Elasticity analysis and design for large metabolic responses produced by changes in enzyme activities

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Metabolic control analysis has been extensively used to describe how the sensitivity properties of the component enzymes in a metabolic pathway (represented by the elasticity coefficients) determine the way in which metabolic variables respond (described by the control coefficients). Similarly, metabolic control design addresses the inverse problem of obtaining the sensitivity properties of the component enzymes that are required for the system to show a pre-established pattern of responses. These formalisms, including what is called elasticity analysis and design, were developed for small, strictly speaking infinitesimal, changes. Here we extend them to large metabolic responses. The new approach can be applied to simple two-step pathways or to any arbitrary metabolic system divided into two groups linked by one intermediate. General expressions that relate control and elasticity coefficients for large changes are derived. Concentration

and flux connectivity relationships are obtained. The relationships for large changes indicate that the pattern of responses is not necessarily the same as the one obtained with the traditional infinitesimal approach, in some cases the patterns being qualitatively different. The general analysis is used to study the control of ketogenesis in rat liver mitochondria, starting from data available in the literature. The control profile of the pathway subject to large changes shows both quantitative and qualitative differences from the one obtained from an analysis that is performed with infinitesimal coefficients. This exemplifies the type of errors that may be introduced when drawing conclusions about large metabolic responses from results obtained with an infinitesimal treatment.

Key words: metabolic control analysis, metabolic control design.

INTRODUCTION

During recent decades a substantial amount of work has been done to describe, quantitatively, the importance that the concentration of enzymes and their regulatory properties have in the way that metabolic pathways respond. Metabolic control analysis (MCA) is one of the tools more extensively developed and used to achieve this aim [1–6]. One of MCA's central results is that the steady-state systemic responses of metabolite concentrations and fluxes to small changes in enzyme activities can be calculated from the responses of reaction rates of component enzymes measured in isolation and the stoichiometric coefficients of the biochemical transformations taking place.

In the framework of MCA, the systemic responses are described by control coefficients. These are defined as the fractional change of the variable (metabolite concentration or flux) divided by the fractional change in the enzyme activity that produced the systemic change. On the other hand, the responses of the individual reaction steps are quantified by the elasticity coefficients, which measure the relative change of the rates per relative change of the internal metabolite concentrations. The control coefficients are constrained by structural relationships (called summation and conservation theorems) and kinetic relationships (called connectivity theorems). From the constraints, the expressions that give the control coefficients in terms of the elasticity and stoichiometric coefficients may be obtained. These show how, at the metabolic level, the sensitivity properties of the network are determined by the sensitivity properties of its components and their interactions [7–11].

The 'inverse' problem to analysing (or predicting) the responses of an existing metabolic system that has components

of known properties, addressed by MCA, is designing (or calculating) the properties of the components that are required to construct a new metabolic network that would present certain desired systemic responses. This problem has been solved, for the case of infinitesimal changes, by the formalism called metabolic control design (MCD) [12–19].

One important limitation of MCA and MCD is that the sensitivity properties of the system and its components correspond to small, strictly speaking infinitesimal, changes in parameters, e.g. enzyme activities. However, many regulatory processes *in vivo* and experiments that involve metabolic perturbations, as well as biotechnological processes of interest, present or require large changes [20–24]. When the changes in enzyme activity are not infinitesimal the definitions and theorems of MCA and MCD do not necessarily hold and, in some situations, the deviations may be important even for rather small changes in parameters [24]. Alternative approaches have been considered to overcome this limitation. Höfer and Heinrich substitute the first-order approximation of MCA with the second-order expansion of the Taylor series [25]. Although this treatment constitutes a quantitative improvement, it still retains the weaknesses of infinitesimal approaches. Small and Kacser [26,27] make the simplifying assumption that the kinetics of all the isolated steps are proportional to the enzyme concentration and vary in a linear way with the metabolite concentrations, resulting in a hyperbolic dependence between flux and enzyme activity. They show that this assumption is in agreement with data from three experimental *in vivo* systems available in the literature. It is, however, clear that the linear kinetic assumption is by no means general. For instance, de Atauri et al. [28] have shown that the control coefficient of hepatic glycogen synthesis flux with respect

Abbreviations used: MCA, metabolic control analysis; MCD, metabolic control design; TDCA, top-down control analysis.

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to glucokinase increases when glucokinase is overexpressed in the presence of high concentrations of its regulatory protein. The behaviour of several glycolytic intermediates in tuber tissue of lines of transgenic potato overexpressing phosphofructokinase also constitutes an example of the breakdown of Small and Kacser's assumption [29]. In a biotechnological context, another strategy to overcome the limitations of infinitesimal changes, called the universal method, has been proposed [21,22]. It was devised to engineer a metabolic system to increase the flux to a product of interest in an, in principle, arbitrary amount. This procedure shows that a simultaneous change in a limited number of specified enzyme concentrations by adequate factors is sufficient to obtain a large arbitrary increase in the steady-state flux of interest, without altering the pools of the metabolic intermediates. The 'exact' application of this method has the advantage that it does not rely on a detailed knowledge of the kinetics of the individual steps. However, it requires that the rates of the isolated steps are proportional to the corresponding enzyme concentrations. Recently, one of us has gone one step further, starting a new approach to the design and analysis of large metabolic changes that makes no assumption about the kinetics of the component enzymes [24]. In this formalism, a control coefficient for large changes, called the mean-sensitivity coefficient, was defined. It was shown that these coefficients are conditioned by structural and kinetic constraints, resulting in the values that some of them can take being limited by the values of the others. Summation and conservation relationships, for non-infinitesimal metabolic changes, were also derived.

