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Priority paper

Taking enzyme kinetics out of control; putting control into regulation

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The matrix formulation of metabolic control analysis, which states that multiplying the elasticity matrix for any system by the corresponding control matrix yields an identity matrix, can be transformed into a statement that multiplying a matrix expressing internal regulatory properties by a matrix expressing external regulatory properties also yields an identity matrix. This transformation supplies the formal basis for metabolic regulation analysis, and provides the key to determining the control structure of a system without the need to know the exact changes in enzyme activities that are made to measure control coefficients.

Since the appearance of the original papers on metabolic control analysis [1, 2], two points have been clear: first, the control structure of a metabolic system is conceptually distinct from the study of the mechanisms and kinetics of its constituent enzymes, and ought to be experimentally separable from it as well; second, a full understanding of metabolic control should lead to an understanding of metabolic regulation. However, although metabolic control analysis can now deal with systems of any complexity [3–6], and is becoming widely applied [7, 8], these specific goals have not been achieved. Determination of the control structure of a system has continued to require detailed knowledge of the kinetic properties of the constituent enzymes, and the relationship of control analysis to the classical study of metabolic regulation has remained more obscure than one would wish. In a previous paper [9] we discussed the differences between regulation and control, setting out some of the regulatory principles that would need to be integrated into a complete theory, but we did not arrive at a full mathematical expression of how these principles were related to control analysis. We now show how an apparently trivial point of matrix algebra allows both goals to be reached. Interposing a matrix product equal to an identity matrix between the two terms in the standard matrix representation of control analysis transforms it into the basis for regulation analysis and obviates the need for measuring the changes in enzyme activities that are made to determine control coefficients experimentally.

THEORY

The relationships between control and elasticity coefficients have been expressed in a number of matrix-equation formats [3–6, 10–15]. A particularly elegant and concise formulation is that of Westerhoff and Kell [13]; in principle it is identical to that obtained by Cascante et al. [5, 6] through implicit differentiation of the steady-state equations:

$$\varepsilon C = I \tag{1}$$

where ε is a square matrix of elasticity coefficients, defined as $\varepsilon^{v_i}_{s_j} = \partial \ln v_i / \partial \ln s_j$, C is a square matrix of control coefficients and I is the identity matrix. A control coefficient is defined in terms of any parameter p that affects enzyme activity as follows:

$$C^x_i = \frac{\partial \ln x / \partial p}{\partial \ln v_i / \partial p} . \tag{2}$$

We define a diagonal matrix D^x that has the control coefficient vector C^x as its diagonal, x being any steady-state variable. Its inverse $(D^x)^{-1}$ is a diagonal matrix containing the reciprocals of the control coefficients. The product $D^x(D^x)^{-1}$ is the identity matrix I.

With these diagonal matrices Eqn (1) can be transformed as follows:

$$\varepsilon D^x (D^x)^{-1} C = I \tag{3}$$

which, if $R^x = \varepsilon D^x$ and $O_x = (D^x)^{-1} C$, becomes

$$R^x O_x = I . \tag{4}$$

R^x is a matrix which, except for its first row of control coefficients [see Eqn (9)], contains rows of what Kahn and Westerhoff [16] have called 'regulatory strengths'. Although we share the misgivings about this name expressed by a referee, we feel it would be misleading if we were to change it here. A regulatory strength of metabolite S_j on variable x via enzyme E_i is defined as follows: [16]

$$^{E_i}R^x_{s_j} = C^x_i \, \varepsilon^{v_i}_{s_j} \tag{5}$$

where C^x_i is the control coefficient of E_i with respect to steady-state variable x, and $\varepsilon^{v_i}_{s_j}$ is the elasticity coefficient of local enzyme rate v_i towards metabolite concentration s_j. Regulatory strengths have been proposed [16] as measures

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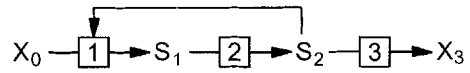

Fig. 1. A metabolic system consisting of three sequentially coupled enzyme reactions with end-product inhibition.

of internal regulation and form the basis of the concept of regulatory potential [17, 18].

