Moiety-conserved cycles and metabolic control analysis: problems in sequestration and metabolic channelling

Herbert M. Sauro

Penrhyn, Pontrhydygroes, Ystrad Mynach, Dyfed SY25 6DP, UK

Received 2 September 1993; revision received 6 January 1994

Abstract

This paper considers certain aspects of the analysis of moiety-conserved cycles in terms of metabolic control analysis. Two response coefficients are discussed: the response coefficient with respect to the total number of moles in a cycle ($R_F^i$), and the response coefficient with respect to perturbations to the internal state of a pathway ($R_S^i$). The relationship between these two different measures is derived and two examples are given to illustrate how the results may be used to simplify the analysis of particular complex pathways. One example considers how metabolite sequestration affects the flux summation theorem for which the analysis confirms the known result that sequestration can depress the value of the summation to below unity. The second example investigates the effect of metabolic channelling on the summation theorems. The analysis indicates that in contrast to metabolite sequestration, metabolic channelling can cause the flux summation theorem to exceed the value of unity. In addition, the maximum value that the summation theorem can reach under these conditions is shown to be equal to 2. Finally, this analysis indicates how one might use control analysis through the use of enzyme titration to determine whether metabolic channelling occurs in real systems or not.

Keywords: Metabolism; Metabolic control analysis; Moiety-conserved cycle; Metabolite sequestration; Channelling

1. Introduction

A visual inspection of one of the commercially available metabolic pathway maps will reveal to the viewer an enormous array of complexity. However, upon closer examination, certain common structural features should become apparent. These features include: linear segments of transformations where a metabolite is transformed via a number of linearly connected reaction steps; branching points where a metabolite can be transformed by two or more different reaction steps; and cycling, where a metabolite, say $S_1$, is transformed by a series of reaction steps such that the final transformation regenerates $S_1$. Cycling structures can in turn be further classified into two groups, namely conserved and non-conserved (see Fig. 1). A conserved cycle is formed (Reich and Sel'kov, 1981) when some chemical grouping is preserved by the cycle so that it suffers neither net synthesis nor...
Non-conserved Cycle. Conserved Cycle.

![Diagram indicating the difference between non-conserved and conserved cycles. In a non-conserved cycle there is free movement of mass into and out of the cycle, whereas in a conserved cycle some particular moiety is conserved, and the mass is only allowed to enter and leave the cycle by multi-molecular reactions.]

breakdown. Such groupings are called moieties and when conserved, the cycles in which they react are termed moiety-conserved cycles. Non-conserved cycles are those where the metabolites of the cycle can leak to or from the cycle so that conservation of any particular grouping is not possible. This paper will be solely concerned with moiety-conserved cycles.

There are many examples of moiety-conserved cycles in metabolism, for instance, the adenine nucleotide and NAD moieties are part of moiety-conserved cycles. The adenine nucleotide is conserved among the transformations that interconvert the forms AMP, ADP and ATP, while NAD is conserved between its two forms, NAD and NADH. Although it was stated that conserved moieties experience neither net synthesis nor breakdown, this situation can be only an approximation because the moiety must at some point have been synthesised to be present in the first place. For this reason, there will in general be a slow metabolic turnover of conserved moiety by other parts of metabolism. If we assume that the moiety interconversions are fast (e.g. NAD $\rightleftharpoons$ NADH) compared with their general turnover, then over a suitably short interval (e.g. the time course of an experiment) the moiety may be considered conserved.

A conserved moiety that is often neglected in studies of metabolism is the enzyme moiety. The conservation of enzyme moiety can be appreciated when one considers the standard Michaelian model of enzymatic catalysis, where free enzyme binds to substrate, effects a chemical transformation, and releases product to regenerate free enzyme. Clearly the enzyme is conserved in this process. The point previously made concerning the approximation inherent in identifying a conserved cycle applies equally to the enzyme cycle since an enzyme (or any protein) is continually being remade and broken down. However, the catalytic cycle is much faster than the general turnover of enzyme so that assigning moiety-conserved cycle status to the enzyme catalytic cycle is quite reasonable.

