Calculation of Control Coefficients of metabolic pathways

A flux-oriented graph-theoretic approach

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Within the premises of the flux-oriented theory of Crabtree & Newsholme [(1987) *Biochem. J.* **247**, 113–120], I have used a graph-theoretic approach for calculating the Control Coefficients of metabolic pathways. It is shown that a directed graph representing the control structure of a metabolic pathway can be constructed in a heuristic manner directly from the reaction diagram of the pathway, without the necessity of writing down the governing equations for the Control Coefficients. The Control Coefficients are derived from an analysis of the topology of the directed graph. The graph-theoretic approach also provides a visual framework for analysing the functional relationships of the individual enzymes. The control structures of the following pathways are examined here: (a) a simple unbranched pathway with four enzymes, (b) a simple branched pathway with three enzymes, and (c) a branched pathway with both carbon and energy (ATP) fluxes.

INTRODUCTION

Metabolic pathways in microbial cells consist of a labyrinth of enzyme-catalysed reactions that transform substrates into products. In a metabolic pathway there are certain key enzymes that control and regulate the fluxes through the different branches and the concentrations of the various metabolites. A study of the regulatory behaviour of these enzymes is of fundamental importance for developing an understanding of the overall metabolism. In order to analyse the control properties of the enzymes in a quantitative manner, three theoretical approaches, known as Biochemical Systems Theory, Flux-Oriented Theory and Metabolic Control Theory, have been used by researchers for many years. The Biochemical Systems Theory was postulated by Savageau [1–5], the Flux-Oriented Theory was developed by Crabtree & Newsholme [6–8] and the Metabolic Control Theory was proposed by Kacser & Burns [9] and Heinrich & Rapoport [10]. Several similarities and differences among these theories have been recently pointed out by Savageau *et al.* [11,12], Sorribas & Savageau [13,14] and Cascante *et al.* [15,16], among others. The primary objective of the Metabolic Control Theory and the Flux-Oriented Theory is to determine the sensitivities of metabolic fluxes and concentrations to variations in enzymic activity in the steady state, whereas the Biochemical Systems Theory, by virtue of its time-dependent formulation, can be used for analysing the steady-state response and the dynamic behaviour of metabolic pathways. A convenient way of expressing the sensitivities of fluxes or concentrations is to use the notion of the so-called Control Coefficients. The Control Coefficient of an enzyme with respect to a flux (or a concentration) is defined as the fractional change in flux (or concentration) in response to a fractional change in enzyme activity. The Control Coefficients are customarily expressed in terms of enzymic parameters that are referred to as Elasticity Coefficients in the Metabolic Control Theory, Intrinsic Sensitivities in the Flux-Oriented Theory and Apparent Kinetic Orders in the Biochemical Systems Theory. The enzymic parameters measure the relative variation in the rate of reaction catalysed by an enzyme with respect to a relative variation in concentration of a metabolite. By examining the Control Coefficients of the enzymes in a given pathway, it is possible to determine the specific enzymes that are most effective in regulating the systemic behaviour of the pathway. A knowledge of these Control Coefficients can be valuable from a

biotechnological point of view, in developing optimal operating strategies for microbial fermentation processes. In particular, knowing the relative magnitudes of the Control Coefficients of the various enzymes in a pathway, one can identify those enzymes whose properties should be altered by mutation or genetic engineering techniques in order to maximize the concentration of a particular metabolite or the flux through a particular segment of the pathway, leading to overproduction. In pharmaceutical applications, the Control Coefficients can be used effectively for identifying the target enzymes for which it would be desirable to design site-directed drugs.

A number of methods have been proposed in the literature for calculating the Control Coefficients of metabolic pathways. Among these methods, the technique using matrix inversion [17–19] is known to be the most convenient to use. In a recent paper [20] I presented a topological approach, within the framework of Metabolic Control Theory, for calculating the Control Coefficients. In this approach, the control structure of a metabolic pathway is represented by a weighted directed graph, known simply as a digraph. A digraph can be drawn in a heuristic manner from the reaction diagram of the pathway, without the necessity of writing down the governing equations for the Control Coefficients. From the digraph, expressions for the Control Coefficients are derived by using concepts of graph theory. The graph-theoretic approach also provides a visual framework for analysing the cause–effect relationships of the individual enzymes in a given pathway. The purpose of the present paper is to apply this topological approach, within the premises of Flux-Oriented Theory, for the calculation of the Control Coefficients of metabolic pathways.

Several alternative approaches are also available for control analysis of metabolic pathways. These include a diagrammatic technique [21], a top-down approach [22], application of signal-flow graphs [23,24], use of spanning trees [25] and electrical analogue circuits [26,27].