Each element of O_x relates the concomitant change in two steady-state variables which results from an activity change of a specified enzyme and is a form of 'derived' sensitivity [19] for which we propose the name 'co-response coefficient'. The co-response coefficient of steady-state variables x and y with respect to a change in the activity of E_i is defined as

$$^{E_i}O_y^x = \frac{C_i^x}{C_i^y}. \tag{6}$$

The similarity of this term to correlation coefficient is deliberate, as this also expresses how two variables vary together in response to some perturbation. However, in statistics the driving variable is normally implicit or even hypothetical, whereas regulation analysis requires the parameter to be specified; it therefore appears as a pre-superscript in the symbol for co-response coefficient. Any co-response coefficient can be calculated as the slope of the tangent, at the steady-state point, to a plot of ln x against ln y obtained by varying the activity of E_i and measuring the steady-state response. A co-response coefficient can be regarded as a quantitative measure of external regulation; conceptually, it is a formalization of ideas expressed in a previous paper on metabolic regulation [9].

As an illustration of the detailed form of these matrix equations, consider the simple linear metabolic pathway with a feedback loop in Fig. 1. The control-matrix Eqn (1) translates to

$$\begin{bmatrix} 1 & 1 & 1 \\ \varepsilon_1^1 & \varepsilon_1^2 & 0 \\ \varepsilon_2^1 & \varepsilon_2^2 & \varepsilon_2^3 \end{bmatrix} \begin{bmatrix} C_1^J & -C_1^{s_1} & -C_1^{s_2} \\ C_2^J & -C_2^{s_1} & -C_2^{s_2} \\ C_3^J & -C_3^{s_1} & -C_3^{s_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}. \tag{7}$$

The transformation described in Eqn (3) is accomplished by

$$\begin{bmatrix} 1 & 1 & 1 \\ \varepsilon_1^1 & \varepsilon_1^2 & 0 \\ \varepsilon_2^1 & \varepsilon_2^2 & \varepsilon_2^3 \end{bmatrix} \begin{bmatrix} C_1^x & 0 & 0 \\ 0 & C_2^x & 0 \\ 0 & 0 & C_3^x \end{bmatrix} \begin{bmatrix} \frac{1}{C_1^x} & 0 & 0 \\ 0 & \frac{1}{C_2^x} & 0 \\ 0 & 0 & \frac{1}{C_3^x} \end{bmatrix} \times$$

$$\times \begin{bmatrix} C_1^J & -C_1^{s_1} & -C_1^{s_2} \\ C_2^J & -C_2^{s_1} & -C_2^{s_2} \\ C_3^J & -C_3^{s_1} & -C_3^{s_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{8}$$

which leads to the R^xO_x formulation

$$\begin{bmatrix} C_1^x & C_2^x & C_3^x \\ ^{E_1}R_{s_1}^x & ^{E_2}R_{s_1}^x & 0 \\ ^{E_1}R_{s_2}^x & ^{E_2}R_{s_2}^x & ^{E_3}R_{s_2}^x \end{bmatrix} \begin{bmatrix} ^{E_1}O_x^J & -^{E_1}O_x^{s_1} & -^{E_1}O_x^{s_2} \\ ^{E_2}O_x^J & -^{E_2}O_x^{s_1} & -^{E_2}O_x^{s_2} \\ ^{E_3}O_x^J & -^{E_3}O_x^{s_1} & -^{E_3}O_x^{s_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}. \tag{9}$$

It is easily verified that the basic equations of control analysis are embedded in Eqn (4). If the diagonal elements of D^x form the control coefficient vector $[C_1^J, C_2^J, C_3^J]$, the first row of R^x is $[C_1^J, C_2^J, C_3^J]$ and the elements of the first column of O_x are all one; for any E_i and x, $^{E_i}O_x^x = 1$, because a variable correlates perfectly with itself. Multiplying the first column vector of O_x into R^x gives the flux-summation and the flux-connectivity equations. Similarly, if the C_i^x elements in D^x are either $C_i^{s_1}$ or $C_i^{s_2}$, the second or third column of O_x, respectively, has the elements -1; multiplication of these column vectors into R^x leads to the concentration-summation and concentration-connectivity equations for this system.

We now consider the implications of various choices of steady-state variable x in D^x.

