling explanation of the lack of intermediates must inevitably involve some degree of dynamic channelling. Models of static and dynamic channels behave rather similarly, but they are very different at the level of mechanistic plausibility, because a dynamic channel requires assumptions about diffusion rates for macromolecules [14,21] that are difficult or impossible to reconcile with the known effects of molecular 'crowding' on the diffusion behaviour of large and small molecules [22,23].

Figure 2

Effect of channel flux on the time course for a channelled intermediate

The factors p and q were varied as in Figure 2 to vary the proportion passing through the channel from 0 to 99.8% while maintaining the net steady-state flux constant.



Most of the intermediates in β-oxidation contain large hydrophobic groups, and their release into free solution is by no means as favourable as it is for most of the disputed examples of channelling in other areas of metabolism. Two-dimensional diffusion on the surface of a static complex could thus be a much faster process than diffusion through the aqueous solution; however, whether this would be metabolite channelling as it is usually understood is another matter.

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