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Co-response Analysis: A New Experimental Strategy for Metabolic Control Analysis*

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The formulation of the standard summation and connectivity relationships as a statement that the matrix of all the elasticities in a system is the inverse of the matrix of all the control coefficients is completely general, provided that only control coefficients for independent fluxes and concentrations are considered, and that the elasticity matrix is written to take account of the stoichiometry of the pathway and the implied dependences between concentrations. This generality implies that co-response analysis is also general, i.e. that all of the elasticities and all of the control coefficients in any system, regardless of branching, feedback effects, moiety conservation or other complications, can be determined by comparing the effects of perturbations of the enzyme activities on the steady-state fluxes and concentrations of the pathway. The approach requires no quantitative information about the magnitudes of the effects on the individual enzyme activities, and consequently no enzymes need to be studied in isolation from the pathway.

Introduction

Henrik Kacser (1956) argued long ago that understanding of genetic mechanisms would not come from studying the properties of individual components of a system but from studying their interactions with one another, in other words from the organization of the system as a whole. Four decades later this idea has yet to find general acceptance in modern biology, where study of components remains a commonplace activity, but it has become the central theme of metabolic control analysis. Even in metabolic control analysis, however, measurement of elasticities has continued to require isolation of the enzymes that compose metabolic pathways so that their kinetic properties can be studied separately from one another. In a sense, therefore, even metabolic control analysis has continued to be the study of the properties of components, and an important part of Kacser's aim to treat systems as systems has remained unrealized.

In the paper that followed the meeting of the Biochemical Society in Nottingham at which one of us (ACB) met Henrik Kacser for the first time, Kacser & Burns (1979) made the first step towards liberating the field from the need to study purified enzymes. They pointed out that observing the effects on the flux and all the intermediate concentrations of perturbing the activities of the first and last enzymes in a linear pathway would, in the absence of feedback effects, allow all of the elasticities to be calculated. This is now known as the double-modulation method. The need to assume that no enzyme was influenced by any metabolite apart from its immediate substrate and product placed a severe limitation on the practical usefulness of this approach, as in reality feedback effects occur in virtually all pathways. The widespread occurrence of moiety-conserved cycles, which provided the context for the other of us (JHSH) to work with Henrik Kacser (Hofmeyr *et al.*, 1986), as well as branching and other complexities, provides further limitations.

* This paper is dedicated to the memory of Henrik Kacser.

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Here we shall show that co-response analysis, previously described (Hofmeyr *et al*., 1993) and tested (Cornish-Bowden & Hofmeyr, 1994) for a restricted class of systems, is completely general. Provided that it is possible to perturb the activities of all of the enzymes in a pathway, comparison of the effects on all the fluxes and concentrations allows all of the control coefficients and elasticities to be determined without needing any enzymes to be isolated (and hence without requiring quantitative information on the magnitude of any perturbation of the enzyme affected), regardless of feedback effects, branching or moiety conservation.

In parallel with our development of co-response analysis, Giersch (1994, 1995) has approached the problem of generalizing the double modulation method from a different point of view. In his multiple modulation method, he has studied how many and which enzymes need to be perturbed in order to take account of any branching and feedback effects that are known to be present. This approach is considered elsewhere in this volume (Giersch & Cornish-Bowden, 1996) and is compared with co-response analysis in the Discussion.

The Control-Matrix Equation $\mathbf{CE} = \mathbf{I}$

The relationships between control and elasticity coefficients have been expressed in a number of matrix-equation formats (Giersch, 1988; Reder, 1988; Cascante *et al*., 1989a, b; Fell & Sauro, 1985; Sauro *et al*., 1987; Small & Fell, 1989; Fell *et al*., 1990; Westerhoff & Kell, 1987; Kacser *et al*., 1990; Sauro & Kacser, 1990). Here we propose what we believe to be a more general and concise formulation than the ones in use up to now. It uses Reder's (1988) formalism as a starting point, but ends up being more similar to the square matrix equations developed by the groups mentioned here. Unlike Reder's (1988) formalism, it is couched in terms of scaled coefficients: unscaled coefficients may be perfectly suitable for mathematical manipulations, but they are of little use for the interpretation of experimental data obtained in control analytical studies (Hofmeyr, 1995).

The summation and connectivity properties of metabolic control analysis (Reder, 1988), can be combined into a single control-matrix equation (Hofmeyr *et al*., 1993):

$$\begin{bmatrix} \bar{\mathbf{C}}^{\mathbf{J}} \\ \bar{\mathbf{C}}^{\mathbf{s}} \end{bmatrix} [\mathbf{K} \quad -\bar{\boldsymbol{\epsilon}}_{\mathbf{s}}\mathbf{L}] = \begin{bmatrix} \mathbf{K} & \mathbf{0} \\ \mathbf{0} & \mathbf{L} \end{bmatrix} \tag{1}$$

where $\bar{\mathbf{C}}^{\mathbf{J}}$ is an $n \times n$ matrix of unscaled flux-control coefficients $\bar{C}^{J_i}_{j} = \partial J_i/\partial v_j$, $\bar{\mathbf{C}}^{\mathbf{s}}$ an $m \times n$ matrix of unscaled concentration-control coefficients $\bar{C}^{s_i}_{j} = \partial s_i/\partial v_j$, and $\bar{\boldsymbol{\epsilon}}_{\mathbf{s}}$ an $n \times m$ matrix of unscaled elasticity coefficients $\bar{\epsilon}^{v_j}_{s_i} = \partial v_j/\partial s_i$; n is the number of reactions and m the number of variable metabolites in the metabolic pathway. The row counter is i and the column counter j.