In the present paper, the theory of large metabolic responses for mean-sensitivity coefficients is extended to include what in the theory of infinitesimal changes is called elasticity analysis and design. In the next section a background is given. In the following sections elasticity coefficients for large changes (mean-elasticity coefficients) are defined and the expressions that relate them with the mean-sensitivity coefficients are obtained. Connectivity theorems for large metabolic changes are deduced and summation theorems 'rediscovered'. Then the elasticity analysis for large metabolic responses is applied to study the control of ketogenesis in isolated rat-liver mitochondria. Finally we discuss some of the implications of the formalism here proposed.

BACKGROUND ON SENSITIVITY ANALYSIS AND DESIGN OF METABOLIC SYSTEMS

Theory of sensitivity coefficients for small changes

In MCA and MCD, the sensitivity of the effect that the change in activity of one enzyme has on a steady-state metabolic variable is quantified by the control coefficient. The control coefficient (C_e^w) is defined as the fractional change of the variable (w) divided by the fractional change of the enzyme activity (e) that produced the systemic alteration, when infinitesimal changes around the operational point are considered [30], i.e.

$$C_e^w = \frac{e}{w} \frac{\partial w}{\partial e} \quad (1)$$

On the other hand, the sensitivity properties of the individual components of a metabolic system are quantified by the elasticity coefficients. The elasticity coefficient (ε_S^v) is the partial derivative of a rate, v , with respect to a metabolite concentration, S , multiplied by the scaling factor S/v :

$$\varepsilon_S^v = \frac{S}{v} \frac{\partial v}{\partial S} \quad (2)$$

Scheme 1 (a) Two-enzyme pathway and (b) metabolic system divided into two groups

Terms are defined in the text.

An important result of MCA and MCD is that the sensitivity properties of the system (flux and concentration control coefficients) can be calculated from the sensitivity properties of the components (elasticity coefficients) and *vice versa* if the stoichiometric coefficients of the transformations are known.

Let us first consider a two-step metabolic pathway consisting of two enzyme-catalysed reactions linked by one metabolite (S), as illustrated in Scheme 1(a). For the sake of simplicity, we will consider that the rates of the isolated steps are proportional to the corresponding enzyme concentrations (e_1 and e_2); the more general case of enzyme–enzyme interactions has been dealt with in the literature [7–9]. This system has two steady-state variables (the intermediate metabolite concentration S and the flux J), two elasticity coefficients (ε_S^{v1} and ε_S^{v2}), two concentration control coefficients (C_{e1}^S and C_{e2}^S) and two flux control coefficients (C_{e1}^J and C_{e2}^J). The control coefficients can be calculated from the elasticity coefficients by the equations:

$$C_{e1}^S = \frac{1}{-\varepsilon_S^{v1} + \varepsilon_S^{v2}} \quad (3a)$$

$$C_{e2}^S = \frac{-1}{-\varepsilon_S^{v1} + \varepsilon_S^{v2}} \quad (3b)$$

$$C_{e1}^J = \frac{\varepsilon_S^{v2}}{-\varepsilon_S^{v1} + \varepsilon_S^{v2}} \quad (3c)$$

$$C_{e2}^J = \frac{-\varepsilon_S^{v1}}{-\varepsilon_S^{v1} + \varepsilon_S^{v2}} \quad (3d)$$

These expressions show how the sensitivity properties of the system depend on the sensitivity properties of the components, for Scheme 1(a) operating at steady state. Using eqns (3) the well-known summation and connectivity theorems can be obtained [1,2,31,32].

The treatment and results described for a two-step pathway can be extended to metabolic systems with an arbitrary number of steps. This is achieved by lumping the reaction rates into two groups that are connected through a single metabolite, S , as is represented in Scheme 1(b). Here, v_{G1} and v_{G2} represent the reaction rates of groups 1 and 2, respectively. We assume that the rates of the component steps in each block are proportional to the corresponding enzyme concentrations. For this system we can define group control coefficients and group elasticity coefficients [33–35]. The flux and concentration group control coefficients, C_{eG1}^J (or C_{eG2}^J) and C_{eG1}^S (or C_{eG2}^S), may be operationally determined by changing all the enzyme concentrations of block 1 (or 2) by the same infinitesimal factor. Owing to the additivity of infinitesimal changes, the group control

coefficient is equal to the sum of the individual control coefficients of the block. The group elasticity coefficients $\varepsilon_S^{v_{G1}}$ and $\varepsilon_S^{v_{G2}}$ describe the sensitivity of the rates of the blocks to changes in the linking metabolite S . Since each group rate represents a portion of the metabolic system (which is composed of several enzymes) the group elasticity coefficients are, in fact, the flux response coefficients to changes in S [30]. The group elasticity coefficients, determined for infinitesimal changes, may therefore be calculated from the elasticity coefficients of the component steps (see, e.g., [36]). Group elasticity and group control coefficients fulfil relationships that are formally identical with eqns (3).

