J. theor. Biol. (1996) **182,** 277–283

How Constrained is Metabolic Control?*

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The response of metabolic variables to small rate changes is quantitatively described by the control coefficients (Kacser & Burns, 1973; Heinrich & Rapoport, 1974). Owing to the existence of structural and kinetic constraints in metabolism, the control coefficients are not all independent. Two quantities are defined to evaluate how constrained metabolic control is: the fraction of independent control coefficients (f_w) and the number of independent control coefficients per independent variable [$N(C/V)$]. It is shown that f_w can be expressed in terms of the fraction of independent metabolite concentrations, the fraction of independent fluxes and the average fraction of independent metabolites affecting each rate. $N(C/V)$ is equal to the average number of metabolite concentrations affecting each rate. The estimation of these quantities using experimental information available leads to the following conclusions concerning cellular metabolism: (i) only a small fraction of the control coefficients are independent; (ii) the number of rates (in average) that independently controls each independent variable is much smaller than its theoretical maximum; and (iii) the kinetic constraints are the main cause of the low value showed by f_w and $N(C/V)$. Finally, some arguments are given that could explain why living organisms do not evolve to less constrained metabolic responses.

1. Introduction

Living organisms maintain a constant composition in a fluctuating environment. To achieve this they respond to external changes in appropriate ways. For instance, a successful organism switches from the consumption of one nutrient to another when availability changes. Many of these adaptive responses rely on the properties of metabolic changes. Understanding quantitative aspects of metabolism is, therefore, an important target. Ideally, a general theory of metabolism would have to be able to deal successfully with any type of changes. Up to now, a theory of large metabolic changes has only been developed in particular cases such as when simultaneous changes in enzymes (Acerenza & Kacser, 1990; Kacser & Acerenza, 1993) or linear rate equations (Small & Kacser, 1993a, b) are considered. In contrast, a theory of small metabolic responses has been extensively developed during the last two decades (Cornish-Bowden & Cárdenas, 1990; Fell, 1992; Liao & Delgado, 1993). One of the formal frameworks describing small changes, known as Metabolic Control Analysis (MCA), was proposed by Kacser & Burns (1973) and Heinrich & Rapoport (1974). In this approach, metabolic changes are described by the control coefficients. They represent the relative change in a variable per relative change in a parameter or rate. Owing to the existing constraints in metabolic systems, the control coefficients are not all independent. Once we assign values to the independent control coefficients, the other control coefficients cannot take arbitrary values. The natural question is what number (or what proportion) of control coefficients are independent? In other words, how constrained is metabolic control?

In the present work we will show that in combining simple theoretical considerations with available experimental data it is possible to give, at least, a qualitative answer to this question.

* Dedicated to the memory of Henrik Kacser, a master and a friend, with whom I enjoyed many years of intellectual adventures. He leaves us a rich legacy of ideas, a cornerstone in the understanding of life.

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2. Metabolic Changes

The metabolic system is represented by a set of differential equations:

$$\frac{dx_j}{dt} = \sum_{l=1}^{r} n_{jl} v_l \quad j = 1, \ldots, m \tag{1}$$

where x_j are the variable metabolite concentrations, v_l the rates and n_{jl} the stoichiometric coefficients. The rates (v_l) depend on the metabolite concentrations (x_j) and on parameters (p). The metabolite concentrations and fluxes at steady state are:

$$S_j = S_j(p) \quad j = 1, \ldots, m \tag{2a}$$

$$J_l = v_l(S_j, p) \quad l = 1, \ldots, r. \tag{2b}$$

We assume that the steady state is stable, and that after a perturbation of the parameters the system reaches a new stable steady state. Although some aspects of temporal metabolic responses have been developed (see e.g., Acerenza *et al*., 1989; Acerenza & Kacser, 1990, Meléndez-Hevia *et al*., 1990; Heinrich & Reder, 1991; Cascante *et al*., 1991), we will restrict our analysis to the steady-state situation.