The **K**-matrix expresses the dependence of the steady-state fluxes on the independent fluxes (Reder, 1988):

$$\mathbf{J} = \mathbf{K}\mathbf{J}_{\mathbf{I}} \tag{2}$$

where **J** is an n-dimensional column vector of all the steady-state fluxes, and $\mathbf{J}_{\mathbf{I}}$ is an $(n - r)$-dimensional column vector of independent fluxes (r is the rank of the stoichiometric matrix). **K** therefore has dimensions $n \times (n - r)$. If **J** is partitioned into $n - r$ independent fluxes $\mathbf{J}_{\mathbf{I}}$ and r dependent fluxes $\mathbf{J}_{\mathbf{D}}$, eqn (2) becomes

$$\begin{bmatrix} \mathbf{J}_{\mathbf{I}} \\ \mathbf{J}_{\mathbf{D}} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_{n-r} \\ \mathbf{K}_0 \end{bmatrix} \mathbf{J}_{\mathbf{I}} \tag{3}$$

where **I** is an $(n - r)$-dimensional identity matrix and $\mathbf{K}_0$ an $r \times (n - r)$-dimensional matrix that expresses the dependent fluxes in terms of the independent fluxes.

The **L**-matrix expresses the dependence of the differential equations on the independent differential equations (Reder, 1988):

$$\frac{\mathrm{d}\mathbf{s}}{\mathrm{d}t} = \mathbf{L}\frac{\mathrm{d}\mathbf{s}_{\mathbf{I}}}{\mathrm{d}t} \tag{4}$$

where $\mathrm{d}\mathbf{s}/\mathrm{d}t$ is an m-dimensional column vector of all time derivatives and $\mathrm{d}\mathbf{s}_{\mathbf{I}}/\mathrm{d}t$ is an r-dimensional column vector of independent time derivatives. **L** therefore has dimensions $m \times r$. If $\mathrm{d}\mathbf{s}/\mathrm{d}t$ is partitioned into r independent time derivatives $\mathrm{d}\mathbf{s}_{\mathbf{I}}/\mathrm{d}t$ and $(m - r)$ dependent time derivatives $\mathrm{d}\mathbf{s}_{\mathbf{D}}/\mathrm{d}t$, eqn (4) becomes

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} \mathbf{s}_{\mathbf{I}} \\ \mathbf{s}_{\mathbf{D}} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_r \\ \mathbf{L}_0 \end{bmatrix} \frac{\mathrm{d}\mathbf{s}_{\mathbf{I}}}{\mathrm{d}t} \tag{5}$$

where **I** is an r-dimensional identity matrix and $\mathbf{L}_0$ an $(m - r) \times r$-dimensional matrix that expresses the dependent time derivatives in terms of the independent time derivatives.

In metabolic control analysis, the control and elasticity coefficients are more useful in their scaled form. To scale the control matrix equation we define the diagonal matrices $\mathbf{D}^{\mathbf{J}}$ and $\mathbf{D}^{\mathbf{s}}$, which respectively have the steady-state fluxes and concentrations on their diagonals. The inverses of these matrices have

inverse fluxes and inverse concentrations on their diagonals. The matrices that occur in the control-matrix equation are transformed as follows:

$$\mathbf{C^J} = (\mathbf{D^J})^{-1}\bar{\mathbf{C}}^{\mathbf{J}}\mathbf{D^J} \tag{6}$$

$$\mathbf{C^s} = (\mathbf{D^s})^{-1}\bar{\mathbf{C}}^{\mathbf{s}}\mathbf{D^J} \tag{7}$$

$$\boldsymbol{\epsilon}_{\mathbf{s}} = (\mathbf{D^J})^{-1}\bar{\boldsymbol{\epsilon}}_{\mathbf{s}}\mathbf{D^s} \tag{8}$$

$$\mathscr{L} = (\mathbf{D^s})^{-1}\mathbf{L}\mathbf{D^{s_I}} \tag{9}$$

$$\mathscr{K} = (\mathbf{D^J})^{-1}\mathbf{K}\mathbf{D^{J_I}}. \tag{10}$$

The control-matrix eqn (1) can now be rewritten in its scaled form

$$\begin{bmatrix}\mathbf{C^J}\\ \mathbf{C^s}\end{bmatrix}[\mathscr{K} \quad -\boldsymbol{\epsilon}_{\mathbf{s}}\mathscr{L}] = \begin{bmatrix}\mathscr{K} & \mathbf{0}\\ \mathbf{0} & \mathscr{L}\end{bmatrix} \tag{11}$$

where $\mathbf{C^J}$ is a matrix of scaled flux-control coefficients $C_j^{J_i} = \partial \ln J_i/\partial \ln v_j$, $\mathbf{C^s}$ a matrix of scaled concentration-control coefficients $C_j^{s_i} = \partial \ln s_i/\partial \ln v_j$, and $\boldsymbol{\epsilon}_{\mathbf{s}}$ a matrix of scaled elasticity coefficients $\epsilon_{s_i}^{v_j} = \partial \ln v_j/\partial \ln s_i$.

The matrices in eqn (11) can be partitioned in terms of independent and dependent variables to give

$$\begin{bmatrix}\mathbf{C^{J_I}}\\ \mathbf{C^{J_D}}\\ \mathbf{C^{s_I}}\\ \mathbf{C^{s_D}}\end{bmatrix}[\mathscr{K} \quad -\boldsymbol{\epsilon}_{\mathbf{s}}\mathscr{L}] = \begin{bmatrix}\mathbf{I}_{n-r} & \mathbf{0}\\ \mathscr{K}_0 & \mathbf{0}\\ \mathbf{0} & \mathbf{I}_r\\ \mathbf{0} & \mathscr{L}_0\end{bmatrix}. \tag{12}$$

Extracting the equations for the independent variables $\mathbf{J_I}$ and $\mathbf{s_I}$ gives:

$$\begin{bmatrix}\mathbf{C^{J_I}}\\ \mathbf{C^{s_I}}\end{bmatrix}[\mathscr{K} \quad -\boldsymbol{\epsilon}_{\mathbf{s}}\mathscr{L}] = \begin{bmatrix}\mathbf{I}_{n-r} & \mathbf{0}\\ \mathbf{0} & \mathbf{I}_r\end{bmatrix}. \tag{13}$$

If $\mathbf{C^I}$ is defined as $[\mathbf{C^{J_I}}\ \mathbf{C^{S_I}}]^T$ and $\mathbf{E}$ as $[\mathscr{K} \quad -\boldsymbol{\epsilon}_{\mathbf{s}}\mathscr{L}]$ this reduces to a particularly elegant form:

$$\mathbf{C^I E} = \mathbf{I}. \tag{14}$$

Both $\mathbf{C^I}$ and $\mathbf{E}$ are invertible $n \times n$ square matrices, i.e., the equation can also be written as $\mathbf{E}\mathbf{C^I} = \mathbf{I}$. This equation is completely general and holds for any network of reactions.