Theory of sensitivity coefficients for large changes

Living organisms regulate the activity of many of their metabolic processes by increasing or decreasing the activity of enzymes to induce large changes in flux [20,23]. The genetic manipulation of organisms to achieve a greater production of a metabolite of interest also requires an increase in the activity of the enzymes of the pathway (or a decrease in the activities of branches that compete with it) by large amounts [21,22].

If a metabolic system undergoes a large change in one of its parameters, e.g. an enzyme concentration (e), the sensitivity of the response of the variable (w) can be quantified by the mean-sensitivity coefficient [24,26,37]:

$$\overline{C}_e^w(x \Rightarrow y) = \frac{(w_y - w_x)/w_x}{(e_y - e_x)/e_x} \quad (4)$$

The mean-sensitivity coefficient represents the relative change of a system variable divided by the relative change in a parameter when the system goes from an initial to a final steady state. The subscripts x and y stand for the initial and final value, respectively, of the parameter e and the steady-state variable w . The definition of control coefficient given in eqn (1) is equal to the definition of the mean-sensitivity coefficient given in eqn (4) in the particular case when e_y tends to e_x . An important difference between the mean-sensitivity coefficient and the control coefficient is that the value of the former depends on the extent of the parameter change.

Similar to what was shown for infinitesimal changes, large metabolic responses are conditioned by structural and kinetic constraints. Concentration and flux mean-sensitivity coefficients are both constrained by summation relationships. For Scheme 1(a) the summation constraints are [24]:

$$\overline{C}_{e_1}^S(e_1 \Rightarrow r \cdot e_1) + \overline{C}_{e_2}^S(e_2 \Rightarrow e_2/r) = 1 - r_s \quad (5a)$$

$$\overline{C}_{e_1}^J(e_1 \Rightarrow r \cdot e_1) + \overline{C}_{e_2}^J(e_2 \Rightarrow e_2/r) = 1 \quad (5b)$$

In these equations the mean-sensitivity coefficients with respect to e_1 represent changes from an initial state x to a final state y where e_1 has been multiplied by a factor r , e_2 being kept constant. The mean-sensitivity coefficients with respect to e_2 represent changes from the same initial state x to a different final state z , where e_2 has been multiplied by $1/r$, e_1 remaining unchanged. $r_s = S_y/S_x$ is the ratio of the concentrations of intermediates corresponding to states y and x . In the flux summation relationship for large changes the 'total control' is constant and equal to 1 (as for infinitesimal changes, $C_{e_1}^J + C_{e_2}^J = 1$). This has the practical consequence that if a parameter of the system is changed in such a way that the control exerted by the up-modulation of one step decreases, then this decrease in control is transformed in an increase in the control exerted by the down-modulation of the other step. In brief, there is a transfer of control from up-modulation of one step to down-modulation of the other. Also, in the concentration summation relationship

one mean-sensitivity coefficient represents an up-modulation and the other a down-modulation, but the difference from flux summation is that for large changes the 'total control' is not constant. This has the consequence that both concentration mean-sensitivity coefficients do not have to simultaneously increase or decrease (in absolute terms) as is required by the summation relationship for infinitesimal changes ($C_{e_1}^S + C_{e_2}^S = 0$). In this respect, the sensitivity properties for large changes may be qualitatively different from those for infinitesimal changes. Eqns (5) reduce to the summation relationships for control coefficients when states y and z tend to state x , i.e. when r and r_s tend to 1. Next we shall comment on how these constraints are deduced. As we shall see, in the following sections the same type of procedure is used to develop an elasticity analysis and design for large changes.

Eqns (5) involve two parameters, the enzyme concentrations e_1 and e_2 , and three steady states, x , y and z . The values of the enzyme concentrations in each state are:

$$e_{1x} = e_1, \quad e_{2x} = e_2 \quad (6a)$$

$$e_{1y} = r \cdot e_1, \quad e_{2y} = e_2 \quad (6b)$$

$$e_{1z} = e_1, \quad e_{2z} = e_2/r \quad (6c)$$

where r is a real number. In words, to go from state x to state y , e_1 is changed by a factor r and e_2 is not changed, whereas, to go from x to z , e_1 is not changed and e_2 is changed by a factor of $1/r$. It is assumed that the rates of steps 1 and 2 are governed by equations of the form: $v_i = e_i \cdot f_i(S)$, where $i = 1$ or 2 , which means that the rates (v_i) are proportional to the corresponding enzyme concentration (e_i) and depend on the metabolite concentration (S) through an arbitrary function (f_i). Under this hypothesis the following relationships hold:

$$S_y = S_z \quad \text{or} \quad S(r \cdot e_1, e_2) = S(e_1, e_2/r) \quad (7a)$$

$$J_y = r \cdot J_z \quad \text{or} \quad J(r \cdot e_1, e_2) = r \cdot J(e_1, e_2/r) \quad (7b)$$

Eqn (7a) shows that changing the concentration of one of the enzymes by a factor of r has the same effect on the concentration of the metabolic intermediate as changing the concentration of the other enzyme by its reciprocal, $1/r$. The same changes in the enzyme concentrations render fluxes that differ by the factor r (eqn 7b).