In Flux-Oriented Theory, the rate of an enzyme-catalysed reaction is modelled by a power equation of the form [6]:

$$v = \lambda S^\alpha P^\beta W^\gamma \quad (1)$$

where S and P are respectively the concentrations of substrate (S) and product (P) (i.e. internal effectors) and W is the concentration of an external regulator (W). The Greek symbols α , β and γ are the so-called **intrinsic sensitivities**; they measure the response of

In these diagrams the intrinsic sensitivity of a metabolite with respect to an enzyme is drawn with a broken line pointing from the metabolite to the enzyme, and the effects of the external regulators W_1 , W_2 , W_3 and W_4 are designated respectively by E_1 , E_2 , E_3 and E_4 . Consider the first reaction. In this reaction note that the intrinsic sensitivity α , which is directed from S to A (or J), points in the reverse (i.e. upstream) direction of the pathway. This intrinsic sensitivity is denoted in Fig. 1 by a directed edge from node S to node J, carrying the weight α . Next we focus our attention on reaction diagram (ii). Here the intrinsic sensitivity γ (directed from I to S) also points in the reverse direction of the pathway; this is represented in Fig. 1 by an edge of weight γ directed from node I to node S. Note, however, that in reaction diagram (ii) the intrinsic sensitivity β (directed from S) points in the forward (i.e., downstream) direction of the pathway; this is designated by a self-loop around the node S in Fig. 1. In general, if an intrinsic sensitivity in any of the above reaction diagrams is directed from a metabolite X towards a metabolite Y, and points in the reverse (i.e. upstream) direction of the pathway, then it is represented in the digraph by a directed edge from node X to node Y. On the other hand, if an intrinsic sensitivity in the reaction diagrams is directed from a metabolite X and points in the forward direction of the pathway, then a self-loop is attached to the node X in the digraph, carrying a weight equal to the

intrinsic sensitivity. By using this convention, the intrinsic sensitivities δ , σ and ω in the reaction diagrams (iii) and (iv) are incorporated into the digraph of Fig. 1. To complete our construction, a source node * is introduced in Fig. 1, and an edge is directed from the source node to the nodes J, S, I and R, with weights E_1 , E_2 , E_3 and E_4 respectively, characterizing the effects of the external regulators W_1 , W_2 , W_3 and W_4 .

From the above digraph, the governing eqns. (6) can be easily derived, if desired. To derive eqn. (6b), for example, consider the node S in the digraph and multiply the weight of every incoming edge of this node by the relative variation of the quantity associated with the node from which the edge originates. (A value of unity is associated with the source node *.) The sum of these products equated to zero leads to eqn. (6b). Eqns. (6a), (6c) and (6d) can be constructed in a similar fashion from a consideration of the nodes J, I and R respectively in Fig. 1.

It is also possible to draw the digraph of Fig. 1 from eqns. (6). A construction procedure using the matrix formulation of these equations is described in Appendix A.

From the topology of the digraph shown in Fig. 1, the Control Coefficients can be evaluated with the aid of the formula:

$$S_{E_i}^r = \frac{N_{r, E_i}}{DE_i}, i = 1, 2, 3, 4 \quad (7)$$

where $S_{E_i}^r$, in general, denotes the Control Coefficient of an enzyme E_i with respect to the concentration of a metabolite or flux Γ ($\Gamma = J, S, I$ or R). The quantity N_{r, E_i} is the sum of the signed gains of all one-connections from the source node to node Γ via the edge of weight E_i , and D is the sum of the signed gains of all connections in the digraph. To familiarize the reader with the present terminology, we first note that a **directed path** from a node j to a node k consists of a sequence of directed edges that originates at node j and terminates at node k , and no edge passes through any node more than once. A **directed circuit** is defined as a directed path whose beginning and ending nodes are the same. A **connection** in the digraph is either a directed circuit or a set of node-disjoint directed circuits (which includes all the nodes) in the subgraph obtained by removing the source node and all its outgoing edges from the digraph. Note that a self-loop around a node is considered as a directed circuit. A **one-connection** from the source node to a node j in the digraph consists of a directed path from the source node to node j and a set of node-disjoint directed circuits that includes all nodes of the digraph, except those contained in the directed path. The gain of a connection (or a one-connection) is equal to the product of the weights on all the edges, including self-loops, constituting that connection (or one-connection). A gain is given a positive sign if the number of

Fig. 1. Digraph of the control structure of pathway (A)

Fig. 2. The four connections in the digraph shown in Fig. 1

Fig. 3. The one-connection from the source node to node J via the edge of weight E_1 in Fig. 1

Fig. 4. The one-connection from the source node to node R via the edge of weight E_1 in Fig. 1

Fig. 5. The set of one-connections from the source node to node I via the edge of weight E_4 in Fig. 1

directed circuits in the connection (or one-connection) is even or zero; otherwise, it is given a negative sign. (See ref. [20] for further details.)

The digraph of Fig. 1 has four connections; these are displayed in Fig. 2. The number of connections present in this Figure is equal to the **permanent** of the adjacency matrix of the subgraph obtained by deleting the source node and all its outgoing edges. The procedure of constructing an adjacency matrix and calculating its permanent is described in Appendix B. By adding the signed gains of these connections, we find:

$$D = -\beta\delta\omega + \alpha\delta\omega - \alpha\gamma\omega + \alpha\gamma\sigma = -(\beta\delta\omega + \bar{\alpha}\delta\omega + \bar{\alpha}\bar{\gamma}\omega + \bar{\alpha}\bar{\gamma}\bar{\sigma}) \quad (8)$$

Since the quantities α , γ and σ represent the intrinsic sensitivities of product inhibition of the enzymes E_1 , E_2 and E_3 respectively, and are negative, I have written in eqn. (8) $\alpha = -\bar{\alpha}$, $\gamma = -\bar{\gamma}$ and $\sigma = -\bar{\sigma}$, where an overbar indicates the absolute value of the intrinsic sensitivity.