Co-response Analysis

Hofmeyr *et al*. (1993) described a transformation of eqn (14) which led to an expression that shows great promise as a general and robust strategy for experimental metabolic control and regulation analysis (Cornish-Bowden & Hofmeyr, 1994). This strategy, which we call *co-response analysis*, has the advantage of not requiring information on the kinetics or activity of the enzymes catalysing the steps in the pathway under study. All that it requires is a means for independently manipulating the activity of each step and for measuring changes in the steady-state fluxes and concentrations. The parameter changes that are available for these activity manipulations are discussed in a later section. In what follows it is shown that the method is applicable to all types of metabolic pathways, including pathways that are branched and contain moiety-conserved cycles.

The control matrix eqn (14) is transformed by using a diagonal matrix $\mathbf{D^C}$ with control coefficients on its diagonal; its inverse $(\mathbf{D^C})^{-1}$ is a diagonal matrix with the reciprocals of the control coefficients on its diagonal. The product $(\mathbf{D^C})^{-1}\mathbf{D^C}$ is the identity matrix $\mathbf{I}$

$$\mathbf{C^I}(\mathbf{D^C})^{-1}\mathbf{D^C E} = \mathbf{I} \tag{15}$$

which, if $\mathbf{O} = \mathbf{C^I}(\mathbf{D^C})^{-1}$ and $\mathbf{R} = \mathbf{D^C E}$, becomes

$$\mathbf{O}\mathbf{R} = \mathbf{I}. \tag{16}$$

Each element of the co-response matrix $\mathbf{O}$ is a *co-response coefficient* (Hofmeyr *et al*., 1993), which relates the concomitant change in two independent steady-state variables when the activity of a step is perturbed. The co-response coefficient of steady-state variables y_1 and y_2 with respect to a change in the local activity of step i is defined as

$$O_i^{y_1,y_2} = \frac{C_i^{y_1}}{C_i^{y_2}} = \frac{\partial \ln y_1}{\partial \ln v_i} \Big/ \frac{\partial \ln y_2}{\partial \ln v_i} = \frac{\partial \ln y_1}{\partial \ln y_2}. \tag{17}$$

Co-response coefficients are determined experimentally by perturbing the activity of each step in the pathway by means of a suitable parameter change, and measuring the resulting changes in steady-state fluxes and concentrations. Co-response coefficients are then determined as the slope of the curve obtained when the logarithm of the two steady-state variables are plotted against each other (Hofmeyr *et al*., 1993; Cornish-Bowden & Hofmeyr, 1994). This procedure follows from the definition in eqn (17). Depending on the content of the diagonal matrix $\mathbf{D^C}$ used in the transformation, the appropriate co-response matrix can then be constructed numerically.

The first $(n–r)$ rows of the internal response matrix $\mathbf{R}$ contain control coefficients, and the remaining rows consist of *internal response coefficients*. Each of these describes the partial effect via step i on a steady-state variable y with respect to a fluctuation in metabolite

concentration s_j and is defined as (Hofmeyr *et al.*, 1993; Kahn & Westerhoff, 1991)

$$^{i}R^{y}_{s_j} = C^{y}_{i}\epsilon^{v_i}_{s_j} \tag{18}$$

where C^{y}_{i} is the y-control coefficient of step i, and $\epsilon^{v_i}_{s_j}$ is the elasticity coefficient of local rate v_i towards metabolite concentration s_j.

From eqn (16) it follows that $\mathbf{R} = (\mathbf{O})^{-1}$, so that a numerical inversion of **O** gives **R**. Each element in a row of **R** contains the corresponding control coefficient in $\mathbf{D}^{\mathbf{C}}$, either on its own or multiplied with a fractional flux or an elasticity coefficient or a linear sum of weighted elasticity coefficients (if there are moiety-conserved cycles). The left-hand n–r columns of **R** are derived from the columns of $\mathscr{K}$ in **E**; their sum is always a column of control coefficients, just as the sum of the columns of $\mathscr{K}$ [constructed as in eqn (3)] is a unit column vector. Dividing the elements of each column by the corresponding element in this vector sum of the left-hand n–r columns of **R** transforms **R** into **E**. In matrix terms this transformation is accomplished by pre-multiplying **R** with a diagonal matrix that contains on the diagonal the reciprocals of the elements of the column vector formed by adding the first n–r columns of **R**. Taking the inverse of **E** then gives $\mathbf{C}^{\mathbf{I}}$ directly, as shown by eqn (14).

The procedure described above will allow control coefficients with respect to all independent variables to be calculated from a co-response analysis. What remains is to show how the control coefficients of the dependent variables can be calculated. The procedure is extremely simple and uses the following two relationships (Acerenza, 1993, 1996):

$$\mathbf{C}^{\mathbf{J_D}} = \mathscr{K}_0\mathbf{C}^{\mathbf{J_I}} \tag{19}$$

$$\mathbf{C}^{\mathbf{s_D}} = \mathscr{L}_0\mathbf{C}^{\mathbf{s_I}} \tag{20}$$

which follow from eqns (3) and (5).