Case 1: D^x contains flux-control coefficients

If the elements of D^x are flux-control coefficients, so that $D^x = D^J$, Eqn (4) becomes

$$R^J O_J = I. \tag{10}$$

Eqn (10) represents the formal basis of what can be called metabolic regulation analysis, and solves the problem of describing external regulatory properties in terms of internal regulatory properties, in the same way that metabolic control analysis solves the problem of describing systemic properties in terms of local enzymic properties.

For the metabolic system in Fig. 1, Eqn (9) can be written as

$$\begin{bmatrix} C_1^J & C_2^J & C_3^J \\ ^{E_1}R_{s_1}^J & ^{E_2}R_{s_1}^J & 0 \\ ^{E_1}R_{s_2}^J & ^{E_2}R_{s_2}^J & ^{E_3}R_{s_2}^J \end{bmatrix} \begin{bmatrix} 1 & -^{E_1}O_J^{s_1} & -^{E_1}O_J^{s_2} \\ 1 & -^{E_2}O_J^{s_1} & -^{E_2}O_J^{s_2} \\ 1 & -^{E_3}O_J^{s_1} & -^{E_3}O_J^{s_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}. \tag{11}$$

As we want to describe co-response coefficients in terms of regulatory strengths, the control coefficients in the first row of R^J must be replaced with regulatory strengths. If the square matrix ε in Eqn (1) is invertible, as it must be if a steady state exists [3], it follows from elementary matrix theory [21] that $C = \varepsilon^{-1}$ and $\varepsilon = C^{-1}$, i. e., control coefficients can be expressed in terms of elasticities and vice versa. It also follows that $C\varepsilon = I$. Eqn (7), can thus be rearranged to

$$\begin{bmatrix} C_1^J & -C_1^{s_1} & -C_1^{s_2} \\ C_2^J & -C_2^{s_1} & -C_2^{s_2} \\ C_3^J & -C_3^{s_1} & -C_3^{s_2} \end{bmatrix} \begin{bmatrix} 1 & 1 & 1 \\ \varepsilon_1^1 & \varepsilon_1^2 & 0 \\ \varepsilon_2^1 & \varepsilon_2^2 & \varepsilon_2^3 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{12}$$

which, when expanded, gives the set of relationships that express flux-control coefficients in terms of products of concentration-control coefficients and elasticity coefficients [4, 22]. The relationships that correspond to the diagonal of the identity matrix contain terms that have been called homeostatic strengths [16]; we prefer just to regard them as regulatory strengths of a metabolite on itself, which are the same apart from signs as homeostatic strengths, e. g., $^{E_i}R_{s_j}^{s_j} = -^{E_i}H_{s_j}$, using the symbol for homeostatic strength proposed by Kahn and Westerhoff [16]. For Eqn (12) these relationships are

$$C_1^J = 1 + C_1^{s_1}\varepsilon_1^1 + C_1^{s_2}\varepsilon_2^1 \tag{13}$$

$$C_2^J = 1 + C_2^{s_1}\varepsilon_1^2 + C_2^{s_2}\varepsilon_2^2 \tag{14}$$

$$C_3^J = 1 + C_3^{s_2}\varepsilon_2^3 \tag{15}$$

which, in terms of self-regulatory strengths, can be rewritten as

$$C_1^J = 1 + {}^{E_1}R_{s1}^{s_1} + {}^{E_1}R_{s2}^{s_2} \quad (16)$$

$$C_2^J = 1 + {}^{E_2}R_{s1}^{s_1} + {}^{E_2}R_{s2}^{s_2} \quad (17)$$

$$C_3^J = 1 + {}^{E_3}R_{s2}^{s_2}. \quad (18)$$

Inserting these relationships into Eqn (11) gives

$$\begin{bmatrix} (1 + {}^{E_1}R_{s1}^{s_1} + {}^{E_1}R_{s2}^{s_2}) & (1 + {}^{E_2}R_{s1}^{s_1} + {}^{E_2}R_{s2}^{s_2}) & (1 + {}^{E_3}R_{s2}^{s_2}) \\ {}^{E_1}R_{s1}^{J} & {}^{E_2}R_{s1}^{J} & 0 \\ {}^{E_1}R_{s2}^{J} & {}^{E_2}R_{s2}^{J} & {}^{E_3}R_{s2}^{J} \end{bmatrix} \times$$

$$\times \begin{bmatrix} 1 & -{}^{E_1}O_J^{s_1} & -{}^{E_1}O_J^{s_2} \\ 1 & -{}^{E_2}O_J^{s_1} & -{}^{E_2}O_J^{s_2} \\ 1 & -{}^{E_3}O_J^{s_1} & -{}^{E_3}O_J^{s_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}. \quad (19)$$

Expanding this equation gives the expressions for metabolite-flux co-response coefficients in terms of regulatory strengths.