In terms of metabolic control analysis, moiety-conserved cycles were first discussed by Westerhoff (1983) and Fell and Sauro (1985) and in much more detail by Hofmeyr et al. (1986). These studies showed that the control properties of moiety-conserved cycles were quite distinct from non-conserved cycles. The reason for this difference is
that moiety-conserved cycles introduce an additional parameter into the metabolic control analysis, namely the total amount of moiety in the cycle. As an illustration consider the simplest possible moiety-conserved cycle where a moiety $S_1$ is transformed into $S_2$, which in turn is transformed by a separate reaction back to $S_1$. By inspection of the cycle (see Fig. 2) it should be apparent that the total number of moles of moiety, $S_1 \cdot \text{Vol}_1 + S_2 \cdot \text{Vol}_2$ is fixed, that is:

$$S_1 \cdot \text{Vol}_1 + S_2 \cdot \text{Vol}_2 = T$$

where $T$ is a constant equal to the total number of moles of moiety in the cycle, $S_1$ and $S_2$ are concentrations, and $\text{Vol}_1$ and $\text{Vol}_2$ are the volumes which $S_1$ and $S_2$ occupy, respectively. Although the levels of metabolites are normally expressed in terms of concentration, it is the number of moles that is conserved and not the concentration, hence the presence of the volume terms in the conservation constraint. However, to keep the following analysis as simple as possible, it is assumed that all metabolites exist in a single unit of volume and therefore the moiety conservation constraint can be expressed in the simpler form

$$S_1 + S_2 = T$$

In the above equation, the volume terms are implied and for the remainder of the discussion it is assumed that all volumes are at constant unit volume.

2. Control analysis

The quantitative analysis of metabolism is usually made in terms of metabolic control analysis (Kacser and Burns, 1973; Heinrich and Rapoport, 1974; Fell, 1992). This method describes metabolism in terms of responses of metabolic variables, such as fluxes and metabolite concentrations, to changes in one or more metabolic parameters, for example, the concentration of an enzyme. The measures that describe such responses are termed control coefficients and a general definition is given by

$$C_p^V = \frac{dV}{dp} \frac{p}{V}$$

where $V$ is some variable and $p$ some parameter. The total derivative is used in the definition to indicate that all metabolites and fluxes of the pathway are allowed to move in response to the perturbation. If the parameter $p$ is some external effector other than the change in the concentration of an enzyme then the coefficients are often termed response coefficients (Kacser and Burns, 1973). Such external effects might include the effect of enzyme inhibitors or changes to the boundary conditions. Of particular interest in this paper is the external parameter related to the total number of moles in a moiety-conserved cycle, namely $T$. Referring again to the example shown in Fig. 2 one could envisage changing the value of $T$ by adding either $S_1$ or $S_2$ and measuring the cycle response. In other words one could define the response coefficient,

$$R_T^V = \frac{dV}{dT} \frac{T}{V}$$

Such a response coefficient was first described in detail by Hofmeyr et al. (1986), where some of its properties and its relation to metabolic control analysis were discussed. One of the important results presented by Hofmeyr et al. was the recognition that the sum of the cycle metabolites act as external parameters to the rest of the system.
Since Hofmeyr et al. (1986) introduced the response coefficient, $R_f^v$, it has been realised that a second, closely related, response coefficient can also be defined (Reder, 1988). Reder introduced an additional class of 'parameter' which was related to changes in the internal state of the pathway. The interpretation of changes to the internal state is, however, more subtle, particularly with regard to the definition of the related response coefficient. For the purposes of this paper the internal state of a pathway will refer to the steady state concentrations of the metabolites; therefore, perturbations to the internal state relate to fluctuations in the steady state metabolite concentrations. Experimentally this might be accomplished by adding to the pathway the particular metabolite of interest and observing the steady state response. Operationally, for metabolites that do not make up moiety-conserved cycles, fluctuations to the internal state will have no net effect on the steady state. This is because the steady state is stable and any fluctuations will decay to zero. However, for metabolites that do form part of moiety-conserved cycles, a fluctuation will have a net effect because the total number of moles in a cycle will have been changed and, as Hofmeyr et al. (1986) have shown, this will cause the steady state variables of the pathway to change.

Operationally, response coefficients measured with respect to fluctuations to the internal state can be described in the following way. Since response coefficients are defined in terms of differential changes, they can be approximately described by

$$R_f^v = \frac{\delta V}{\delta S} = \frac{\delta V}{V} \left/ \frac{\delta S}{S} \right.$$  

where the $\delta$ signifies a 'small' change, e.g. $\delta S$ signifies a 'small' change in the metabolite $S$.