We have thus solved the control analysis part of co-response analysis, and must now consider the elasticity analysis. If $\mathbf{L} = \mathbf{I}$, i.e., if there are no conservation constraints, the job has already been done—the right-hand r columns of **E** form the elasticity matrix $-\boldsymbol{\epsilon}_{\mathbf{s}}$ and thus contain the values of the elasticity coefficients. However, if $\mathbf{L} \neq \mathbf{I}$, some elements in the right-hand r columns of **E** contain linear functions of elasticity coefficients, and more information is needed to solve for the individual elasticities. This extra information can only be obtained by perturbing the conservation sums in the column vector **T** and measuring the resulting steady-state changes in *all* the fluxes and concentrations. A matrix equation to solve for $\boldsymbol{\epsilon}_{\mathbf{s}}$ is constructed on the basis of the general relationship (Acerenza, 1993):

$$\mathbf{R}^{\mathbf{J}}_{\mathbf{T}} = \boldsymbol{\epsilon}_{\mathbf{s}}\mathbf{R}^{\mathbf{s}}_{\mathbf{T}} \tag{21}$$

where $\mathbf{R}^{\mathbf{J}}_{\mathbf{T}}$ is an $n \times (m - r)$ matrix of scaled flux-response coefficients $R^{J_i}_{T_j} = \partial \ln J_i / \partial \ln T_j$ and $\mathbf{R}^{\mathbf{s}}_{\mathbf{T}}$ an $n \times (m - r)$ matrix of scaled concentration-response coefficients $R^{s_i}_{T_j} = \partial \ln s_i / \partial \ln T_j$.

Both sides of the equation are now augmented with the matrix $\boldsymbol{\epsilon}_{\mathbf{s}}\mathscr{L}$, which has already been calculated during the co-response analysis:

$$[\boldsymbol{\epsilon}_{\mathbf{s}}\mathscr{L} \quad \mathbf{R}^{\mathbf{J}}_{\mathbf{T}}] = [\boldsymbol{\epsilon}_{\mathbf{s}}\mathscr{L} \quad \boldsymbol{\epsilon}_{\mathbf{s}}\mathbf{R}^{\mathbf{s}}_{\mathbf{T}}] \tag{22}$$

which can be re-arranged to solve for $\boldsymbol{\epsilon}_{\mathbf{s}}$:

$$\boldsymbol{\epsilon}_{\mathbf{s}} = [\boldsymbol{\epsilon}_{\mathbf{s}}\mathscr{L} \quad \mathbf{R}^{\mathbf{J}}_{\mathbf{T}}][\mathscr{L} \quad \mathbf{R}^{\mathbf{s}}_{\mathbf{T}}]^{-1}. \tag{23}$$

The $n \times n$ matrix $[\mathscr{L} \ \mathbf{R}^{\mathbf{s}}_{\mathbf{T}}]$ has been proved to be invertible (Acerenza, 1993).

An Illustrative Example

To make the rather abstract nature of the above reasoning more transparent we now analyse the specific model in Fig. 1. It has been constructed to be simple but yet contain all the features necessary to demonstrate the procedure of co-response analysis, i.e., it has a branched flux and a moiety-conserved cycle. The calculations are rather simple, and can be done with any modern spreadsheet program, or with dedicated mathematics programs such as Mathematica^{R}, Maple^{R} or MathCad^{R}.

The stoichiometric matrix for this sytem is augmented with an identity matrix and then subjected

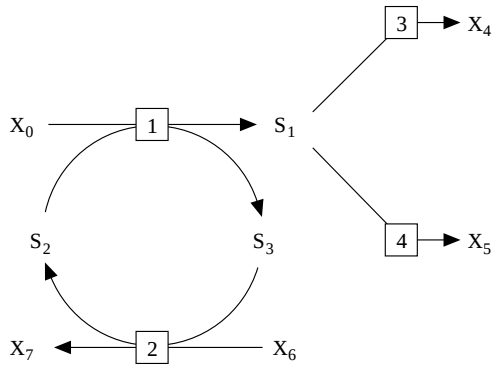

FIG. 1. A metabolic pathway with a branched flux and a moiety-conserved cycle.

to Gaussian elimination until the row echelon form is obtained:

	R_1	R_2	R_3	R_4	$\dot{s}_1$	$\dot{s}_2$	$\dot{s}_3$
S_1	1	0	−1	−1	1	0	0
S_2	−1	1	0	0	0	1	0
S_3	1	−1	0	0	0	0	1

		R_1	R_2	R_3	R_4	$\dot{s}_1$	$\dot{s}_2$	$\dot{s}_3$
→	S_1	1	0	−1	−1	1	0	0
	S_2	0	1	−1	−1	1	1	0
	S_3	0	0	0	0	0	1	1

(24)

The rank of the stoichiometric matrix is 2 and there is one conservation relationship $s_2 + s_3 = T$, where T is the conserved sum. There are two independent fluxes and two independent metabolites.

Choosing J_3 and J_4 [the R_3 and R_4-columns without pivots in the reduced stoichiometric matrix on the r.h.s. of eqn (24)] as the independent fluxes, the **K**-matrix follows from the flux-relationships

$$\begin{bmatrix} J_3 \\ J_4 \\ J_1 \\ J_2 \end{bmatrix} = \begin{bmatrix} J_3 \\ J_4 \\ J_3 + J_4 \\ J_3 + J_4 \end{bmatrix} = \begin{bmatrix} 1 \\ 0 \\ 1 \\ 1 \end{bmatrix} J_3 + \begin{bmatrix} 0 \\ 1 \\ 1 \\ 1 \end{bmatrix} J_4 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 1 & 1 \\ 1 & 1 \end{bmatrix} \begin{bmatrix} J_3 \\ J_4 \end{bmatrix}. \quad (25)$$

K is scaled to $\mathscr{K}$ as follows:

$$\begin{bmatrix} \frac{1}{J_3} & 0 & 0 & 0 \\ 0 & \frac{1}{J_4} & 0 & 0 \\ 0 & 0 & \frac{1}{J_1} & 0 \\ 0 & 0 & 0 & \frac{1}{J_2} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 1 & 1 \\ 1 & 1 \end{bmatrix} \begin{bmatrix} J_3 & 0 \\ 0 & J_4 \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ \frac{J_3}{J_1} & \frac{J_4}{J_1} \\ \frac{J_3}{J_2} & \frac{J_4}{J_2} \end{bmatrix}. \quad (26)$$