Eqn (12) also supplies other expressions for each flux-control coefficient, which can be used to replace them in R^J. The $C_i^s\varepsilon_s^{v_i}$ in these equations are, however, not regulatory strengths, but rather the unscaled form of what Sauro [23] called partitioned response coefficients; to obtain these coefficients the $C_i^s\varepsilon_s^{v_i}$ terms were scaled by dividing them with the relevant flux-control coefficient.

Recently, we proposed the concept of regulatory potential of an internal metabolite for describing the degree to which a metabolic system senses a perturbation in the metabolite concentration [17, 18]. In any flux-connectivity equation the regulatory strengths sum to zero. This means that some regulatory strengths of internal metabolites must be positive, while the others must be negative. The sum of positive regulatory strengths must cancel the sum of negative regulatory strengths. Either sum gives an indication of how sensitive the flux, J, is to a perturbation in the concentration of the metabolite. We have termed the sum of positive regulatory strengths the regulatory potential of the metabolite [17]. This argument also holds for the concentration-connectivity equations where the regulatory strengths of the metabolite refer to other metabolites. In general, for a steady-state variable x that fulfils these conditions, the definition of the regulatory potential of metabolite S_k is

$$P_{sk}^x = \sum_{\text{positive}} {}^{E_i}R_{sk}^x = -\sum_{\text{negative}} {}^{E_j}R_{sk}^x = \sum_{\text{positive}} C_i^x\varepsilon_{sk}^{v_i} = -\sum_{\text{negative}} C_j^x\varepsilon_{sk}^{v_j} \quad (20)$$

where each E_i is an enzyme through which S_k exerts positive effects on x, while each E_j is an enzyme through which S_k exerts negative effects on x.

Inserting, where possible, flux-regulatory potentials into the R^J-matrix of Eqn (19) gives

$$\begin{bmatrix} (1 + {}^{E_1}R_{s1}^{s_1} + {}^{E_1}R_{s2}^{s_2}) & (1 + {}^{E_2}R_{s1}^{s_1} + {}^{E_2}R_{s2}^{s_2}) & (1 + {}^{E_3}R_{s2}^{s_2}) \\ -P_{s1}^J & P_{s1}^J & 0 \\ {}^{E_1}R_{s2}^J & {}^{E_2}R_{s2}^J & P_{s2}^J \end{bmatrix}. \quad (21)$$

It can be shown that the determinant of this matrix, and, therefore, the denominator of all co-response coefficients, is the product $-P_{s1}^J P_{s2}^J$, although some of these regulatory potentials are usually cancelled by regulatory potentials in the numerator. This demonstrates that regulatory potentials emerge as natural entities of metabolic regulation analysis, as do regulatory strengths and co-response coefficients.

Case 2: D^x contains concentration-control coefficients

If the elements of D^x are concentration-control coefficients, so that $D^x = D^{s_j}$, Eqn (4) becomes

$$R^{s_j}O_{s_j} = I. \quad (22)$$

For example, if s_1-control coefficients are chosen, Eqn (9) becomes

$$\begin{bmatrix} C_1^{s_1} & C_2^{s_1} & C_3^{s_1} \\ {}^{E_1}R_{s1}^{s_1} & {}^{E_2}R_{s1}^{s_1} & 0 \\ {}^{E_1}R_{s2}^{s_1} & {}^{E_2}R_{s2}^{s_1} & {}^{E_3}R_{s2}^{s_1} \end{bmatrix} \begin{bmatrix} {}^{E_1}O_{s1}^J & -1 & -{}^{E_1}O_{s1}^{s_2} \\ {}^{E_2}O_{s1}^J & -1 & -{}^{E_2}O_{s1}^{s_2} \\ {}^{E_3}O_{s1}^J & -1 & -{}^{E_3}O_{s1}^{s_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}. \quad (23)$$

The first row of R^{s_j} consists of concentration-control coefficients and the other rows of regulatory strengths of S_j on its own steady-state concentration or on other steady-state concentrations. If the objective of metabolic regulation analysis were to describe the effects of metabolites on metabolites, then this formulation would be its basis. However, the concentration-control coefficients in the first row of R^{s_j} cannot be expressed purely in terms of regulatory strengths, as was possible in R^J.