By way of illustration, we first calculate the Flux Control Coefficient S'_{E_1} . For this purpose we need to find all the one-connections from the source node to node J in Fig. 1 via the directed edge of weight E_1 . Notice that there is a directed path from the source node to node J consisting of the edge $(*, J)$. There is only one one-connection associated with this directed path, as shown in Fig. 3. The signed gain of this one-connection is:

$$N_{J,E_1} = -\beta\delta\omega E_1 \quad (9)$$

By using the results from eqns. (8) and (9) in eqn. (7), we obtain:

$$S'_{E_1} = \beta\delta\omega / (\beta\delta\omega + \bar{\alpha}\delta\omega + \bar{\alpha}\bar{\gamma}\omega + \bar{\alpha}\bar{\gamma}\bar{\sigma}) \quad (10)$$

As another example, the Control Coefficient of, say, the enzyme E_1 with respect to the concentration of the metabolite R is derived from the observation that in Fig. 1 there is a one-

connection from the source node to node R via the edge of weight E_1 (see Fig. 4). This one-connection has a signed gain equal to $-\beta\delta E_1$. Accordingly:

$$S'_{E_1} = \beta\delta / (\beta\delta\omega + \bar{\alpha}\delta\omega + \bar{\alpha}\bar{\gamma}\omega + \bar{\alpha}\bar{\gamma}\bar{\sigma}) \quad (11)$$

Finally, suppose that we want to calculate the Control Coefficient of E_4 with regard to the concentration of I . Notice that in Fig. 1 there are two one-connections from the source node to node I via the edge of weight E_4 ; these are depicted in Fig. 5. The sum of the signed gains of these one-connections is equal to $(\alpha - \beta)\sigma E_4$. Therefore S'_{E_4} has the value:

$$S'_{E_4} = -(\bar{\alpha} + \beta)\bar{\sigma} / (\beta\delta\omega + \bar{\alpha}\delta\omega + \bar{\alpha}\bar{\gamma}\omega + \bar{\alpha}\bar{\gamma}\bar{\sigma}) \quad (12)$$

The remaining Control Coefficients can be derived in a similar manner by examining all the one-connections from the source node to the respective nodes via the appropriate directed edges (of weight E_1 , E_2 , E_3 or E_4) from the source node. For the sake of brevity, these results are not given here.

As mentioned before, the sensitivity of a flux or a metabolite concentration in response to an external regulator can be calculated as the product of its Control Coefficient with respect to the enzyme affected by the external regulator and the intrinsic sensitivity of the enzyme towards the regulator. For instance, sensitivity of the flux (J) with respect to the regulator W_1 is given by:

$$S'_{W_1} = \phi S'_{E_1} = \phi \beta\delta\omega / (\beta\delta\omega + \bar{\alpha}\delta\omega + \bar{\alpha}\bar{\gamma}\omega + \bar{\alpha}\bar{\gamma}\bar{\sigma}) \quad (13)$$

ϕ being the intrinsic sensitivity of E_1 towards W_1 .

We now demonstrate how the cause-effect relationships of the various enzymes can be analysed directly from the digraph of Fig. 1, in certain limiting cases. To begin with, consider the situation when the enzyme E_1 is devoid of product inhibition, i.e.

$\alpha = 0$. In this case the directed edge ($\bar{S}, \bar{J}$) should be deleted from Fig. 1. It is then clear that there will be no directed path (and hence no one-connection) from the source node to node $\bar{J}$ via any of the edges of weight $\bar{E}_2$, $\bar{E}_3$ or $\bar{E}_4$. Accordingly, the Flux Control Coefficients $S_{E_2}^J$, $S_{E_3}^J$ and $S_{E_4}^J$ must be zero. There will be, however, a directed path (and a one-connection) from the source node to node $\bar{J}$ via the edge of weight $\bar{E}_1$ (see Fig. 3). As indicated above, this one-connection has a signed gain equal to $-\beta\delta\omega\bar{E}_1$. Note also that when $\alpha = 0$ the digraph of Fig. 1 has only one connection with a signed gain of $-\beta\delta\omega$ [see Fig. 2(i)]. Therefore $S_{E_1}^J = 1$. It follows that when the first enzyme is free of product inhibition the control of flux through the pathway is governed entirely by this enzyme.

It is also apparent in Fig. 1 that if $\alpha = 0$ there is no directed path from the source node to node $\bar{I}$ or node $\bar{R}$ via the edge of weight $\bar{E}_2$; thus $S_{E_2}^I = S_{E_2}^R = 0$. Furthermore, no directed path exists from the source node to node $\bar{R}$ via the edge of weight $\bar{E}_3$; therefore $S_{E_3}^R = 0$. These results indicate that when $\alpha = 0$ the control of concentration of the last effector $\bar{R}$ is shared only by the enzymes $\bar{E}_1$ and $\bar{E}_4$; the intervening enzymes $\bar{E}_2$ and $\bar{E}_3$ do not play a regulatory role. The concentration control of the effector $\bar{I}$, on the other hand, is governed by the enzymes $\bar{E}_1$, $\bar{E}_3$ and $\bar{E}_4$, with $\bar{E}_2$ remaining inactive.