Either S_2 or S_3 can be chosen as the dependent metabolite. We choose S_3. The **L**-matrix follows from the relationships between the time derivatives, which are read off from the last row of the right-hand matrix in eqn (24):

$$\begin{bmatrix} \dot{s}_1 \\ \dot{s}_2 \\ \dot{s}_3 \end{bmatrix} = \begin{bmatrix} \dot{s}_1 \\ \dot{s}_2 \\ -\dot{s}_2 \end{bmatrix} = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} \dot{s}_1 + \begin{bmatrix} 0 \\ 1 \\ -1 \end{bmatrix} \dot{s}_2 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & -1 \end{bmatrix} \begin{bmatrix} \dot{s}_1 \\ \dot{s}_2 \end{bmatrix}. \quad (27)$$

L is scaled to $\mathscr{L}$ as follows:

$$\begin{bmatrix} \frac{1}{s_1} & 0 & 0 \\ 0 & \frac{1}{s_2} & 0 \\ 0 & 0 & \frac{1}{s_3} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & -1 \end{bmatrix} \begin{bmatrix} s_1 & 0 \\ 0 & s_2 \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & \frac{-s_2}{s_3} \end{bmatrix}. \quad (28)$$

The matrix product $\boldsymbol{\epsilon}_s\mathscr{L}$ for this system is

$$\begin{bmatrix} \epsilon_{s_1}^{v_3} & 0 & 0 \\ \epsilon_{s_1}^{v_4} & 0 & 0 \\ \epsilon_{s_1}^{v_1} & \epsilon_{s_2}^{v_1} & \epsilon_{s_3}^{v_1} \\ 0 & \epsilon_{s_2}^{v_2} & \epsilon_{s_3}^{v_2} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & -\frac{s_2}{s_3} \end{bmatrix} = \begin{bmatrix} \epsilon_{s_1}^{v_3} & 0 \\ \epsilon_{s_1}^{v_4} & 0 \\ \epsilon_{s_1}^{v_1} & (\epsilon_{s_2}^{v_1} - \epsilon_{s_3}^{v_1}\frac{s_2}{s_3}) \\ 0 & (\epsilon_{s_2}^{v_2} - \epsilon_{s_3}^{v_2}\frac{s_2}{s_3}) \end{bmatrix}. \quad (29)$$

Note that the re-ordering of fluxes in the $\mathscr{K}$-matrix is taken into account.

We now prepare for co-response analysis by constructing the $\mathbf{C}^{\mathbf{I}}\mathbf{E} = \mathbf{I}$ control-matrix equation using $\mathscr{K}$ and $-\boldsymbol{\epsilon}_s\mathscr{L}$:

$$\begin{bmatrix} C_3^{J_3} & C_4^{J_3} & C_1^{J_3} & C_2^{J_3} \\ C_3^{J_4} & C_4^{J_4} & C_1^{J_4} & C_2^{J_4} \\ C_3^{s_1} & C_4^{s_1} & C_1^{s_1} & C_2^{s_1} \\ C_3^{s_2} & C_4^{s_2} & C_1^{s_2} & C_2^{s_2} \end{bmatrix} \times \begin{bmatrix} 1 & 0 & -\epsilon_{s_1}^{v_3} & 0 \\ 0 & 1 & -\epsilon_{s_1}^{v_4} & 0 \\ \frac{J_3}{J_1} & \frac{J_4}{J_1} & -\epsilon_{s_1}^{v_1} & (\epsilon_{s_3}^{v_1}\frac{s_2}{s_3} - \epsilon_{s_2}^{v_1}) \\ \frac{J_3}{J_2} & \frac{J_4}{J_2} & 0 & (\epsilon_{s_3}^{v_2}\frac{s_2}{s_3} - \epsilon_{s_2}^{v_2}) \end{bmatrix}$$

$$= \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}. \quad (30)$$

The co-response matrix is constructed with reference to J_4, although any of the set of independent variables could have been chosen, even a mixture (Hofmeyr *et al*., 1993; Cornish-Bowden & Hofmeyr, 1994):

$$\begin{bmatrix} C_3^{J_3} & C_4^{J_3} & C_1^{J_3} & C_2^{J_3} \\ C_3^{J_4} & C_4^{J_4} & C_1^{J_4} & C_2^{J_4} \\ C_3^{s_1} & C_4^{s_1} & C_1^{s_1} & C_2^{s_1} \\ C_3^{s_2} & C_4^{s_2} & C_1^{s_2} & C_2^{s_2} \end{bmatrix} \begin{bmatrix} \frac{1}{C_3^{J_4}} & 0 & 0 & 0 \\ 0 & \frac{1}{C_4^{J_4}} & 0 & 0 \\ 0 & 0 & \frac{1}{C_1^{J_4}} & 0 \\ 0 & 0 & 0 & \frac{1}{C_2^{J_4}} \end{bmatrix}$$

$$= \begin{bmatrix} O_3^{J_3,J_4} & O_4^{J_3,J_4} & O_1^{J_3,J_4} & O_2^{J_3,J_4} \\ 1 & 1 & 1 & 1 \\ O_3^{s_1,J_4} & O_4^{s_1,J_4} & O_1^{s_1,J_4} & O_2^{s_1,J_4} \\ O_3^{s_2,J_4} & O_4^{s_2,J_4} & O_1^{s_2,J_4} & O_2^{s_2,J_4} \end{bmatrix} = \mathbf{O}. \quad (31)$$

The internal response matrix **R** is constructed using the same control coefficients. For purposes of calculation it is not necessary to construct this matrix, but it is done here to demonstrate some of its properties.