Case 3: D^x contains a mixture of control coefficients: making Kacser's dream a reality

Up to now we have considered instances of D^x where x referred to one specific variable. This does not have to be so. Each control coefficient in D^x can refer to any variable, in fact the diagonal elements can be any number at all except zero. D does not even have to be diagonal, but we do not examine non-diagonal cases because they do not at present appear to lead to useful results. We shall only consider a mixture of control coefficients, and leave it to others to be more creative. The possibility of mixing control coefficients has important implications for experimental control analysis. Consider an example of mixing variables in Eqn (9) by forming the diagonal of D^x from the vector $[C_1^{s_1}, C_2^{s_2}, C_3^J]$:

$$\begin{bmatrix} C_1^{s_1} & C_2^{s_2} & C_3^J \\ {}^{E_1}R_{s1}^{s_1} & {}^{E_2}R_{s1}^{s_2} & 0 \\ {}^{E_1}R_{s2}^{s_1} & {}^{E_2}R_{s2}^{s_2} & {}^{E_3}R_{s2}^{J} \end{bmatrix} \begin{bmatrix} {}^{E_1}O_{s1}^J & -1 & -{}^{E_1}O_{s1}^{s_2} \\ {}^{E_2}O_{s2}^J & -{}^{E_2}O_{s2}^{s_1} & -1 \\ 1 & -{}^{E_3}O_J^{s_1} & -{}^{E_3}O_J^{s_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}. \quad (24)$$

Provided each of the three enzymes can be perturbed and the fluxes and concentrations measured, all of the entries in O_x can be found without knowing any of the kinetic details, and thus all of the entries in R^x can be calculated because $R^x = (O_x)^{-1}$. The best choice of D^x is dictated by the characteristics of the steady state, as discussed below. The control coefficient at the top of each column in R^x occurs in the regulatory strengths underneath it; therefore all the elasticity coefficients can be calculated and the control problem is solved, as the elasticity coefficients can be used to calculate other control coefficients with Eqn (1).

This procedure therefore allows the experimental determination of regulatory strengths, and effectively removes a major limitation of the experimental application of classical control analysis, namely, that to measure a control coefficient the value of the fractional change in the enzyme activity must

be known. Until now, if inhibitors were used to modulate enzyme activity, it was usually necessary to know the type of inhibition, compartmental inhibitor concentration, inhibition constants, etc. Using the $R^xO_x = I$ formalism, all that is required is to be able to modulate each enzyme activity in turn around its normal value and measure the steady-state flux and concentration changes; knowledge of actual enzyme activities is unnecessary. With inhibitor studies it is only necessary to know that the inhibitor acts on one particular enzyme. All co-response coefficients for a specific enzyme modulation can be obtained by plotting all combinations of fluxes and concentrations against one another in logarithmic space. How $R^xO_x = I$ is constructed to process these results is dictated by the co-response profile. For example, for a steady state in which the last step dominates the control of flux so that $C_3^J \approx 1$, plots of ln s_1 and ln s_2 against ln J should give usable slopes. This implies that C_3^J should be incorporated into D^x so that $-^{E_3}O_J^{s_1}$ and $-^{E_3}O_J^{s_2}$ appear in the last row of O_x. However, for modulations in the activities of E_1 and E_2, plots of either ln s_1 or ln s_2 against ln J would give statistically unreliable slopes, as both C_1^J and C_2^J are very small. This implies that D^x should contain either $C_1^{s_1}$ or $C_1^{s_2}$ as its first element and either $C_2^{s_1}$ or $C_2^{s_2}$ as its second element. The choice would be decided by plotting ln s_1 and ln s_2 against each other for the E_1 and E_2 modulations and observing where measurable slopes occur. If it so happens that $C_1^{s_1}$ and $C_2^{s_2}$ are the relevant control coefficients, the expression in Eqn (24) is used.