Taking into account that $\overline{C}_{e_1}^S(e_1 \Rightarrow r \cdot e_1)$ and $\overline{C}_{e_1}^J(e_1 \Rightarrow r \cdot e_1)$ describe the change from x to y and that $\overline{C}_{e_2}^S(e_2 \Rightarrow e_2/r)$ and $\overline{C}_{e_2}^J(e_2 \Rightarrow e_2/r)$ describe the change from x to z , and using eqns (7) the summation relationships for large metabolic changes given in eqns (5) are easily derived.

The results obtained for large metabolic changes in a two-step pathway can be extended to metabolic systems with an arbitrary number of steps. This is done in a similar way to that described above, by lumping the reaction rates into two groups that are connected through one metabolite, S (Scheme 1b). v_{G1} and v_{G2} are the reaction rates of groups 1 and 2, respectively. We assume that the rates of all the individual steps belonging to each block are proportional to the corresponding enzyme concentrations. Mean-sensitivity coefficients for groups of enzymes have been defined previously (appendix A in [24]). These coefficients may be operationally determined by changing all the enzyme concentrations of the corresponding block by the same arbitrary factor. They satisfy the summation relationships given in eqns (5), if the changes of the enzyme concentrations in the blocks are carried out as indicated by eqns (6). Contrary to what happens in the case of infinitesimal changes, for large changes in enzyme concentrations responses are not additive and, therefore, the group mean-sensitivity coefficients cannot be expressed generally

as the sum of the mean-sensitivity coefficients corresponding to the individual enzymes in the block.

Large metabolic responses are conditioned by the kinetic constraints implicit in the particular form of the rate equations involved. In the design procedure developed in a previous contribution [24], it was shown how the kinetic parameters of the rate equations are calculated from the pre-established values of the mean-sensitivity coefficients. The requirement that the values of these parameters have physical meaning (e.g. they are positive) restricts the range of values that the mean-sensitivity coefficients can take. As a consequence, the kinetic constraints impose additional restrictions on the values of the mean-sensitivity coefficients to those in eqns (5).

Next we shall show that the kinetic constraints can, alternatively, be described in terms of elasticity coefficients for large changes, which we shall call ‘mean-elasticity coefficients’. This new approach will allow us to develop a formalism equivalent to what in the theory of infinitesimal changes is called elasticity analysis and design.

DEFINITION OF ELASTICITY COEFFICIENTS FOR LARGE CHANGES

Elasticity coefficients were defined above to quantify the sensitivity of an isolated enzyme reaction rate to an infinitesimal change in one of the metabolite concentrations (eqn 2). Here we will extend this definition to deal with large changes in metabolite concentrations. We define the mean-elasticity coefficient as the relative change of the isolated rate divided by the non-infinitesimal relative change in the metabolite concentration that produced the rate change:

$$\overline{\varepsilon}_S^v(x \Rightarrow y) = \left(\frac{v_y - v_x}{v_x} \right) / \left(\frac{S_y - S_x}{S_x} \right) \quad (8)$$

The subscripts x and y indicate the initial and final states, respectively, of the rate, v , and the metabolite concentration, S . Note that when S is changed all the other metabolite concentrations are kept at a constant value. When y tends to x eqn (8) returns to eqn (2). To exemplify the calculation of a mean-elasticity coefficient we shall consider an irreversible Michaelis-Menten rate equation, i.e. $v = V_S \cdot S / (K_S + S)$. The rate at the initial (or final) steady state x (or y) is $v_x = v(S_x)$ [or $v_y = v(S_y)$], where S_x (or S_y) is the substrate concentration at the initial (or final) state. Using the definition of the mean-elasticity coefficient given in eqn (8) the following expression is obtained: $\overline{\varepsilon}_S^v(x \Rightarrow y) = K_S / (K_S + S_y)$. It can be immediately seen that, as y tends to x , $\overline{\varepsilon}_S^v(x \Rightarrow y)$ tends to ε_S^v (evaluated at the operation point x), which is given by: $\varepsilon_S^v = K_S / (K_S + S_x)$.

RELATIONSHIPS BETWEEN MEAN-SENSITIVITY AND MEAN-ELASTICITY COEFFICIENTS

In this section, we shall obtain the relationship between the sensitivity properties of the system variables and the sensitivity properties of the component rates, when large metabolic responses are considered. In the terminology of mean coefficients, we shall find the functional relationship between mean-sensitivity and mean-elasticity coefficients.