$$\begin{bmatrix} C_3^{J_4} & 0 & 0 & 0 \\ 0 & C_4^{J_4} & 0 & 0 \\ 0 & 0 & C_1^{J_4} & 0 \\ 0 & 0 & 0 & C_2^{J_4} \end{bmatrix}$$

$$\times \begin{bmatrix} 1 & 0 & -\epsilon_{s_1}^{v_3} & 0 \\ 0 & 1 & -\epsilon_{s_1}^{v_4} & 0 \\ \frac{J_3}{J_1} & \frac{J_4}{J_1} & -\epsilon_{s_1}^{v_1} & (\epsilon_{s_3}^{v_1}\frac{s_2}{s_3} - \epsilon_{s_2}^{v_1}) \\ \frac{J_3}{J_2} & \frac{J_4}{J_2} & 0 & (\epsilon_{s_3}^{v_2}\frac{s_2}{s_3} - \epsilon_{s_2}^{v_2}) \end{bmatrix}$$

$$= \begin{bmatrix} C_3^{J_4} & 0 & -C_3^{J_4}\epsilon_{s_1}^{v_3} & 0 \\ 0 & C_4^{J_4} & -C_4^{J_4}\epsilon_{s_1}^{v_4} & 0 \\ \frac{J_3}{J_1}C_1^{J_4} & \frac{J_4}{J_1}C_1^{J_4} & -C_1^{J_4}\epsilon_{s_1}^{v_1} & C_1^{J_4}(\epsilon_{s_3}^{v_1}\frac{s_2}{s_3} - \epsilon_{s_2}^{v_1}) \\ \frac{J_3}{J_2}C_2^{J_4} & \frac{J_4}{J_2}C_2^{J_4} & 0 & C_2^{J_4}(\epsilon_{s_3}^{v_2}\frac{s_2}{s_3} - \epsilon_{s_2}^{v_2}) \end{bmatrix} = \mathbf{R}. \quad (32)$$

TABLE 1
Kinetic properties of the model in Fig. 1

Rate Equations
$v_1 = e_1(10x_0s_2 - s_1s_3)/(1 + x_0 + s_2 + s_1 + s_3 + x_0s_2 + s_1s_3)$
$v_2 = e_2(10x_6s_3 - x_7s_2)/(1 + s_3 + s_2 + x_6 + x_7 + s_3x_6 + s_2x_7)$
$v_3 = e_3(50s_1 - x_4)/(1 + s_1 + x_4)$
$v_4 = e_4(10s_1 - x_5)/(1 + s_1 + x_5)$

Enzyme concentrations e_i were set to 1. The fixed external concentrations were $x_0 = 10$, $x_4 = x_5 = 0$, and $x_6 = x_7 = 1$. The conservation sum $T = s_2 + s_3 = 5$. Concentration units are arbitrary.

Note that, as $J_3 + J_4 = J_1 = J_2$, addition of the left-hand two columns of **R** gives a column vector of the four J_4-control coefficients.

To demonstrate the calculation procedure we simulated the steady-state of the model in Fig. 1. The simulation conditions are given in Table 1 and the results in Table 2.

The simulation yielded the following co-response matrix **O**:

$$\mathbf{O} = \begin{bmatrix} -0.2025 & -0.1995 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1.0935 & -0.2181 & 1.0931 & 1.0931 \\ 0.0094 & -0.0019 & -4.4979 & 1.0058 \end{bmatrix}. \quad (33)$$

Inverting **O** numerically gives:

$$\mathbf{R} = \begin{bmatrix} -0.8313 & 0 & 0.7605 & 0 \\ 0 & 0.8337 & -0.7624 & 0 \\ 0.1506 & 0.0301 & 0.0019 & -0.1817 \\ 0.681 & 0.1362 & 0 & 0.1817 \end{bmatrix}. \quad (34)$$

The two left-hand columns of **R** are summed to give the column vector of J_4-control coefficients $[-0.8313, 0.8834, 0.1807, 0.8172]^T$. **R** is then pre-multiplied with a diagonal matrix that contains the reciprocals of the elements of this vector on its

TABLE 2
Steady-state properties of the model in Fig. 1

Flux		Concentration	
J_1	5.1132	s_1	0.0932
J_2	5.1132	s_2	0.8268
J_3	4.2610	s_3	4.1732
J_4	0.8522		

Calculations were done with MetaModel (Cornish-Bowden & Hofmeyr, 1991) and checked with Scamp (Sauro, 1993). Flux and concentration units are arbitrary.

diagonal to give **E**. This operation divides the elements in each column of **R** by the corresponding elements in the control coefficient vector.

$$\begin{bmatrix} \frac{1}{-0.8313} & 0 & 0 & 0 \\ 0 & \frac{1}{0.8334} & 0 & 0 \\ 0 & 0 & \frac{1}{0.1807} & 0 \\ 0 & 0 & 0 & \frac{1}{0.8172} \end{bmatrix} \times \begin{bmatrix} -0.8313 & 0 & 0.7605 & 0 \\ 0 & 0.8337 & -0.7624 & 0 \\ 0.1506 & 0.0301 & 0.0019 & -0.1817 \\ 0.681 & 0.1362 & 0 & 0.1817 \end{bmatrix}$$

$$= \begin{bmatrix} 1 & 0 & -0.9148 & 0 \\ 0 & 1 & -0.9148 & 0 \\ 0.8333 & 0.1667 & 0.0105 & -1.0057 \\ 0.8333 & 0.1667 & 0 & 0.2223 \end{bmatrix} = \mathbf{E}. \tag{35}$$

E is then inverted to give the control coefficient matrix:

$$\mathbf{C^I} = \begin{bmatrix} 0.1683 & -0.1663 & 0.1807 & 0.8172 \\ -0.8313 & 0.8334 & 0.1807 & 0.8172 \\ -0.9091 & -0.1818 & 0.1975 & 0.8933 \\ -0.0078 & -0.0016 & -0.8126 & 0.8220 \end{bmatrix}. \tag{36}$$

The rows sum to the column vector $[1, 1, 0, 0]^T$, in accordance with the summation properties of control coefficients (Kacser & Burns, 1973).