Next we examine the effect of $\gamma = 0$, which corresponds to the enzyme $\bar{E}_2$ lacking product inhibition. Under this condition the directed edge ($\bar{I}, \bar{S}$) should be deleted from Fig. 1. Clearly, since no directed path now exists from the source node to any of the nodes $\bar{J}$, $\bar{S}$ and $\bar{R}$ via the edge of weight $\bar{E}_3$, the result is: $S_{E_3}^J = S_{E_3}^S = S_{E_3}^R = 0$. There is also no directed path from the source node to node $\bar{J}$ or node $\bar{S}$ via the edge of weight $\bar{E}_4$; as a consequence, $S_{E_4}^J = S_{E_4}^S = 0$. Finally we investigate the situation when $\sigma = 0$, i.e. the enzyme $\bar{E}_3$ is free of product inhibition. It is easy to see from Fig. 1 that in this case $S_{E_4}^J = S_{E_4}^S = S_{E_4}^R = 0$. Combining all these results the following assertion can be made. In a linear pathway every enzyme located downstream from an enzyme with no product inhibition has a zero sensitivity towards (a) the pathway flux and (b) the concentrations of all internal effectors in the pathway except those effectors that lie between the enzyme under consideration and the enzyme lacking product inhibition. It should be emphasized that these conclusions are reached directly from the digraph shown in Fig. 1, without the necessity of calculating any of the Control Coefficients. These conclusions have been further confirmed by analysing the control structure of a linear pathway with six enzymes.

The digraph of Fig. 1 is also convenient to use for studying the effect of enzyme saturation in pathway (A). Consider, for instance, that the enzyme $\bar{E}_2$ is saturated, i.e. $\beta = 0$. In this case there will be no self-loop around node $\bar{S}$ in Fig. 1. Let us examine the control coefficient $S_{E_1}^J$. Note that in Fig. 1 there are two directed paths from the source node to node $\bar{J}$ via the edge of weight $\bar{E}_1$ (one connecting the nodes $\ast, \bar{J}$ and $\bar{R}$ and $\bar{I}$, and the other joining the nodes $\ast, \bar{J}$ and $\bar{I}$); however, since node $\bar{S}$ is devoid of a self-loop, neither of these directed paths can form a one-connection. Therefore $S_{E_1}^J$ must be zero. In a similar manner it can be shown that when $\beta = 0$, $S_{E_1}^J = S_{E_1}^S = 0$. Next we examine the case when $\delta = 0$, i.e. the enzyme $\bar{E}_3$ (instead of $\bar{E}_2$) is saturated. Under this condition it is easily seen from Fig. 1 that $S_{E_1}^J = S_{E_1}^S = S_{E_2}^J = S_{E_2}^S = S_{E_2}^R = 0$. Similarly if $\bar{E}_4$ is saturated, i.e. $\omega = 0$, we find $S_{E_1}^J = S_{E_1}^S = S_{E_2}^J = S_{E_2}^S = S_{E_3}^J = S_{E_3}^S = S_{E_3}^R = 0$. Collectively, these results indicate that (a) all enzymes located upstream from a saturated enzyme have a zero sensitivity towards the pathway flux and the control of flux is distributed among the saturated enzyme and the enzymes lying further downstream,

and (b) every enzyme preceding (i.e. upstream from) a saturated enzyme has no sensitivity towards all internal effectors with the exception of those effectors that lie between the enzyme under consideration and the saturated enzyme.

In the foregoing discussion we considered the mathematically limiting cases of no product inhibition and complete saturation of the various enzymes. Although such extreme situations do not occur *in vivo*, their analysis helps us intuitively to understand the trend in the overall systemic behaviour.

Effects of feedback or feedforward regulation can also be incorporated into a directed graph. Consider, as an example, that the first enzyme ($\bar{E}_1$) in pathway (A) is inhibited by the last internal effector ($\bar{R}$). A directed graph for this feedback-inhibited pathway can be readily constructed from that of the unregulated pathway (i.e. Fig. 1) by adding a directed edge from node $\bar{R}$ to node $\bar{J}$. If instead the effector $\bar{R}$ inhibits the enzyme $\bar{E}_2$, then an edge should be directed from node $\bar{R}$ to node $\bar{S}$ in Fig. 1. In general, when an effector, say $\bar{I}$, inhibits the activity of an enzyme $\bar{E}_i$, then if $\bar{E}_i$ is the first enzyme in the pathway we must draw a directed edge from node $\bar{I}$ to node $\bar{J}$ (i.e. the flux node); if $\bar{E}_i$ is not the first enzyme, then a directed edge should be drawn from node $\bar{I}$ to the node that represents the relative variation in concentration of the effector immediately preceding the enzyme $\bar{E}_i$.