The response of the steady-state variables to changes in parameters are quantified by the control coefficients:

$$C_p^W = \frac{p}{W} \frac{\partial W}{\partial p} \tag{3}$$

where W stands for S_j and J_l. According to the response theorem (Kacser & Burns, 1973; Kacser *et al*., 1990; Sauro & Kacser, 1990), i.e.,

$$C_p^W = C_v^W \pi_p^v, \tag{4}$$

the effect that a parameter has on a variable (C_p^W) depends on two factors: the effect of the parameter on the rates (π_p^v) and the effect of the rates on the variable (C_v^W). The π-elasticity coefficients (π_p^v) depend on which parameter changes and, in this sense, they are associated to the conditions of the milieu. We could call π_p^v the "environmental factor". On the other hand, C_v^W is the same for everyone of the parameters affecting the rate. It is an "intrinsic factor". For instance, in the extreme case where C_v^W is virtually zero, whatever the parameter that changes, the variable will not respond to its change. Since our interest is to study the intrinsic constraints in metabolism, the following analysis will be based on the rate control coefficients (C_v^W).

3. Constraints in the Variables

Steady-state metabolic fluxes are always subject to conservation relationships. For instance, in an unbranched chain of steps all the fluxes are identical. Owing to these conservation constraints one can classify the fluxes into two groups, namely, the independent fluxes (J_I) and the dependent fluxes (J_D). The dependent fluxes can be expressed as a function (F_J) of the independent fluxes:

$$J_D = F_J(J_I). \tag{5}$$

In addition, metabolite concentrations may also be subject to conservation constraints (e.g. in conserved cycles). In this case, the dependent concentrations (S_D) may be calculated from the independent concentrations (S_I):

$$S_D = F_S(S_I). \tag{6}$$

We represent the number of independent fluxes, dependent fluxes, independent concentrations and dependent concentrations by r_I, r_D, m_I and m_D respectively. As an immediate consequence [see eqn (2)]:

$$r = r_I + r_D \tag{7a}$$

$$m = m_I + m_D. \tag{7b}$$

4. Structural Constraints in the Control Coefficients

The control coefficients of a metabolic system are subject to two types of constraints: structural and kinetic constraints (the discussion of the kinetic constraints is postponed until Section 6).

The structural constraints are the well-known conservation and summation relationships (Kacser & Burns, 1973; Heinrich & Rapoport, 1974; Reder, 1988). As a consequence of these relationships, any flux (concentration) control coefficient can be calculated from the values of the control coefficients of the independent fluxes (concentrations) with respect to the dependent rates (see Acerenza, 1993), i.e.,

$$C_v^J = H_J(C_{v_D}^{J_I}) \tag{8a}$$

$$C_v^S = H_S(C_{v_D}^{S_I}). \tag{8b}$$

Note that the independent (dependent) rates are the rates of the steps associated to the independent (dependent) fluxes. The $C_{v_D}^{J_I}$ and $C_{v_D}^{S_I}$ are the only coefficients that remain independent after consideration of the structural constraints.

5. Consequences of the Structural Constraints

In this section we shall analyse the consequences of the structural constraints on the number of independent control coefficients.

The maximum number of flux and concentration control coefficients that can be defined in the system are:

$$N(C_v^J) = r^2 \tag{9a}$$

$$N(C_v^S) = mr. \tag{9b}$$

Here (and in what follows) the operator $N(\ )$ applied to a coefficient means "number of". The number of control coefficients that remain independent after introducing the structural constraints are:

$$N(C_{v_D}^{J_I}) = r_I r_D \tag{10a}$$

$$N(C_{v_D}^{S_I}) = m_I r_D. \tag{10b}$$

The quantities on the r.h.s. of eqns (9) and (10) fulfil eqns (7) and the following relationship (Reder, 1988):

$$m_I = r_D. \tag{11}$$

It is useful to define the fraction of independent fluxes (y) and the fraction of independent metabolite concentrations (z):

$$y \equiv \frac{r_I}{r} \tag{12a}$$

$$z \equiv \frac{m_I}{m}. \tag{12b}$$

Introducing eqns (7), (11) and (12) into eqns (9) and (10) it is easy to show that:

$$N(C_v^J) = r^2 \tag{13a}$$

$$N(C_v^S) = \frac{r^2(1-y)}{z} \tag{13b}$$

$$N(C_{v_D}^{J_I}) = r^2 y(1-y) \tag{13c}$$

$$N(C_{v_D}^{S_I}) = r^2(1-y)^2. \tag{13d}$$

The fractions of independent control coefficients can be calculated from eqns (13) as follows:

$$f_J = \frac{N(C_{v_D}^{J_I})}{N(C_v^J)} = y(1-y) \tag{14a}$$

$$f_S = \frac{N(C_{v_D}^{S_I})}{N(C_v^S)} = z(1-y) \tag{14b}$$

$$f_{JS} = \frac{N(C_{v_D}^{J_I}) + N(C_{v_D}^{S_I})}{N(C_v^J) + N(C_v^S)} = \frac{z(1-y)}{z+1-y} \tag{14c}$$