The term, $\delta S/S$ in the above definition requires special consideration. Usually a $\delta S/S$ term represents an effect which signifies the steady state change in the variable, $S$, as a result of a parameter perturbation. However, in the above definition the $\delta S/S$ term means operationally something different. The $\delta S/S$ term in fact relates to the externally imposed $\delta S$ change on $S$ before $S$ or any other metabolite or flux has had time to begin to evolve to a new steady state, in other words it represents a cause. This interpretation resolves the apparent inconsistency of a control coefficient relating the change in one variable to a change in another variable because the $\delta S/S$ in the denominator is effectively being treated as a parameter, i.e. an experimentally controllable quantity. Experimentally, the addition of a small quantity of $S$ cannot, however, be easily measured because the new concentration of $S$ will begin to change immediately. Instead, $\delta S/S$ can be measured from a knowledge of the initial steady state concentration in $S$ and the $\delta S$ added by the experimenter. Bringing these points together we arrive at a more meaningful symbolic definition by using the notation:

$$R_s^v = \frac{(\delta V/V)_{\text{final}}}{(\delta S/S)_{\text{initial}}} \quad (2)$$

We have thus two types of response coefficient that can be used to describe the responses of moiety-conserved cycles. One response coefficient that refers to changes in the total number of moles of a moiety ($R_f^v$ — Eq. 1) and a second that reflects the effect of changes to the individual members of a cycle ($R_s^v$ — Eq. 2). One of the aims of this paper is to show how one can relate these two response coefficients. At this point the reader may be wondering what could the purpose be of relating these two coefficients other than to have the satisfaction of filling an algebraic gap in the theoretical analysis of metabolism. Two reasons can be given as to why it is important from a biological point of view to relate these two measures. Since Reder published her work on the $R_s^v$ coefficient much of MCA that has since been developed now relates to this coefficient. As a result, the $R_f^v$ coefficient of Hofmeyr et al. is somewhat isolated from mainstream MCA. However, the $R_f^v$ coefficient is a much more accessible coefficient from an experimentalist's point of view. It would thus seem appropriate to be able to relate the two coefficients. The second reason for relating these two coefficients is that it enables one to tackle certain problems in metabolism much more easily and the examples towards the end of this paper are intended to demonstrate this point.
For organisational purposes, the following discussion is in two parts. The first part describes the relationship between \( R_v^v \) and \( R_v^y \) in simple cycles where each moiety in a conserved cycle has no association with any other conserved cycle, and a second part where the more complex situation of linked cycles are dealt with. Finally, two examples are given that illustrate how the relationships might be usefully applied.

### 3. Simple moiety-conserved cycles

The first case considered is the 'simple' case where a conserved moiety is common to only a single cycle, for example the cycle shown in Fig. 2. When one discusses the effect of changing the total number of moles of metabolite, \( T \), in a conserved cycle, it is important to remember that there is no metabolite called 'total'. Instead, the total is a function of the 'real' metabolite concentrations that make up the conservation laws. Often the relationship between the total and individual metabolite concentrations takes the simple form:

\[
T = \sum_i S_i
\]

In other words the total number of moles of moiety is simply the sum of the participating members of the cycle. The only way of changing the total \( T \) is by changing the amount of one or more of the \( S_i \) metabolites and relating this to the change in \( T \). Thus, given an initial change \( \delta S_i \), and with all other metabolite concentrations remaining unchanged, the effect on \( \delta T \) is, by mass conservation, given simply by \( \delta T = \delta S_i \). Thus, in terms of fractional changes, the change in \( S_i \) can be related to the change in \( T \) by the relation:

\[
\frac{\delta T}{T} = \left( \frac{\delta S_i}{S_i} \right)_{\text{initial}} \frac{S_i}{T}
\]

(3)

where \( \delta S_i/S_i \) is the initial fractional change in \( S_i \), \( \delta T/T \) the fractional change in \( T \) and \( S_i/T \) is the mole fraction of \( S_i \) in the conserved total. According to the operational definition of the internal state response coefficient, namely,

\[
R_{v}^{v} = \frac{\left( \delta V/V \right)_{\text{final}}}{\left( \delta S_i/S_i \right)_{\text{initial}}}
\]

(4)

the \( \delta S_i/S_i \) that occurs in Eq. 4 is the same \( \delta S_i/S_i \) as given in Eq. 3, and by substitution a relationship between the two different coefficients is obtained, thus:

\[
R_{v}^{v} = R_{v}^{v} \frac{S_i}{T}
\]

(5)

A further relationship between \( R_{v}^{v} \) and \( R_{v}^{v} \) can also be derived as follows. The general form of a metabolite conservation law is given by:

\[
T = \sum_{i=1}^{m} n_i S_i
\]

(6)

where \( n_i \) relates to the stoichiometric amounts of each metabolite that contribute to the total (often \( n_i = 1 \)) and \( m \) equals the number of participating metabolites. Eq. 5 can now be generalised to:

\[
R_{v}^{v} = R_{v}^{v} n_i \frac{S_i}{T}
\]

Summing over all \( i \) metabolites that are members of the moiety-conserved cycle leads to

\[
\sum_i R_{v}^{v} = R_{v}^{v} \sum_i \left( n_i \frac{S_i}{T} \right)
\]

but since from Eq. 6

\[
\sum_i n_i \frac{S_i}{T} = 1
\]

therefore:

\[
R_{v}^{v} = \sum_i R_{v}^{v}
\]

(7)

Since this section only deals with simple cycles, Eq. 7 does not apply if any of the metabolites, \( S_i \), is a common metabolite to more than one conserved cycle. For simple cycles, the relationship is...
clearly very straightforward; the response to a change in the total is equal to the sum of the responses with respect to each metabolite in the cycle.