Next we examine the effect of feedforward activation. First suppose that the effector $\bar{S}$ in pathway (A) activates the last enzyme $\bar{E}_4$. In this case a directed edge from node $\bar{S}$ to node $\bar{R}$ should be added in Fig. 1 to account for the feedforward effect. In general, if an internal effector, say $\bar{A}$, activates an enzyme $\bar{E}_i$ in pathway (A), then in Fig. 1 an edge should be directed from node $\bar{A}$ to the node representing the relative variation in concentration of the effector immediately preceding the activated enzyme $\bar{E}_i$.

A simple branched pathway

We now examine the diverging pathway shown below in which the flux $\bar{J}$ divides into the fluxes $\bar{J}_a$ and $\bar{J}_b$ along the two branches:

A digraph characterizing the control structure of this pathway is constructed in the following manner. First we draw three nodes $\bar{J}$, $\bar{J}_a$ and $\bar{J}_b$ representing the relative variations of the fluxes $\bar{J}$, $\bar{J}_a$ and $\bar{J}_b$ respectively, and a node $\bar{S}$ designating the relative variation of concentration of the branch-point metabolite $\bar{S}$ (see Fig. 6). An additional node $\ast$ (i.e. a source node) is included in this Figure. A self-loop of weight -1 is attached to each of the three flux-nodes. To join the various nodes, we consider the individual reactions at the branch point as shown in the following diagrams:

Fig. 9. The one-connection from the source node to node S via the edge of weight E_3 in Fig. 6

path from the source node to node 1 via the edges of weight E_2 and E_3 , we conclude that $S'_{E_2} = S'_{E_3} = 0$. In other words, the flux J has zero sensitivity towards E_2 and E_3 ; this result is also intuitively clear. Similarly it can be seen that if $\beta = 0$ then J_a has no sensitivity towards E_1 and E_3 . Finally, if $\gamma = 0$ then the sensitivity of J_b towards E_1 and E_2 becomes zero. Note, however, that whether or not α or β or γ is zero, the concentration of the metabolite S remains sensitive to any change in activity of the enzymes E_1 , E_2 and E_3 .

The graph-theoretic techniques described above can be readily extended when a branched pathway contains two or more enzymes in one (or both) of its branches. A procedure for constructing the digraphs for such pathways is described in Appendix C.

A branched pathway with carbon and energy fluxes

In order to explain some of the basic principles that may be involved in the regulation of glycolysis by ATP and other metabolites, Crabtree & Newsholme [8] examined the following branched pathway:

where Pyr represents pyruvate.

A characteristic feature of this pathway is that it contains both carbon and energy fluxes. Each of these fluxes is indicated by a single reaction catalysed by a single enzyme; the symbols m and n represent the fraction of the fluxes J_a (of lactate production) and J_b (pyruvate oxidation) respectively that are transferred by the corresponding reactions. When m and n are not equal, then a complex feedback process is set up in this pathway as follows. A change in J_a or J_b changes the net yield of ATP by glycolysis, which in turn alters the total rate of glycolysis. The Control Coefficients of the enzymes depend on the fluxes J , J_a , J_b , U and T , the intrinsic sensitivities α , β , δ and ϵ (written inside square brackets), the parameter γ shown by a broken line (and, of course, m and n). The parameter γ is not, in general, an intrinsic sensitivity since it includes interactions of ATP with other regulators of glycolysis such as ADP, AMP and P_i ; it represents an intrinsic sensitivity to ATP only when the concentrations of these other external regulators remain constant.

Fig. 10. Digraph of the control structure of pathway (C)

By using the graph-theoretic approach, the Control Coefficients of the various enzymes can be evaluated as follows. The digraph for the control structure is shown in Fig. 10, which is constructed in the manner described in the previous section. In particular, the relative variations of the fluxes (U , T , J , J_a and J_b) and the concentrations (A and P) are designated as nodes; node $*$ is the source node. Here A and P denote the concentrations of ATP and pyruvate respectively. The intrinsic sensitivity α (of E_2 to ATP) is represented by a directed edge from node A to node T in Fig. 10. The other intrinsic sensitivities (and γ) are portrayed in this Figure in a similar fashion. The requirement of flux conservation at each of the branch points in the pathway is incorporated in Fig. 10 by using the convention adopted in the previous section. For example, the fluxes at the pyruvate branch point are designated in Fig. 10 by directed edges to node P from the nodes J , J_a and J_b , with weights J , $-J_a$ and $-J_b$ respectively. Similarly, in view of flux conservation at the ATP branch point, directed edges are drawn from the nodes U , T , J_a and J_b to the node A , carrying weights U , $-T$, mJ_a and nJ_b respectively.