First, we shall illustrate the procedure using the simple two-step pathway represented in Scheme 1(a). As discussed above, we assume that the rates of steps 1 and 2 are governed by rate laws of the form: $v_i = e_i \cdot f_i(S)$, where $i = 1$ or 2, and the enzyme concentrations e_i are parameters. We also consider three steady states, x , y and z , whose enzyme concentration values are related

according to eqns (6). Using eqns (7) and the definitions given in eqns (4) and (8) it may be shown that:

$$\overline{C}_{e1}^J(x \Rightarrow y) = 1 + r \cdot \overline{\varepsilon}_S^{v1}(x \Rightarrow y) \overline{C}_{e1}^S(x \Rightarrow y) \quad (9a)$$

$$\overline{C}_{e1}^J(x \Rightarrow y) = \overline{\varepsilon}_S^{v2}(x \Rightarrow y) \overline{C}_{e1}^S(x \Rightarrow y) \quad (9b)$$

$$\overline{C}_{e2}^J(x \Rightarrow z) = 1 + \frac{1}{r} \overline{\varepsilon}_S^{v2}(x \Rightarrow y) \overline{C}_{e2}^S(x \Rightarrow z) \quad (9c)$$

$$\overline{C}_{e2}^J(x \Rightarrow z) = \overline{\varepsilon}_S^{v1}(x \Rightarrow y) \overline{C}_{e2}^S(x \Rightarrow z) \quad (9d)$$

Note that $\overline{\varepsilon}_S^{v1}(x \Rightarrow y) = \overline{\varepsilon}_S^{v1}(x \Rightarrow z)$ and $\overline{\varepsilon}_S^{v2}(x \Rightarrow y) = \overline{\varepsilon}_S^{v2}(x \Rightarrow z)$ because mean-elasticity coefficients describe the effect on the rate of changes on the concentration of the intermediate only and at states y and z these concentrations are identical. Eqns (9) can be solved to obtain the mean-sensitivity coefficients as a function of the mean-elasticity coefficients, which we shall call ‘elasticity analysis for large metabolic responses’. The resulting expressions are:

$$\overline{C}_{e1}^S(x \Rightarrow y) = \frac{1}{-r \cdot \overline{\varepsilon}_S^{v1}(x \Rightarrow y) + \overline{\varepsilon}_S^{v2}(x \Rightarrow y)} \quad (10a)$$

$$\overline{C}_{e2}^S(x \Rightarrow z) = \frac{-r}{-r \cdot \overline{\varepsilon}_S^{v1}(x \Rightarrow y) + \overline{\varepsilon}_S^{v2}(x \Rightarrow y)} \quad (10b)$$

$$\overline{C}_{e1}^J(x \Rightarrow y) = \frac{\overline{\varepsilon}_S^{v2}(x \Rightarrow y)}{-r \cdot \overline{\varepsilon}_S^{v1}(x \Rightarrow y) + \overline{\varepsilon}_S^{v2}(x \Rightarrow y)} \quad (10c)$$

$$\overline{C}_{e2}^J(x \Rightarrow z) = \frac{-r \cdot \overline{\varepsilon}_S^{v1}(x \Rightarrow y)}{-r \cdot \overline{\varepsilon}_S^{v1}(x \Rightarrow y) + \overline{\varepsilon}_S^{v2}(x \Rightarrow y)} \quad (10d)$$

These equations show that the mean-sensitivity coefficients depend, apart from on the mean-elasticity coefficients, on the extent of the change given by factor r (compare eqns 10 with eqns 3). Operating on eqns (10) the concentration and flux summation relationships for large metabolic responses, given in eqns (5), may be obtained in an alternative way.

Eqns (10) can be used to obtain concentration and flux connectivity relationships for large metabolic responses, i.e.

$$r \overline{C}_{e1}^S(x \Rightarrow y) \overline{\varepsilon}_S^{v1}(x \Rightarrow y) + \frac{1}{r} \overline{C}_{e2}^S(x \Rightarrow z) \overline{\varepsilon}_S^{v2}(x \Rightarrow y) = -1 \quad (11a)$$

$$r \overline{C}_{e1}^J(x \Rightarrow y) \overline{\varepsilon}_S^{v1}(x \Rightarrow y) + \overline{C}_{e2}^J(x \Rightarrow z) \overline{\varepsilon}_S^{v2}(x \Rightarrow y) = 0 \quad (11b)$$

Eqns (9)–(11) reduce to the well-known relationships for infinitesimal changes when $r \rightarrow 1$ (i.e. y and $z \rightarrow x$).

In the context of large responses, we can also solve the inverse problem to ‘elasticity analysis’, namely ‘elasticity design’. This consists of obtaining the mean-elasticity coefficients as a function of the mean-sensitivity coefficients. The design expressions for large metabolic changes deduced from eqns (9) are:

$$\overline{\varepsilon}_S^{v1}(x \Rightarrow y) = \frac{\overline{C}_{e2}^J(x \Rightarrow z)}{\overline{C}_{e2}^S(x \Rightarrow z)} \quad (12a)$$

$$\overline{\varepsilon}_S^{v2}(x \Rightarrow y) = \frac{\overline{C}_{e1}^J(x \Rightarrow y)}{\overline{C}_{e1}^S(x \Rightarrow y)} = r \frac{\overline{C}_{e2}^J(x \Rightarrow z) - 1}{\overline{C}_{e2}^S(x \Rightarrow z)} \quad (12b)$$

The elasticity analysis and design for large responses that we have developed for a two-step pathway may be extended readily to a metabolic system having an arbitrary number of steps. This is done by lumping the reaction rates of the system into two groups that are connected through a single metabolite, S (Scheme 1b). The group rates and group mean-sensitivity coefficients were defined above. In addition, the enzyme concentrations in the

blocks are co-ordinately changed, as indicated in eqns (6), i.e. all enzyme concentrations in group 1 are changed by a factor r to obtain steady state y from x , and all enzyme concentrations in group 2 are changed by a factor of $1/r$ to obtain steady z from x . The group mean-elasticity coefficients are conceptually similar to mean-elasticity coefficients (see above), the only difference being that they quantify the sensitivity of the group rates, and not of an individual rate, to changes in the linking metabolite S . Since group rates correspond to a portion of a metabolic system, group mean-elasticity coefficients represent the response of the flux in the group to large changes in S . Note that, due to the intrinsic non-linear nature of large metabolic changes, the group mean-elasticity coefficients may not be calculated from the mean-elasticity coefficients of the component steps of the blocks (which could be done in the infinitesimal approach). It may be shown that group mean-elasticity coefficients and group mean-sensitivity coefficients fulfil relationships that are formally equivalent to eqns (9)–(12).