4. Linked moiety-conserved cycles

There are many instances in metabolism where a metabolite carries more than one kind of moiety so that the metabolite is shared by more than one conserved cycle (Sauro et al., 1987). Consider a metabolic system which has two conserved cycles with the following molar constraints:

\[ A + B = T_1 \]
\[ A + S_1 + S_2 = T_2 \]

The shared metabolite is clearly metabolite \( A \). \( T_1 \) and \( T_2 \) are the total amounts of moiety in each respective cycle. If an initial perturbation is made to metabolite \( B \), then this will cause only \( T_1 \) to change, without any alteration to total \( T_2 \) because \( B \) only carries moiety for the first cycle. If, however, an initial perturbation is made instead to metabolite \( A \), then both \( T_1 \) and \( T_2 \) will change because \( A \), being the shared metabolite, must be carrying both moieties. Using the law of mass conservation the perturbation in \( A \) can be represented by the identities:

\[ \delta T_1 = \delta A \]
\[ \delta T_2 = \delta A \]

or in fractional terms:

\[ \frac{\delta T_1}{T_1} = \left( \frac{\delta A}{A} \right) \frac{A}{T_1} \]
\[ \frac{\delta T_2}{T_2} = \left( \frac{\delta A}{A} \right) \frac{A}{T_2} \]

where the subscript \( I \) denotes 'initial'. Once the perturbation in \( A \) has been made the pathway will begin to evolve to a new steady state and all system variables, such as other metabolites and fluxes, will be affected. There are two ways in which one can express how \( A \) affects a system variable, either via the \( R_S^V \) coefficient or \( R_F^V \) coefficient. In terms of the change in both totals, \( T_1 \) and \( T_2 \), the change in a system variable can be expressed using the \( R_F^V \) coefficients as the sum of the effects:

\[ \frac{\delta V}{V} = R_F^V \frac{\delta T_1}{T_1} + R_F^V \frac{\delta T_2}{T_2} \]

In terms of the change that was made to \( A \), the change in the system variable can be expressed in terms of the \( R_A^V \) coefficient as

\[ \frac{\delta V}{V} = R_A^V \frac{\delta A}{A} \]

Equating these two changes, since they are equivalent, and using the fractional relations (Eq. 8), one obtains

\[ R_A^V = R_F^V \frac{A}{V_1} + R_F^V \frac{A}{T_2} \]

Eq. 9 represents a relationship between the two coefficients. In more general terms, it is straightforward to show that

\[ R_{Si}^V = \sum_i R_{Si}^F n_{ik} \frac{S_i}{T_i} \]

where \( n_{ik} \) is the stoichiometry of moiety \( k \) occurring in cycle \( i \). A matrix formulation can also be derived by inspection of the scalar form (Eq. 10) and in terms of the specific variables, flux and metabolite concentrations, one can show that:

\[ R_S^F = R_F^F (\text{diag } T)^{-1} \gamma \text{ diag } J \]
\[ R_S^S = R_F^S (\text{diag } T)^{-1} \gamma \text{ diag } S \]

where \( R_S^F \) is an \( n \) (number of reactions) by \( m \) (number of metabolites) matrix containing \( R_S^F \) elements; \( R_F^F \) is an \( n \) by \( N_t \) (number of conserved cycles) matrix; \( \text{diag } T \) is an \( N_t \) by \( N_t \) square matrix with all off-diagonal elements equal to zero, \( \text{diag } T_{ij} = T_{ii} \); \( \text{diag } S \) is an \( m \) by \( m \) square matrix with all off-diagonal elements equal to zero, and \( \text{diag } S_{ii} = S_i \); \( R_S^S \) is an \( m \) by \( m \) square matrix; and \( R_S^S \)
is an $m$ by $N_i$ matrix. Finally, $\gamma$ is the stoichiometric conservation matrix ($N_i$ by $m$) that relates the kind and stoichiometry of metabolites that enter each conserved cycle of the pathway (Clarke, 1980). Using $\gamma$, Eq. 6 can be generalised to matrix form as:

$$\gamma S = T$$

where $S$ is the $m$-dimensional vector of metabolites, and $T$ is the $N_i$-dimensional vector of total molar masses. $\gamma$ can be computed from the $L_o$ matrix as defined by Reder (1988) using the construction

$$\gamma = [-L_o I]$$

5. Applications

Two examples are now given of how one might use the relationships given above. One of the concerns of MCA is to discover under what conditions do the enzyme-dependent flux and metabolite summation theorems breakdown, i.e. when $\Sigma_i C_i^{E'} \neq 1$ and $\Sigma_i C_i^{S'} \neq 0$. A number of metabolic mechanisms are known that can potentially cause such a violation, in particular metabolite sequestration, metabolic channelling, and group transfer. Proving theoretically that such violations can occur, however, is not straightforward, but by using the relationships introduced in this paper the task becomes much simpler.

The first example illustrates how one can easily show that metabolite sequestration in a moiety-conserved cycle results in a depression of the flux summation theorem. Although this conclusion is not new, the approach used is sufficiently novel to warrant discussion. The second example is an investigation of how metabolic channelling affects the summation theorems and for this the conclusion is new.

5.1. Metabolite sequestration

In traditional control analysis one assumes that in a moiety-conserved cycle, all the mass of metabolites is to be found in free bulk-phase form. In other words, the enzymes that catalyse the individual reactions of the cycle are assumed only to sequester an insignificant amount of free bulk-phase metabolite. If for some reason the enzymes do sequester a significant proportion of the free bulk-phase metabolites, then it has been shown that the enzyme flux and metabolite summation theorems no longer hold (Fell and Sauro, 1990; Kholodenko et al., 1992). For example, the enzyme flux summation theorem will be depressed to below the expected value of 1.

The simplest model that exhibits the phenomenon of metabolite sequestration is a two-membered conserved cycle (Fig. 3), where $S_1$ is transformed to $S_2$, and $S_2$ transformed by a different reaction back to $S_1$. One of the enzyme-catalysed steps shown in the figure is considered in its full mechanistic form so that the effect of
sequestration can be followed. To simplify the following discussion the other step of the cycle is considered not to sequester free metabolites to any significant degree. A numerical simulation of this system can be found in Fell and Sauro (1990) and Sauro (1993).

The molar constraints for this system can easily be written down by simple inspection of the pathway. They are:

\[ E + ES = T_1 \]
\[ S_1 + S_2 + ES = T_2 \] (11)

where \( T_1 \) is the total number of moles of enzyme one, and \( T_2 \) the total number of moles of metabolite. Note how \( T_2 \) is made up of both free pools, \( S_1 \) and \( S_2 \), and the complexed form \( ES \). \( ES \) is therefore a bridging species, linking two moiety-conserved cycles.

The aim of the following analysis is to compute the enzyme flux control coefficient for the step made up of the explicit enzyme catalytic cycle. In a 'normal' control analysis this step would be condensed into a single reaction and the individual steps that make up the catalytic cycle would be hidden from the analysis. In such a case the enzyme flux control coefficient would be denoted by the symbol, \( C_e^I \). However, in this model the enzyme flux control coefficient can be described by the response coefficient, \( R_{T_1}^I \). Operationally, \( C_e^I \) and \( R_{T_1}^I \), are equivalent, and that computing \( R_{T_1}^I \), is the same as computing \( C_e^I \), except that by computing \( R_{T_1}^I \) we gain a greater understanding of the mechanics of the system through knowledge of the internal enzyme mechanism. The following analysis will therefore concentrate on evaluating \( R_{T_1}^I \), on the understanding that it is equivalent to \( C_e^I \). There are two ways to write equations involving \( R_{T_1}^I \) one in terms of \( R_{E}^I \) and another in terms of \( R_{ES}^I \). Using the results presented previously (Eq. 10) one can show that

\[ R_{E}^I = R_{T_1}^I \frac{E}{T_1} \] (12)
\[ R_{ES}^I = R_{T_1}^I \frac{ES}{T_1} + R_{T_2}^I \frac{ES}{T_2} \]

Summing these equations gives

\[ R_{E}^I + R_{ES}^I = R_{T_1}^I \frac{ES}{T_2} \] (13)