It is not difficult to see that Fig. 10 has six connections; these are shown in Fig. 11. The number of connections can be established *a priori* by calculating the permanent of the adjacency matrix associated with the digraph of Fig. 10 (see Appendix B). As usual, the Control Coefficients of the various enzymes are derived from eqn. (7) in which D is given by the sum of the signed gains of the above six connections. We have:

$$D = -J\gamma enJ_b - \beta U\delta J_a - \beta U\epsilon J_b + \alpha T\delta J_a + \alpha T\epsilon J_b - J\gamma\delta mJ_a \quad (17)$$

which can be re-arranged to:

$$D = (\alpha T - \beta U)(\delta J_a + \epsilon J_b) - J\gamma(\delta mJ_a + \epsilon nJ_b) \quad (18)$$

consistent with the result obtained by Crabtree & Newsholme [8] (see Table 1 of their paper).

I now illustrate the calculation of some of the Control Coefficients. To find S'_{E_1} , for example, notice that in Fig. 10 there are two one-connections from the source node to node J , via the edge of weight E_1 ; these are shown in Fig. 12. From the sum of the signed gains of these one-connections, we obtain:

$$S'_{E_1} = U\gamma(\delta J_a + \epsilon J_b)/D \quad (19)$$

where D has the value according to eqn. (18). Clearly, in the limiting case when $\gamma = 0$, eqn. (19) shows that $S'_{E_1} = 0$. This may also be deduced by noting that when $\gamma = 0$ there is no directed path (and therefore no one-connection) from the source node to node J in Fig. 10 (see also Fig. 12). The response of the flux J to

Fig. 11. The six connections in the digraph of Fig. 10

an external regulator can be obtained as a product of S'_{E_1} and the intrinsic sensitivity of E_1 towards the external regulator.

As a second example, S'_{E_4} is obtained by observing that in Fig. 10 there are two one-connections from the source node to node J via the edge of weight E_4 ; these are displayed in Fig. 13. From the signed gains of these one-connections, it follows that:

$$S'_{E_4} = (m-n)\gamma\epsilon J_a J_b / D \quad (20)$$

which shows that sensitivity of the flux J with respect to E_4 is proportional to the difference $(m-n)$. According to eqn. (20), $S'_{E_4} = 0$ when either $\gamma = 0$ or $\epsilon = 0$; this is also apparent from the fact that if $\gamma = 0$ or $\epsilon = 0$ there is no one-connection from the source node to node J in Fig. 10 (see also Fig. 13).

Finally, suppose that it is desired to find S'_{E_3} . In Fig. 10 we see that there are four one-connections from the source node to node J via the edge of weight E_3 . For the sake of brevity, these one-

connections are not shown here. The sum of their signed gains yields:

$$S'_{E_3} = (\alpha T - U\beta)(\delta J_a + \epsilon J_b) / D \quad (21)$$

Consider now the situation when $\alpha = 0$, i.e. E_2 is saturated with ATP, and that $U = 0$, i.e. all the ATP is derived from J . This may be the case, for instance, in the flight muscles of some insects such as flies and bees [28,29]. Under these circumstances eqn. (21) shows $S'_{E_3} = 0$, implying that E_3 has no control over the flux J . However, Crabtree & Newsholme [8] argued that this result is paradoxical and that E_3 continues to exert its control on J via ATP even when $\alpha = U = 0$. I wish to point out that $S'_{E_3} = 0$ is not a paradox but a correct reflection of the fact that changing the activity of E_3 cannot possibly have any influence on the flux J at steady state, under the present conditions. The steady state must be consistent with T (a constant) $= mJ_a + nJ_b$. This has a unique solution for J_a and J_b because they are not independently

Fig. 12. The set of one-connections from the source node to node J via the edge of weight E_1 in Fig. 10

Fig. 13. The set of one-connections from the source node to node J via the edge of weight E_4 in Fig. 10

adjustable variables; in this model they are both functions of a single variable, [pyruvate]. Since these fluxes are determined by the value of T , so also is J , which is their sum at steady state. The interaction of ATP and E_3 is still operative, and the [ATP] will adjust to whatever concentration is necessary to secure the rate that is demanded. Therefore it does not seem necessary to invoke the distinction between partially and totally external regulators as Crabtree & Newsholme [8] do in order to deal with this point.

The calculation procedure described above can be simplified considerably if the Control Coefficients are evaluated of one enzyme at a time by reducing the topology of the digraph in Fig. 10 to a simpler form. Consider, for instance, evaluating the Control Coefficients of enzyme E_4 . For this purpose we first delete the directed edges with weights E_1 , E_2 , E_3 and E_5 in Fig. 10, which gives rise to Fig. 14. The topology of Fig. 14 can be simplified by eliminating the appropriate nodes from the digraph. The node-elimination technique was illustrated in an earlier paper [20] in the context of simplifying the structure of a metabolic pathway by grouping several enzymes together, and is reproduced here for completeness.

In the digraph of Fig. 14, consider a non-source node k that has a self-loop of weight w_{kk} . We wish to eliminate this node. For this purpose, we examine the following two cases. First, suppose that there are two other nodes i and j , and (i,k) and (k,j) are two directed edges with weights w_{ik} and w_{kj} respectively, which are incident to and from the node k . For each such pair of edges we form a weight c_{ij} equal to $-w_{ik}w_{kj}/w_{kk}$. If there are no edges from node i to node j in the digraph, then we introduce a directed edge (i,j) and assign to it the weight c_{ij} . If a directed edge (i,j) is originally present in the digraph with, say, a weight w_{ij} , we replace w_{ij} by $(w_{ij} + c_{ij})$. Finally, we delete all the edge pairs such as (i,k) and (k,j) , and remove the node k from the digraph.