The general formalism that we have presented may be used to study certain features of particular metabolic systems. For example, in the next section we shall apply the new theoretical approach to a study of the control of ketogenic flux in liver of normal fed adult rats from palmitoyl-CoA reported in the literature [38,39]. From the analysis we shall obtain two types of information: the systematic error that is introduced into the control coefficients (of the theory of infinitesimal changes) when these are determined by increasing the enzyme activities by large (non-infinitesimal) amounts and the changes on the profile of mean-sensitivity coefficients produced by large enzyme concentration changes.

EXPERIMENTAL CASE

In this section, we use data obtained by applying top-down control analysis (TDCA) to ketogenesis in isolated rat liver mitochondria [38,39] to illustrate the usefulness of the formalism developed above. This TDCA strategy aimed to determine the control coefficients (of the theory of infinitesimal changes) from measurements of the elasticity coefficients. It consisted of putting the reactions of β -oxidation in one group and those of ketogenesis in another group, the intermediate of the two processes being the ratio of the concentrations of acetyl-CoA and CoA. The overall process is described by Scheme 1(b), with v_{G1} and v_{G2} representing the rates of β -oxidation and ketogenesis respectively and S the acetyl-CoA/CoA ratio. The group elasticity coefficients, $\varepsilon_{S^{G1}}$ and $\varepsilon_{S^{G2}}$, were determined as follows. The concentration of acetyl-CoA was set to different levels by adding pyruvate to the system. The rates of the groups are measured as a function of the concentration of acetyl-CoA. The authors [38,39] find that, within the limits of experimental error, v_{G1} and v_{G2} change linearly with S , for values of S between 1.25 and 4.75. Therefore, in this range of S values, the group elasticity coefficients defined by eqn (2) (corresponding to one operation point x in the interval) may be determined, without systematic error, using the definition of mean-elasticity coefficient (eqn 8; as long as the final state y belongs to the range). The values of the elasticity coefficients obtained depend on the operation point x that is considered and are independent of the final state y that is chosen. At the state x corresponding to $S = 1.25$ (where pyruvate is absent and v_{G1} is not significantly different from v_{G2}) the values of the group elasticity coefficients are $\varepsilon_{S^{G1}} = -0.26$ and $\varepsilon_{S^{G2}} = 0.10$. Introducing these values into eqns (3) the following values of the group control coefficients are obtained: $C_{eG1}^J = 0.28$, $C_{eG2}^J = 0.72$, $C_{eG1}^S = 2.8$ and $C_{eG2}^S = -2.8$. It is important to note that since there was no systematic error introduced into the de-

termination of the elasticity coefficients because large (non-infinitesimal) changes in the ratio acetyl-CoA/CoA were used, the same applies to the values of the control coefficients obtained by this TDCA procedure.

The TDCA procedure that we have described is an indirect way to obtain control coefficients, because these are calculated from the elasticity coefficients, which are the ones experimentally determined. An alternative method consists of directly manipulating the enzyme concentrations. Next we shall estimate, using the elasticity analysis for large responses developed here, the systematic error that would have been introduced if the control coefficients were determined by manipulating the enzymes of the groups. For this purpose we shall use eqns (10). We shall consider the range of acetyl-CoA/CoA ratios between 1.25 and 4.75, where the elasticity coefficients take the constant values $\varepsilon_{S^{G1}} = -0.26$ and $\varepsilon_{S^{G2}} = 0.10$. If we introduce these elasticity coefficients into eqns (10) the mean sensitivity coefficients obtained represent approximate values for the control coefficients, with the goodness of the approximation depending on the factor r [remember that r (or $1/r$) is the factor by which the enzyme concentrations of group 1 (or 2) are changed].

$$\overline{C_{e1}^S}(x \Rightarrow y) = \frac{1}{r \cdot 0.26 + 0.10} \quad (13a)$$

$$\overline{C_{e2}^S}(x \Rightarrow z) = \frac{-r}{r \cdot 0.26 + 0.10} \quad (13b)$$

$$\overline{C_{e1}^J}(x \Rightarrow y) = \frac{0.10}{r \cdot 0.26 + 0.10} \quad (13c)$$

$$\overline{C_{e2}^J}(x \Rightarrow z) = \frac{r \cdot 0.26}{r \cdot 0.26 + 0.10} \quad (13d)$$

With these equations the percentage error in the control coefficients as a function of r can be calculated. For instance, it may be shown that if the final state y is obtained from the initial state x by a 20 % increase in the enzyme concentrations of group 1, the systematic error introduced into the corresponding flux control coefficient because of using non-infinitesimal changes is a 13 % decrease. Note that this systematic error adds to the random error involved. In addition, if the elasticity coefficients were not constant in the range of values of the acetyl-CoA/CoA ratio considered, the group control coefficients determined by the TDCA procedure would also involve a systematic error originating from the change of the values of the elasticity coefficients.