To simplify this equation further an equation that relates to the \( R_{E}^I \) and \( R_{ES}^I \) terms must be used. Reder (1988) has shown that flux response coefficients with respect to perturbations to the internal state are of the following form:

\[ R_S^I = \sum_i C_{v_i}^I \epsilon_{\xi}^{v_i} \] (14)

where the sum is over all reactions that interact with \( S \) and \( C_{v_i}^I \) is the parameter-independent flux control coefficient (Heinrich and Rapoport, 1974; Sauro and Kacser, 1990) and \( \epsilon_{\xi}^{v_i} \) the elasticity. Using Eq. 14, \( R_{E}^I \) and \( R_{ES}^I \) can be expanded to

\[ R_{E}^I = C_{v_2}^I \epsilon_{\xi}^{v_2} + C_{v_3}^I \epsilon_{\xi}^{v_3} \]
\[ R_{ES}^I = C_{v_1}^I \epsilon_{\xi}^{v_1} + C_{v_4}^I \epsilon_{\xi}^{v_4} \] (15)

Since the reactions of an enzyme mechanism are all first order, the elasticities in the above expressions are all equal to unity, therefore Eq. 15 simplifies to

\[ R_{E}^I = C_{v_2}^I + C_{v_3}^I \]
\[ R_{ES}^I = C_{v_1}^I + C_{v_4}^I \]

Inserting these identities on the right-hand side of Eq. 13 and adding the control coefficient of the non-sequestering step \( (C_{v_1}^I) \) to both sides yields

\[ C_{v_1}^I + C_{v_2}^I + C_{v_3}^I + C_{v_4}^I + C_{v_5}^I = R_{T_1}^I \]

\[ + R_{T_2}^I \frac{ES}{T_2} + C_{v_5}^I \]

but since by definition:

\[ C_{v_1}^I + C_{v_2}^I + C_{v_3}^I + C_{v_4}^I + C_{v_5}^I = 1 \]
therefore we arrive at a summation theorem that is modified:

$$ R_{T_1}^{I} + C_{v_5}^{I} = 1 - R_{T_2}^{I} \frac{ES}{T_2} $$

Eq. 16 was previously shown to be true by Fell and Sauro (1990) and more explicitly by Kholodenko et al. (1992). A similar approach can be used to derive the analogous equation in terms of \( R_{T_2}^{I} \). The left-hand side of Eq. 16 is the usual summation theorem written in terms of the concentration of enzyme one and the activity of enzyme two. The equation essentially shows how much the summation theorem deviates from unity depending on the response of the system to changes in the total metabolite concentration and the ratio of enzyme complex to total metabolite concentration. It is also worth noting that since \( R_{T_2}^{I} \), is positive, the equation shows in a simple way that the sequestration of free metabolites in moiety-conserved cycles leads to a depression of the summation theorem to below unity.

5.2. Metabolic channelling

The traditional view of metabolism is one of a bulk-phase aqueous solution containing a homogeneous distribution of enzymes. In this scheme, each enzyme is considered to act in a catalytically independent manner, binding to substrates from a 'well mixed' bulk-phase and subsequently releasing product immediately back to the bulk-phase ready for the 'next' enzyme to catalyse the following step. The term 'channelled system' has been used by many authors to signify an alternative and somewhat speculative form of metabolic organisation. In the channelled scheme, enzymes catalysing different steps associate to form heterologous complexes. It is presumed that in the most extreme form of this view, the complexes are able to catalyse a series of reaction steps without releasing any of the intermediate metabolites into the bulk-phase. The pathway shown in Fig. 4 shows the model considered here and corresponds closely to the models studied by Cornish-Bowden and Cárdenas (1993) and Mendes et al. (1992). The pathway consists of two enzymes catalysing a two-step linear sequence. However, unlike the usual linear sequence, the enzyme-substrate complex of the first enzyme is able to interact with unbound enzyme from the second step and in the process release free enzyme one and form the second enzyme-substrate complex. This interchange step thereby bypasses the normal route for a metabolite, namely via the bulk-phase intermediate denoted by \( S \). The channel step could be made more realistic by adding an intermediate complex,

Fig. 4. A complex set of two linked enzyme cycles forming a metabolic channel. \( E_1 \) and \( E_2 \) denote the two free enzyme forms in the model and \( ES_1 \) and \( ES_2 \) are the enzyme-substrate complexes for each enzyme. \( S \) represents the free bulk-phase metabolite and reaction \( v_5 \) represents the metabolite channel diverting flux away from the 'normal' bulk-phase route. A control analysis of this model reveals that at intermediate channelling the summation theorems are violated by a positive deviation, thus \( \sum C_{v_i}^{I} \geq 1 \). At low and high channelling, there is no violation.
say \( E_1 S_1 E_2 \), but this was omitted because its presence was found not to be important to the conclusions that follow.