The values of dependent flux-control coefficients are calculated using the relationship $\mathbf{C^{J_D}} = \mathscr{K}_0\mathbf{C^{J_I}}$, here

$$\begin{bmatrix} C_3^{J_1} & C_4^{J_1} & C_1^{J_1} & C_2^{J_1} \\ C_3^{J_2} & C_4^{J_2} & C_1^{J_2} & C_2^{J_2} \end{bmatrix} = \begin{bmatrix} \frac{J_3}{J_1} & \frac{J_4}{J_1} \\ \frac{J_3}{J_2} & \frac{J_4}{J_2} \end{bmatrix} \begin{bmatrix} C_3^{J_3} & C_4^{J_3} & C_1^{J_3} & C_2^{J_3} \\ C_3^{J_4} & C_4^{J_4} & C_1^{J_4} & C_2^{J_4} \end{bmatrix}. \tag{37}$$

The calculation gives:

$$\mathbf{C^{J_D}} = \begin{bmatrix} 0.0017 & 0.0003 & 0.1807 & 0.8172 \\ 0.0017 & 0.0003 & 0.1807 & 0.8172 \end{bmatrix} = \begin{bmatrix} 0.8333 & 0.1667 \\ 0.8333 & 0.1667 \end{bmatrix} \times \begin{bmatrix} 0.1683 & -0.1663 & 0.1807 & 0.8172 \\ -0.8313 & 0.8334 & 0.1807 & 0.8172 \end{bmatrix}. \tag{38}$$

As $J_1 = J_2$, the two rows of dependent flux-control coefficients are identical.

The values of the dependent s_3-control coefficients are calculated using the relationship $\mathbf{C^{S_D}} = \mathscr{L}_0\mathbf{C^{S_I}}$, here

$$[C_3^{s_3} \quad C_4^{s_3} \quad C_1^{s_3} \quad C_2^{s_3}] = \begin{bmatrix} 0 & -\frac{s_2}{s_3} \end{bmatrix} \begin{bmatrix} C_3^{s_1} & C_4^{s_1} & C_1^{s_1} & C_2^{s_1} \\ C_3^{s_2} & C_4^{s_2} & C_1^{s_2} & C_2^{s_2} \end{bmatrix} \tag{39}$$

which, in this specific case, simplifies to:

$$[C_3^{s_3} \quad C_4^{s_3} \quad C_1^{s_3} \quad C_2^{s_3}] = -\frac{s_2}{s_3}[C_3^{s_2} \quad C_4^{s_2} \quad C_1^{s_2} \quad C_2^{s_2}]. \tag{40}$$

The calculation gives

$$\mathbf{C^{S_D}} = [0.0015 \quad 0.0003 \quad 0.1610 \quad -0.1628] = (-0.1981)[-0.0078 \quad -0.0016 \quad -0.8126 \quad 0.822]. \tag{41}$$

It now remains to calculate the individual elasticity coefficients using eqn (23). There is only one conserved sum $s_2 + s_3 = T$. The matrix product $\boldsymbol{\epsilon}_s\mathbf{L}$ has already been calculated in the course of the co-response analysis. For this system the detailed matrix equation is:

$$\begin{bmatrix} \epsilon_{s_1}^{v_3} & 0 & 0 \\ \epsilon_{s_1}^{v_4} & 0 & 0 \\ \epsilon_{s_1}^{v_1} & \epsilon_{s_2}^{v_1} & \epsilon_{s_3}^{v_1} \\ 0 & \epsilon_{s_2}^{v_2} & \epsilon_{s_3}^{v_2} \end{bmatrix} = \begin{bmatrix} \epsilon_{s_1}^{v_3} & 0 & R_T^{J_3} \\ \epsilon_{s_1}^{v_4} & 0 & R_T^{J_4} \\ \epsilon_{s_1}^{v_1} & (\epsilon_{s_2}^{v_1} - \epsilon_{s_3}^{v_1}\frac{s_2}{s_3}) & R_T^{J_1} \\ 0 & (\epsilon_{s_2}^{v_2} - \epsilon_{s_3}^{v_2}\frac{s_2}{s_3}) & R_T^{J_2} \end{bmatrix} \times \begin{bmatrix} 1 & 0 & R_T^{s_1} \\ 0 & 1 & R_T^{s_2} \\ 0 & -\frac{s_2}{s_3} & R_T^{s_3} \end{bmatrix}. \tag{42}$$

The numerical details are

$$\begin{bmatrix} 0.9148 & 0 & 0 \\ 0.9148 & 0 & 0 \\ -0.0105 & 0.9534 & -0.2639 \\ 0 & -0.1235 & 0.4989 \end{bmatrix}$$

$$= \begin{bmatrix} 0.9148 & 0 & 0.4314 \\ 0.9148 & 0 & 0.4314 \\ -0.0105 & 1.0057 & 0.4314 \\ 0 & -0.2223 & 0.4314 \end{bmatrix}$$

$$\times \begin{bmatrix} 1 & 0 & 0.4715 \\ 0 & 1 & 0.7482 \\ 0 & -0.1981 & 1.0499 \end{bmatrix}. \qquad (43)$$

Which Parameters Should be Varied in a Co-responsive Analysis?

Up to now we have assumed that the activity of each step in the system can be manipulated independently, without directly affecting the activity of other steps. Other steps are, however, ultimately affected, but only through systemic effects, i.e., through changes in the metabolite pools that link the reactions. In order to change selectively the activity of a step in a metabolic system, one of that step's enzyme parameters should be altered. Typically, the options are to change the total number of enzyme molecules (the enzyme concentration), either by titration (in a cell free extract) or by varying the expression of its gene, or to change the activity of the existing enzyme molecules by adding, for example, a specific inhibitor or activator of the enzyme (or, where applicable, an externally buffered substrate or product).