Eqns (13) may also be used to perform a sensitivity analysis of the metabolic system, when subject to large changes in the enzyme concentrations or activities. From this point of view, the values -0.26 and 0.10 that we have replaced on the right-hand side of the equations represent the mean-elasticity coefficients, which are constant in the range of S values considered. The mean-sensitivity coefficients only depend on the value of the factor r , the coefficients for infinitesimal changes being the values for $r = 1$. An increase in r , corresponding to an increase in the enzyme concentrations of group 1 (e_{G1}), results in a decrease in the control exerted by e_{G1} in the flux (Figure 1, top panel). Similarly, an increase in r , which corresponds to a decrease in the enzyme concentrations of group 2 (e_{G2}), results in an increase in the control exerted by e_{G2} in the flux (Figure 1, top panel). As r increases, the control of flux is transferred from up-modulation of the β -oxidation group to down-modulation of the ketogenesis group. This transfer is constrained by the summation relationship given in eqn (5b), according to which the 'total control' of the flux is constant and equal to 1. The control of the intermediate S

Figure 1 Dependence of the mean-sensitivity coefficients on the factor r

(Top panel) Flux mean-sensitivity coefficients and (bottom panel) concentration mean-sensitivity coefficients were generated using eqns (13) with $\varepsilon_{S^{G1}}^{x \Rightarrow y} = -0.26$ and $\varepsilon_{S^{G2}}^{x \Rightarrow y} = 0.10$. The summation relationships are also shown.

behaves with r in a similar way as the flux, i.e. an increase in r decreases the control by up-modulation of e_{G1} and increases (in absolute terms) the control by down-modulation of e_{G2} (Figure 1, bottom panel). This concentration sensitivity profile is different from the one found for infinitesimal changes, because the concentration summation relationship ($C_{e1}^S + C_{e2}^S = 0$) implies that the absolute value of both coefficients (which have opposite signs) either increases or decreases when a parameter of the system is changed. Therefore, the fact that for large responses the

Figure 2 Dependence of the mean-sensitivity coefficients on the factor α by which the enzymes of the groups are changed

Dependence of (top panel) the flux mean-sensitivity coefficients and (bottom panel) the concentration mean-sensitivity coefficients on α . The mean-sensitivity coefficients of groups 1 and 2 were calculated, using eqns (13), with $\alpha = r$ and $\alpha = 1/r$, respectively. Solid lines represent the mean-sensitivity coefficients profile in the experimental range and dashed lines give the values extrapolated outside this range, assuming that the mean-elasticity coefficients are constant (as given for Figure 1). The operational point ($\alpha = 1$) is indicated by ●.

‘total control’ of the intermediate concentration S is not constant (eqn 5a) allows a richer repertoire of sensitivity profiles.

In the experimental case that we have studied, the ratio acetyl-CoA/CoA was increased (but not decreased) starting from the operation value $S = 1.25$. This is why we could only calculate

the mean-sensitivity coefficients corresponding to up-modulations of the enzymes of group 1 and down-modulations of the enzymes of group 2 (Figure 1). To have a full picture of the control for large responses in the variables we would need to know the effect of up- and down-modulations of both groups. Since this experimental information is not available, we shall assume that the constancy of the mean-elasticity coefficients in the region experimentally determined is valid outside this region. In particular, we will assume that between $r = 0.1$ and $r = 6$, $\varepsilon_s^{vG1}(x \Rightarrow y) = -0.26$ and $\varepsilon_s^{vG2}(x \Rightarrow y) = 0.10$. In addition, for the sensitivity analysis to be transparent it is desirable to compare the effect of an increase (or decrease) in group 1 with the effect of an increase (or decrease) in group 2. For this reason, in eqns (13a) and (13c), corresponding to changes in group 1, we shall replace r with α , and in eqns (13b) and (13d), corresponding to changes in group 2, we will change r to $1/\alpha$; α is the factor by which e_{G1} and e_{G2} are increased (or decreased; these changes are of course not simultaneous). In Figure 2 we plot the flux and concentration mean-sensitivity coefficients as a function of α inside (Figure 2, solid curves) and outside (Figure 2, dashed curves) the region determined experimentally. We see that the acetyl-CoA consumption block always has a greater control on the flux than the production block. In contrast, which block shows the greatest concentration sensitivity depends on the value of α . For $\alpha > 1$, a change in group 1 produces a greater effect on S than group 2, whereas for $\alpha < 1$ group 2 has the greatest effect.