To simplify the analysis, assume that all steps are irreversible. The aim of the analysis is to derive the enzyme flux summation theorem and to determine whether any violation occurs. The coefficients of interest are \( R_j^I \) and \( R_j^F \), where \( T_1 \) refers to the total amount of enzyme one and \( T_2 \) refers to the total amount of enzyme two. The mass constraint equations for this system are given by

\[
T_1 = E_1 + E S_1 \\
T_2 = E_2 + E S_2
\]

This system is simpler to analyse than the previous sequestration model because the cycles are not linked and as a result one can use Eq. 7. Concentrating on the flux variable and applying Eq. 7 one obtains:

\[
R_j^T_1 = R_j^E_1 + R_j^E S_1 \\
R_j^T_2 = R_j^E_2 + R_j^E S_2
\]

Using Eq. 14 one can expand as before the coefficients on the right-hand side so that:

\[
R_j^E_1 = C_j^{E^I} \\
R_j^E S_1 = C_j^{E^I} + C_j^{E^S} \\
\]

and for the second step

\[
R_j^E_2 = C_j^{E^I} + C_j^{E^S} \\
R_j^E S_2 = C_j^{E^S}
\]

In each case one can now compute the response coefficient for each enzyme by summing the appropriate parameter independent control coefficients. Thus for \( T_1 \) and \( T_2 \):

\[
R_j^T_1 = C_j + C_j^{E^I} + C_j^{E^S} = C_j^{E^I} \\
R_j^T_2 = C_j + C_j^{E^I} + C_j^{E^S} = C_j^{E^I}
\]

The terms \( C_j^{E^I} \) and \( C_j^{E^F} \) are shown to emphasise that they are equivalent to \( R_j^{I_1} \) and \( R_j^{I_2} \) coefficients. The point to take of note is that the parameter independent control coefficient for step five \( (C_j^{E^S}) \), the 'channelling' step, is counted twice. If the two response coefficients are summed one obtains the flux summation theorem as

\[
R_j^{I_1} + R_j^{I_2} = 1 + C_j^{E^I}
\]

Clearly the summation theorem (Eq. 17) is violated in this system by an amount given by the parameter independent control coefficient for the fifth step, i.e. \( C_j^{E^S} \). This control coefficient can be expanded using the control analysis approach given by Fell and Sauro (1985). Rather than perform the necessary algebra by hand, the required matrix construction and inversion was performed by the program METACON (Thomas and Fell, 1993) with subsequent checking and confirmation by the simulation program SCAMP (Sauro, 1993). This approach yielded the following expression for \( C_j^{E^S} \):

\[
C_j^{E^S} = \frac{\alpha E_2 E S_1}{E_2 T_1 + \alpha E S_1 E S_2}
\]

where \( \alpha \) is the ratio of channel flux \( \langle v_5 \rangle \) to the net pathway flux \( \langle v_1 \rangle \), so that when \( \alpha = 0 \), the channelled route is not active, while at \( \alpha = 1 \) (its maximum value) all flux goes through the channelling step. The first point to note from the above expression is that \( C_j^{E^S} \) must always be positive which means that the summation theorem will always show a positive deviation. This is an interesting contrast to the previous sequestration model where the deviation was negative. The second point is that at both extreme values of \( \alpha \) (0 and 1), \( C_j^{E^S} \) tends to zero and this implies a bell shaped response of \( C_j^{E^S} \) versus \( \alpha \). Unfortunately it is difficult to obtain from this equation the exact shape of the response curve because the concentration terms \( E_1, ES_1 \) and \( ES_2 \) are themselves a function of \( \alpha \) and this function is not known. The only way to obtain the response curve is by simulation. Fig. 5 shows one such simulation which plots the value of the summation theorem against \( \alpha \) (the degree of
Fig. 5. A computer simulation showing the amount of deviation of the flux summation theorem versus the degree of channelling (ratio of channel flux to net flux, altered by increasing the value of $k_3$) in the metabolic channelling model described in Fig. 4. Note how maximum deviation occurs at intermediate channelling rates while at very low and very high channelling, no deviation is observed. The simulation was carried out using the SCAMP simulation package (Sauro, 1993). Parameters for the model were: $k_1 = 2.3$, $k_2 = 0.3$, $k_3 = 1.2$, $k_4 = 3.3$, $k_5 = 8.9$, $X_0 = 1$ and $X_1 = 0.0$. $T_1 = E_1 + ES_1 = 2.5$ and $T_2 = E_2 + ES_2 = 1.2$.