Next we consider the case in which there is a pair of directed edges (i,k) and k,i between the nodes i and k . To eliminate node k from the digraph, we simply attach a self-loop to node i with

Fig. 14. The digraph of Fig. 10 after deletion of the directed edges of weight E_1 , E_2 , E_3 and E_5

Fig. 15. The digraph of Fig. 14 after elimination of the nodes J and T

a weight c_{ii} equal to $-w_{ik}w_{ki}/w_{kk}$, and delete the edges (i,k) and (k,i) together with the node k . If node i has a pre-existing self-loop with, say, a weight w_{ii} , then the weight c_{ii} is added to w_{ii} ; subsequently, the directed edges (i,k) and (k,i) and the node k are deleted from the digraph. When the node to be eliminated does not possess a self-loop, a slightly different method should be used; the interested reader is referred to Coates' original paper [30] for details.

To calculate the Control Coefficients of enzyme E_4 , we first eliminate the nodes J and T from Fig. 14, by using the node-elimination technique just described; the reduced digraph is shown in Fig. 15. The nodes U and J_b are subsequently eliminated from Fig. 15, giving rise to Fig. 16. From these Figures the Control Coefficients of enzyme E_4 are determined with the aid of eqn. (7). Consider Fig. 16 first, from which $S_{E_4}^A$, $S_{E_4}^P$ and $S_{E_4}^{J_4}$ can

Fig. 16. The digraph of Fig. 15 after elimination of the nodes U and J_b

be found. It is easy to see that there are four connections in this Figure. The sum of the signed gains of these connections yields the expression for D given by eqn. (18). As an example, let us evaluate $S_{E_4}^A$. Note that in Fig. 16 there are two directed paths from the source node $*$ to node A . By using the signed gains of the two one-connections associated with these directed paths we find from eqn. (7):

$$S_{E_4}^A = (m-n)\epsilon J_a J_b / D \quad (22)$$

Expressions for $S_{E_4}^A$ and $S_{E_4}^P$ can be derived from Fig. 16 in a similar manner by examining the one-connections from the source node to node J_a and node P respectively. Of the remaining Control Coefficients of enzyme E_4 , $S_{E_4}^U$ and $S_{E_4}^b$ may be found from Fig. 14 or Fig. 15 as follows. To find $S_{E_4}^U$, for instance, we form the product of the weight of each incoming edge of node U in Fig. 14 or Fig. 15; the sum of these products equated to zero yields the relation $\dot{U} = \beta A$; as a consequence, we have $S_{E_4}^U = \beta S_{E_4}^A$, with $S_{E_4}^A$ given by eqn. (22). Finally, to calculate $S_{E_4}^J$ and $S_{E_4}^T$ we must return to Fig. 14. From node T in this Figure it follows that:

$$S_{E_4}^T = \alpha S_{E_4}^A = (m-n)\alpha \epsilon J_a J_b / D \quad (23)$$

Similarly, by using the relation $S_{E_4}^J = \gamma S_{E_4}^A$ at node J , we find $S_{E_4}^J$, as given by eqn. (20) above.

CONCLUDING REMARKS

I have used a topological approach for analysing the regulatory behaviour of metabolic pathways. The analysis was carried out within the premises of the Flux-Oriented Theory of Crabtree & Newsholme [8]. It is shown that, by using the topological approach, the Control Coefficients of a metabolic pathway can be calculated from a weighted directed graph representing the control structure of the pathway. A directed graph can be constructed in a heuristic manner directly from the reaction diagram of the pathway without the necessity of writing down the governing equations for the Control Coefficients. In addition, the topological method is found to be very convenient for analysing the cause-effect relationships of the individual enzymes in a given pathway. Three different types of pathways were examined. These are: (a) a simple linear pathway with four enzymes, (b) a simple branched pathway with three enzymes, and (c) a branched pathway with both carbon and energy fluxes. In order to simplify the calculation of the Control Coefficients,

a step-by-step node elimination technique is described for simplifying the topology of the directed graphs.

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APPENDIX A

I show below how a directed graph representing the control structure of a metabolic pathway can be constructed from a matrix formulation of the governing equations. For definiteness, consider the simple linear pathway (A) for which the governing equations are written in the following matrix form:

$$\begin{array}{c|c|c|c|c} \begin{array}{c} \overset{r}{J} \\ \overset{r}{S} \\ \overset{r}{I} \\ \overset{r}{R} \end{array} & \begin{array}{c} \overset{r}{J} \\ \overset{r}{S} \\ \overset{r}{I} \\ \overset{r}{R} \end{array} & \begin{array}{c} \overset{r}{I} \\ \overset{r}{S} \\ \overset{r}{I} \\ \overset{r}{R} \end{array} & \begin{array}{c} \overset{r}{R} \\ \overset{r}{S} \\ \overset{r}{I} \\ \overset{r}{R} \end{array} & \begin{array}{c} \overset{r}{J} \\ \overset{r}{S} \\ \overset{r}{I} \\ \overset{r}{R} \end{array} \\ \hline \begin{array}{c} -1 \\ -1 \\ -1 \\ -1 \end{array} & \begin{array}{c} \alpha \\ \beta \\ 0 \\ 0 \end{array} & \begin{array}{c} 0 \\ \gamma \\ \delta \\ 0 \end{array} & \begin{array}{c} 0 \\ 0 \\ \sigma \\ \omega \end{array} & \begin{array}{c} \overset{r}{J} \\ \overset{r}{S} \\ \overset{r}{I} \\ \overset{r}{R} \end{array} + \begin{array}{c} \overset{r}{E_1} \\ \overset{r}{E_2} \\ \overset{r}{E_3} \\ \overset{r}{E_4} \end{array} = \begin{array}{c} 0 \\ 0 \\ 0 \\ 0 \end{array} \end{array} \quad (A1)$$

For convenience, the components $\overset{r}{J}$, $\overset{r}{S}$, $\overset{r}{I}$ and $\overset{r}{R}$ of the variables vector are written horizontally above the matrix of intrinsic sensitivities as shown. I refer to them as elements of the row vector.

From eqn. (A1), the digraph of Fig. 1 of the main paper can be formed as follows. First, four nodes are drawn representing the variables $\overset{r}{J}$, $\overset{r}{S}$, $\overset{r}{I}$ and $\overset{r}{R}$. Next, an edge is directed from each node representing the variables in the row vector to each node representing the variables in the column vector ($\overset{r}{J}$, $\overset{r}{S}$, $\overset{r}{I}$ and $\overset{r}{R}$); each of these directed edges carries a weight equal to the corresponding matrix element. For example, the -1 element in the (1,1) position corresponds to a self-loop around the node $\overset{r}{J}$, the element α in the (1,2) position is represented by an edge from

node $\bar{S}$ to node $\bar{J}$, and so on. The zeros in this matrix, of course, lead to no edges in the digraph. Finally, a source node $*$ is introduced, and edges are directed from the source node to the

variable nodes $\bar{J}$, $\bar{S}$, $\bar{I}$ and $\bar{R}$ with weights $\bar{E}_1$, $\bar{E}_2$, $\bar{E}_3$ and $\bar{E}_4$ respectively, corresponding to the elements in the last column vector of the left-hand side of eqn. (A1).

APPENDIX B

The adjacency matrix of an n -node directed graph is an $n \times n$ matrix $\mathbf{H}$ such that $H_{ij} = 1$ if and only if there is an edge directed from node i to node j in the digraph; $H_{ij} = 0$ if there is no edge directed from node i to node j .

It can be shown [1] that the number of connections in a digraph is equal to the permanent of its adjacency matrix. The permanent of a square matrix is defined in precisely the same fashion as its determinant except that all the terms are taken with a positive sign.

Consider the digraph of Fig. 1 in the main paper without the source node and all its outgoing edges. The adjacency matrix of this digraph is given by:

$$\mathbf{H} = \begin{vmatrix} 1 & 1 & 1 & 1 \\ 1 & 1 & 0 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 0 & 1 & 1 \end{vmatrix} \quad (\text{B1})$$

It is easy to check that the permanent of this matrix is 4. Accordingly, there are exactly four connections in the above digraph.

In Appendix A, recall that the equations governing the control structure of pathway (A) of the main paper were written in a matrix form. The adjacency matrix $\mathbf{H}$, given by eqn. (B1) above, is the transpose of the matrix obtained from the matrix of intrinsic sensitivities [see eqn. (A1)] by replacing each of its non-zero entries by 1.

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APPENDIX C

Consider a branched pathway with two enzymes in the upper branch as shown below:

As usual, the various fluxes are written in parentheses and the intrinsic sensitivities of the enzymes are given in square brackets. A directed graph of the control structure of this pathway can be constructed in the following manner. First, we ignore the segment of the upper branch containing the enzyme E_4 and the effector I , i.e. consider the simple branched pathway (B) analysed in the main paper. A directed graph for this simplified pathway is drawn. Next we introduce a node $\bar{I}$, designating the relative variation in concentration of the effector I . In order to connect this node to the remaining nodes, we treat the effector I as part of a linear pathway transmitting the flux J_a , and containing the enzymes E_2 and E_4 . Accordingly, as done in Fig. 1 of the main paper, an edge with weight -1 is directed from the flux-node $\bar{J}_a$

Fig. C1. Digraph of the control structure of the branched pathway considered in Appendix C

to node $\bar{I}$, an edge with weight δ is directed from node $\bar{I}$ to node $\bar{J}_a$, and a self-loop carrying the weight ϵ is drawn around node $\bar{I}$. Finally, a directed edge of weight $\bar{E}_4$ is drawn from the source node $*$ to node $\bar{I}$. The complete digraph is shown in Fig. C1.

Presence of additional enzymes in the upper branch can be incorporated into this digraph in a similar fashion by considering them and the adjoining effectors as part of a linear pathway transmitting the flux J_a . An analogous procedure can be used when there are two or more enzymes in the lower branch in addition to those in the upper branch.