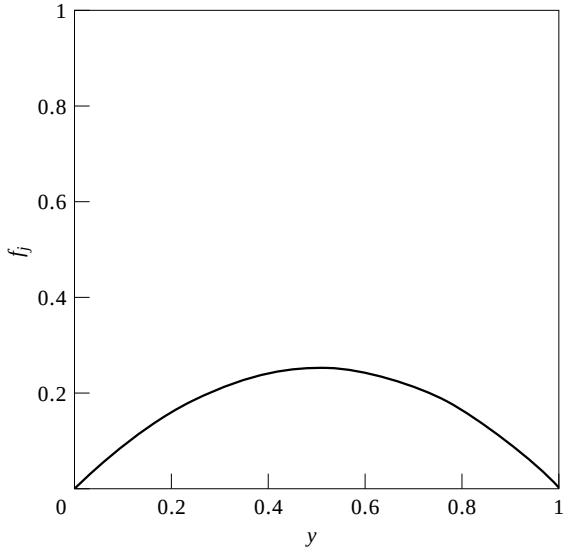

FIG. 1. f_J vs. y. y is defined by r_I/r. It is calculated assuming $r = 2000$ and r_I ranging from 1 to 1999.

f_J, f_S and f_{JS} are represented in Figs 1–3. Their values depend on the structural parameters z and y. The different portions of the graphs could therefore be associated to different types of structure. For instance, if we consider a network with thousands of isomerisation reactions ($r \sim 2000, z \approx 1$) $y \approx 0$ is equivalent to an unbranched chain of steps, $y \approx 1/2$ is equivalent to a binary tree structure and $y \approx 1$ is equivalent to a star structure (i.e. all the rates around a single metabolite).

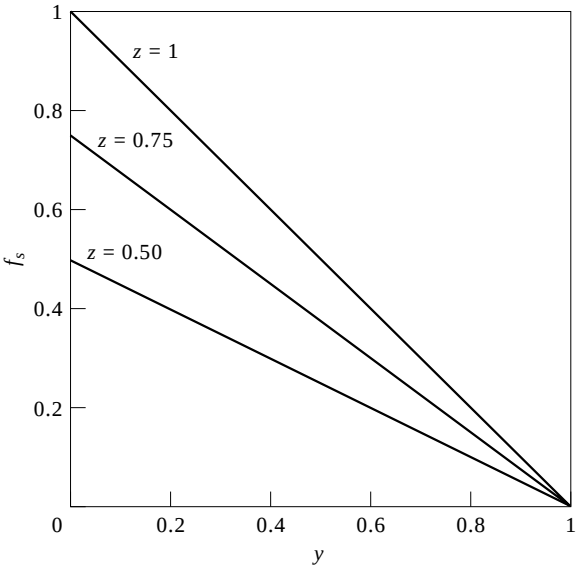

FIG. 2. f_S vs. y. See comments in Fig. 1.

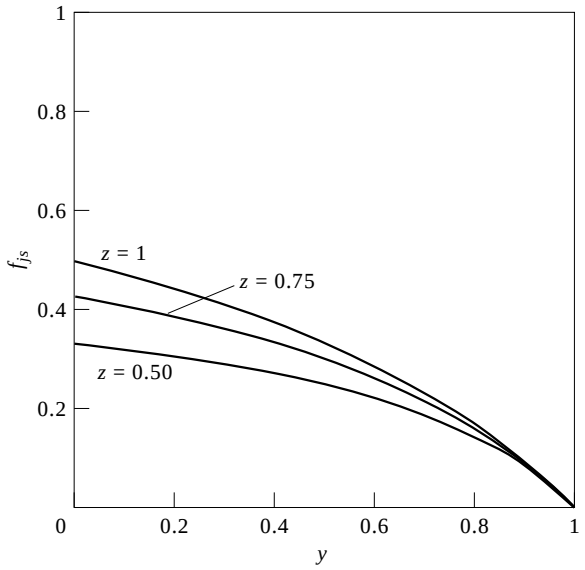

FIG. 3. f_{JS} vs. y. See comments in Fig. 1.