channelling). The simulation was performed using the metabolic simulation package SCAMP (available by ftp from the Oxford metabolic programs archive bmsdarwin.brookes.ac.uk (161.73.104.10)). Details of the values of the parameters used in the simulation can be found in the legend of Fig. 5. However, the main point to observe is the bell shaped response, so that deviation of the summation theorem from the expected value of 1 only occurs at intermediate channelling rates. At low and very high rates of channelling the summation theorem reverts to the classical expectation, namely $\Sigma C_r = 1$. The other statement one can make about $C_r$ is that its value cannot exceed unity, therefore the maximum deviation the summation theorem can achieve is 2. It is interesting to note that the maximum deviation of 2 is identical to the maximum deviation reported (Van Dam et al., 1993) for group transfer reactions. In fact an analysis of group transfer systems using the method described here, confirms their result (Kholodenko, Sauro, and Westerhoff, in press). If the channel is made reversible, two additional effects emerge. The effect on the summation theorem is to add the control coefficient for the reverse step to $C_r$. If, for the sake of argument the reverse step is designated $v_6$, then the summation theorem becomes modified to:

$$R_{T_1}^L + R_{T_2}^L = 1 + (C_{v_6} + C_{v_6})$$

An inspection of $C_{v_6}$ however, will reveal that it has a negative value, in other words, increasing the activity of the reverse step reduces the net flux. The effect therefore of $C_{v_6}$ is to reduce the deviation on the summation theorem. Under the condition when the forward and reverse rates for the channelled reaction are equal then $C_{v_6}$ and $C_{v_6}$ are equal in magnitude but opposite in sign and cancel any deviation so that the summation theorem equals 1.
Finally, under the unusual condition that the reverse rate, \( r_6 \), should exceed the forward rate, \( r_5 \), the net deviation becomes negative and the summation theorem is depressed to below unity. However, under these conditions, the enzyme system begins to exhibit ‘futile’ cycling.

A similar analysis can be done to show that the metabolite summation theorem will also be violated. For example, the metabolite summation theorem with respect to the bulk-phase metabolite, \( S \), can be readily shown to be:

\[
\sum_i R^S_i = C^S_y
\]

i.e. the summation shows a deviation equal to \( C^S_y \). One can also show by simulation or by computing \( C^S_y \) algebraically that its value is positive. This means of course that increasing the efficiency of the channelled step causes the free bulk-phase intermediate to increase in concentration. This result is in contrast to the recent claim (Mendes et al., 1992) that channelling causes a decrease in pool size (however, see Cornish-Bowden and Cárdenas (1993) for further arguments on this point).

6. Discussion

One of the primary aims of metabolic control analysis has been to devise methods that enable one to describe the steady state response of an intact metabolic pathway in terms of the properties of the constituent parts. The response of a pathway can be any measurable quantity, but generally what is measured is the steady state pathway flux or floating metabolite concentrations. The properties of the constituent parts relate to the behaviour of the enzymes that make up pathways. The usual enzyme properties that are considered are the concentration of enzyme and the kinetic constants. The ability to relate an intact pathway property to the basic properties of the enzymes is probably the major advance made by MCA, especially since before the advent of MCA the only way of achieving such a feat was by brute force simulation of particular cases with particular parameter combinations.

In this paper some novel approaches of MCA to certain problems in metabolic biochemistry are described. In particular, the relationship between two response coefficients are described and it is illustrated how the relationship may be used to understand certain problems that occur with metabolite sequestration and metabolic channelling. The two response coefficients in question are the response of a pathway to changes in the internal state of a pathway (termed \( R^S_y \)) and the response of a pathway to changes in the total amount of moiety in a moiety-conserved cycle (termed \( R^y_f \)). Two cases are considered, simple moiety-conserved cycles where the moiety carrying metabolites are not shared by any other cycle and linked cycles where two or more moiety-conserved cycles may share a common metabolite. By analysing such systems in terms of the two response coefficients a novel approach to deriving the summation theorem for pathways where the enzymes are considered in their full mechanistic form is obtained. To illustrate this approach two examples are chosen, the first example is one where an enzyme is considered to sequestor free metabolite; as a result, the flux summation theorem, which is classically expected to equal unity, is found to deviate below unity. The second example that is considered is a channelled system, where it is found that the flux summation theorem, in contrast to the sequestration model, shows a positive deviation from unity. It is of particular interest that a channelled system should show positive deviation of the flux summation theorem because this result offers an experimental method by which the occurrence of channelling can be detected, namely by titration of the pathway with free enzyme and measuring the flux response. This approach has been discussed before by Sauro and Kacser (1990) and the results presented here reinforce this point further.

Acknowledgements

I would like to thank Rankin Small for useful comments on the manuscript and for giving me access to his computer. I would also like to thank the anonymous reviewers whose comments helped to improve the manuscript.
References