DISCUSSION

In the present article the definition of elasticity coefficient and the theory to perform an elasticity analysis and design for infinitesimal changes were extended to deal with arbitrarily large metabolic responses. The general relationships obtained (eqns 9–12) may be applied to simple two-step pathways or to any metabolic system whose steps are grouped into two blocks linked by a single metabolite (Scheme 1). In the latter case, the mean-elasticity and mean-sensitivity coefficients represent group coefficients equivalent to the ones used in infinitesimal TDCA [33,34]. The central relationships to perform an elasticity analysis of large metabolic responses are given in eqns (10). These allow us to predict, starting from the factor r by which the enzyme concentrations are changed and the sensitivity properties of the component rates represented by the mean-elasticity coefficients, how the systemic variables respond. On the other hand, the solution to the problem of elasticity design of large metabolic responses is given in eqns (12). In these expressions, the sensitivity properties of the component rates that are required to obtain a pre-established profile of responses in the variables are calculated from the mean-sensitivity coefficients describing this profile and the factor r .

The infinitesimal point of view of ketogenesis showed that the control of the flux is shared among the blocks of production and consumption of acetyl-CoA [38,39]. Roughly speaking, one-quarter of the control is in the production and three-quarters are in the consumption block. When changes in the enzyme concentrations by large factors are considered, the control changes dramatically with the factor α , by which the enzyme concentrations are multiplied (Figure 2, top panel). In our case, an increase in α results in a decrease in the control of the flux. This type of behaviour, i.e. the decrease of the flux control exerted by a block of enzymes when their concentration is increased, is the one usually found. However, it is important to keep in mind that there are some situations where the increase in the activity of one block results in an increase in the control that it exerts, what is called paradoxical control behaviour [28,40]. On the other hand,

the control of the acetyl-CoA/CoA ratio for infinitesimal changes is equally shared by the consumption and production blocks. This is an inevitable consequence of the summation relationship that states that the control of the blocks must be equal in absolute terms. In contrast, large changes usually result in an unevenly shared control of the intermediate (eqn 5a), as in the experimental case under consideration. As we shall see next, this asymmetry in the intermediate concentration control, appearing for large changes, is an additional element to be considered when it is required that fluxes and concentrations of a cell simultaneously respond according to a particular control profile.

We shall analyse two possible physiological situations and best way for the cell to solve them. Let us first consider that the hepatocyte has to increase the ketogenic flux, keeping the ratio acetyl-CoA/CoA as constant as possible. We saw in the previous section that for independent increases of the enzyme concentrations of the blocks by the same factor, $\alpha > 1$, the enzymes in the ketogenesis block (block 2) produce a greater increase in flux and a smaller change in the ratio acetyl-CoA/CoA than the enzymes in the β -oxidation block (block 1; Figure 2). Consequently, in this situation the solution of increasing the enzymes in the ketogenic block is the best.

Another situation would be that the cell has to decrease the ketogenic flux, also while keeping the acetyl-CoA/CoA ratio as constant as possible. For independent decreases of the enzyme concentrations of the blocks by the same factor ($\alpha < 1$), the enzymes in the ketogenesis block (block 2) produce a greater decrease in flux than the enzymes in the β -oxidation block (block 1; Figure 2a) but also produce the greater change in acetyl-CoA/CoA. The question still remains of what block to change to obtain the best solution to the situation. To give an answer, we have considered the case that block 1 is multiplied by a factor of α_1 and block 2 by α_2 such that $\overline{C_{eG1}^J}$ and $\overline{C_{eG2}^J}$ take the same value. Under these conditions we find that, for the range of α values explored, $\overline{C_{eG1}^S} > |\overline{C_{eG2}^S}|$. In conclusion, for the same decrease in the flux to ketogenesis, the decrease of the enzymes in the ketogenic block produces a smaller change in the intermediate acetyl-CoA/CoA ratio than the decrease in the enzymes of the β -oxidation block. The best solution to this situation would therefore be to decrease the activity of the enzymes in the ketogenic block. In summary, the two possible physiological situations analysed would be better solved by the hepatocyte changing the ketogenic block than the β -oxidation block. In the terminology of Hofmeyr and Cornish-Bowden, the regulatory enzyme(s) controlling the ketogenic pathway should be in the demand block [36].

In the particular experimental case analysed the group mean-elasticity coefficients are constant, but, the formalism here proposed remains valid for systems where the values of these coefficients depend on the extent of the change. The limitations in the application of elasticity analysis for large metabolic responses to intact systems are similar to those of the infinitesimal approach [34,41]. Importantly, the groups defined should not present significant interactions through intermediates that are internal to the groups. This may be confirmed using several combinations of modulations that change both explicit and implicit intermediates in independent ways [42]. Determination of the elasticity coefficients with different sets of modulations must give similar values. The only restriction of the analysis for large changes not present in the infinitesimal treatment is that the system can only be divided in two groups at a time. This does not allow simultaneous division in several modules, such as was done, for example, for hepatocyte metabolism by Ainscow and Brand [43]. However, in this system some divisions in two groups could be tested separately to assess the control distribution for

large changes of particular metabolic processes taking place. For instance, one could divide cellular metabolism in two groups, one being glycogen degradation, lactate production or ATP consumption and the other the rest of the network. This restriction, characteristic of elasticity analysis for large responses, is a direct consequence of the non-linear nature of large changes, which is precisely what we aim to describe.

The general elasticity analysis and design presented here show that the control profile for large metabolic responses is not necessarily equal to the one for infinitesimal changes. This draws attention on the risk implied in extrapolating conclusions from an infinitesimal analysis to large metabolic responses.

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