DISCUSSION

Eqn (1) and Eqn (4) are valid for systems of any complexity. However, the occurrence of branchpoints or moiety-conserved cycles necessitates modification of the elasticity and control matrices. The existence of branchpoints, with unitary or non-unitary stoichiometry, or of substrate loops, introduces relationships between fluxes and control coefficients into ε, and columns of control coefficients with respect to the distribution of fluxes at branchpoints into C [4, 10–13]. The existence of moiety-conserved cycles introduces connectivity relationships that refer to pairs of cycle metabolites [10, 11, 20] or to ratios of cycle metabolites [17]. We leave it to the reader to apply the analysis to specific systems. In the Appendix we show how the transformation in Eqns (1–4) can be applied to Reder's general matrix expression for control analysis [3].

We have intentionally defined the control coefficients in control matrix C in a general way that does not depend on enzyme concentration. If control coefficients are defined as $C_{e_i}^x = \partial \ln x / \partial \ln e_i$, the diagonal elements of the identity matrix in Eqn (1) and Eqn (4) must be replaced by elasticity coefficients that describe the sensitivity of each rate towards its own enzyme concentration; only when such elasticity coefficients equal one, i. e., rate is proportional to enzyme concentration, does the identity matrix reappear. If enzyme-enzyme interactions occur, the off-diagonal elements in I must be replaced by elasticity coefficients that describe the sensitivity of a rate towards another enzyme concentration [5, 6, 14, 15]. This does not affect the transformation described in Eqn (3), but it does mean that the elasticity and regulatory strength matrices are no longer simple inverses of their respective control and co-response matrices, and that these additional enzyme-enzyme elasticity coefficients must be known for the matrix equation to be numerically solvable.

We have shown that the simple transformation described in Eqns (1–4) has important implications both for the quantitative understanding of metabolic regulation and for experimental control analysis. First, it forms the basis for a theoretical framework called metabolic regulation analysis, which uses regulatory strengths, regulatory potentials and co-response coefficients to quantify and relate the regulatory and regulated properties of metabolic systems. Second, it is also now clear that valuable experimentally-obtained information has thus far not been used to full effect in control analysis. We have shown how this information can be used to obtain co-response coefficients and how this simplifies experimental control analysis. In essence, the proposed experimental analysis is a generalisation of the double-modulation method described by Kacser and Burns [24] and approaches their ideal of separating control analysis from the detailed kinetic and mechanistic properties of the enzymes and transport systems that constitute metabolic systems. We are, of course, aware of the many potential pitfalls in the experimental application of such an analysis, such as domination of results by measurement errors (cf. [25]). Nevertheless, we believe that the matrix transformation described here may have many uses.

APPENDIX

The R^xO_x transformation in Reder's notation

Reder [3] has given a mathematically rigorous derivation of the relationships of control analysis. She uses unscaled control coefficients and elasticities, i. e., she defines them as simple derivatives, not logarithmic ones, and in her notation Eqn (1) can be written as follows:

$$\begin{bmatrix} C' \\ \Gamma \end{bmatrix} [D_\chi vL\ K] = \begin{bmatrix} 0 & K \\ -L & 0 \end{bmatrix} \tag{1}$$

where
C' = matrix of unscaled flux-control coefficients
Γ = matrix of unscaled concentration-control coefficients
$D_\chi vL$ = matrix of unscaled elasticity coefficients
K = null-space of the stoichiometric matrix (flux relationships)
L = link matrix (conservation relationships)

Define a diagonal matrix Δ of unscaled control coefficients. Use Δ to transform Eqn (1) as follows:

$$\begin{bmatrix} C' \\ \Gamma \end{bmatrix} [\Delta^{-1}] [\Delta] [D_\chi vL\ K] = \begin{bmatrix} 0 & K \\ -L & 0 \end{bmatrix} \tag{2}$$

which becomes

$$\begin{bmatrix} C'\Delta^{-1} \\ \Gamma\Delta^{-1} \end{bmatrix} [\Delta D_\chi vL\ \Delta K] = \begin{bmatrix} 0 & K \\ -L & 0 \end{bmatrix}. \tag{3}$$

The lefthand matrix is now the unscaled co-response matrix. Its multiplier contains the unscaled regulatory strength matrix $\Delta D_\chi vL$ and a matrix of unscaled control coefficients ΔK.

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