It remains to show under which conditions the co-response coefficients with respect to perturbations in parameters are the same as the co-response coefficients with respect to perturbations in the activities of steps, i.e., those coefficients on which co-response analysis as described in this paper is based and which are defined in eqn (17).

The response of a steady state to a parameter perturbation depends not only on the control coefficients of the steps directly affected by the parameter, but also on the elasticity coefficients of these steps with respect to the parameter change. This response is quantified in terms of a response coefficient, $R_p^y = \partial \ln y / \partial \ln p$, where y is a steady-state variable and p a parameter. According to the combined response property of metabolic control analysis (Kacser & Burns, 1973; Kholodenko, 1988) a response coefficient can in general be expressed as

$$R_p^y = \sum_{i=1}^{n} C_i^y \epsilon_p^{v_i}. \qquad (44)$$

A co-response coefficient with respect to parameter p is then defined as

$$\Omega_p^{y_1,y_2} = \frac{R_p^{y_1}}{R_p^{y_2}} = \frac{\sum_{i=1}^{n} C_i^{y_1} \epsilon_p^{v_i}}{\sum_{i=1}^{n} C_i^{y_2} \epsilon_p^{v_i}}, \qquad (45)$$

which, if p acts only on a single step i, reduces to

$$\Omega_p^{y_1,y_2} = \frac{C_i^{y_1} \epsilon_p^{v_i}}{C_i^{y_2} \epsilon_p^{v_i}} = \frac{C_i^{y_1}}{C_i^{y_2}} = O_i^{y_1,y_2}. \qquad (46)$$

In conclusion, therefore, co-response analysis requires the perturbation of a set of parameters chosen in such a way that there is one for each step, which affects only that step.

Discussion

This paper describes a strategy for the experimental control analysis called co-response analysis. Its advantages are that it is general for metabolic systems of arbitrary structural complexity and that it requires no quantitative information about the magnitudes of the effects on the individual enzyme activities, and consequently no detailed kinetic information about the enzymes in the pathway. It is informative to contrast co-response analysis to other general experimental strategies that have recently been proposed, notably the multiple modulation method (Giersch, 1994, 1995), the top-down method (Brown *et al*., 1990), and the modular method (Kahn & Westerhoff, 1991; Schuster *et al*., 1993). In addition to what follows the discussion by Rohwer *et al*. (1996) should also be consulted.

Giersch (1994, 1995) has developed an extension of the double modulation method of Kacser & Burns (1979) for determining elasticities of individual enzymes, which is based, like co-response analysis, on the measurement of steady-state metabolite concentrations and fluxes. Giersch also addresses the specific problem of identifying the size and composition of the minimal set of independent modulations that have to be made to allow a complete elasticity analysis. In Giersch (1994) only a linear chain of reactions is considered, but the method has since been extended to include more complex pathways containing

feedback loops, branches and moiety-conserved cycles (Giersch & Cornish-Bowden, 1996). Giersch (1994) compared his method to ours, but the two differences that he noted are somewhat misleading and require some comment. First, confusion can result from the statement that the $\mathbf{C}^{\mathrm{I}}\mathbf{E} = \mathbf{I}$ formalism on which co-response analysis is based is not derived from the differential equations of the metabolic system, and therefore implies different mathematics for its solution. The equation $\mathbf{C}^{\mathrm{I}}\mathbf{E} = \mathbf{I}$ as used in this paper is constructed from general connectivity and summation relationships that derive directly from implicit differentiation of the steady-state system equations. Although Reder (1988) did not follow this route in her original treatment, Heinrich & Reder (1991) subsequently showed that the same equations arise from differentiation. Second, co-response analysis does not require the measurement of the slopes $\partial J/\partial p_r$ and $\partial s_j/\partial p_r$ where p_r is the parameter used to modulate the activity of step r. Giersch (1994) is quite correct to state that specific parameter modulations are required in co-response analysis, one for each step as described in the previous section, even though they do not appear explicitly in the equations. Nevertheless, the co-response coefficients are calculated directly from log-log plots of one steady-state variable against another; measurement of the exact magnitude of the change in parameter, or of $\partial J/\partial p_r$, etc., is unnecessary.

Nevertheless, an attractive feature of the multiple modulation method is that it identifies a minimal set of parameter perturbations necessary for a full control analysis. As described here, co-response analysis seems to require a full set of perturbations. However, as noted in Cornish-Bowden & Hofmeyr (1994), it is not always necessary to perturb all the steps to fill in the whole co-response coefficient matrix. This aspect has recently been investigated by Rohwer *et al*. (1996). They found rules for identifying subsystems within metabolic systems that function as units with respect to their effect on the remainder of the system and showed that the co-response of two steady-state variables outside of that subsystem to a perturbation of the subsystem does not depend on which step in the subsystem is perturbed. Such "monofunctional units" must fulfill three criteria: (i) the reactions outside the subsystem are not affected directly by metabolites belonging to the subsystem, (ii) there are no conservation relations linking the subsystem to the rest, and (iii) the subsystem is linked to the remainder of the system only via one degree of freedom in fluxes. Analysing a system into its monofunctional units should greatly aid and simplify co-response analysis. The work of Rohwer *et al*. (1996) also relates co-response analysis to the top-down approach (Brown *et al*., 1990) and the modular approach (Kahn & Westerhoff, 1991; Schuster *et al*., 1993), both of which depend on the division of the system into blocks or modules.

The aim of this paper is to generalise co-response analysis as a frame-work for experimental control analysis, but there is an additional use for co-response and internal response coefficients that makes this approach even more attractive. These coefficients can be interpreted as measures of external and internal regulatory aspects of metabolic systems (Hofmeyr & Cornish-Bowden, 1991, 1993, 1994; Hofmeyr, 1995). In fact, it was out of the search for a relationship between co-response and internal response coefficients and, therefore, a basis for metabolic regulation analysis, that co-response analysis originally arose (Hofmeyr *et al*., 1993). This aspect will be pursued in a further series of publications.

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