6. Kinetic Constraints

In the framework of MCA the sensitivity properties of the isolated steps are described by the elasticity coefficients (Kacser & Burns, 1973). The ϵ-elasticity coefficients are defined by:

$$\epsilon_S^v = \frac{S}{v}\frac{\partial v}{\partial S} \tag{15}$$

where S is the steady-state metabolite concentration and v is the rate. An important result in MCA is that the v-type control coefficients may be expressed in terms of the ϵ-elasticity coefficients (Kacser, 1983; Fell & Sauro, 1985; Kacser *et al*., 1990; Sauro & Kacser, 1990). Recently a procedure that achieves the inverse has been developed, i.e., the ϵ-elasticity coefficients are calculated from the control coefficients. This procedure is part of a general strategy that allows the design of a metabolic system with desired profile of control coefficients. It is called Metabolic Control Design (MCD) (Acerenza, 1993). One important result of MCD is that the ϵ-elasticity coefficients with respect to the independent concentrations ($\epsilon_{S_I}^v$) can be expressed as a function (Q) of the control coefficients that stay independent after considering the structural constraints, i.e.,

$$\epsilon_{S_I}^v = Q(C_{v_D}^{J_I}, C_{v_D}^{S_I}). \tag{16}$$

The existence of restrictions in the ϵ-elasticity coefficients will, therefore, impose constraints to the control coefficients. We call these type of constraints: kinetic constraints. We distinguish three types of kinetic constraints: cybernetic, mechanistic and external.

CYBERNETIC CONSTRAINTS

The ϵ-elasticity coefficient for a metabolite concentration that does not affect the rate is zero. According to eqn (16), for each ϵ-elasticity coefficient equal to zero there is one control coefficient to which we cannot assign an arbitrary value. Owing to this type of constraint some of the $C_{v_D}^{J_I}$ and/or $C_{v_D}^{S_I}$ are not independent.

MECHANISTIC CONSTRAINTS

The elasticity coefficients associated to one rate are not completely independent. For instance, the substrate and product elasticities (ϵ_S^v and ϵ_p^v respectively) of a Michaelis–Menten rate law satisfy: $0 \leqslant \epsilon_S^v + \epsilon_p^v \leqslant 1$. This type of inequality restrict the values that the control coefficients can take [eqn (16)].

EXTERNAL CONSTRAINTS

The elasticity coefficients depend on the values of parameters such as external metabolite concentrations. Let us consider the last step of a metabolic pathway catalysed by a Michaelis–Menten rate equation. If the product concentration is maintained at a value of zero the substrate elasticity can only take values between zero and one (and not greater than one as in the general case).

A difference between the cybernetic constraints and the other kinetic constraints is that while the cybernetic constraints decrease the number of independent control coefficients, the other constraints, in most cases, restrict the range of values that the control coefficients can take. Since we are interested in the effect of the constraints on the number of independent control coefficients, in what follows, the cybernetic constraints are the only type of kinetic constraints that will be considered.

7. Consequences of the Cybernetic Constraints

In a real metabolic system one would expect that each rate would be directly affected by a small proportion of the metabolite concentrations. A typical metabolic step could have two substrates, one product and four effectors (activators and inhibitors). Since each rate is affected by a different number of metabolites, it will be useful to introduce m_I^*, the mean number of independent metabolites affecting the rates. Similarly, the mean fraction of independent metabolites affecting the rates (c) is:

$$c \equiv \frac{m_I^*}{m_I}. \tag{17}$$

The maximum number of $\epsilon^v_{S_I}$ that can be defined in a metabolic system is rm_I (note that $N(C^{J_I}_{v_D}) + N(C^{S_I}_{v_D}) = rm_I$). After introducing the cybernetic constraints, the number of non-zero $\epsilon^v_{S_I}$ is rm_I^*. As a consequence of eqn (16), the number of control coefficients that stay independent after consideration of the structural and cybernetic constraints ($N(C^{W_*}_{v_*})$) is also equal to rm_I^*. Following a similar procedure as the one described in Section 5 we can write:

$$N(C^{W_*}_{v_*}) = rm_I^* = cr^2(1 - y). \tag{18}$$

The fraction of independent control coefficients is:

$$f_w = \frac{N(C^{W_*}_{v_*})}{N(C^J_v) + N(C^S_v)} = \frac{cz(1 - y)}{z + 1 - y}. \tag{19}$$

Note that the effect of introducing the cybernetic constraints is to reduce the number of independent control coefficients in a fraction c [compare eqns (14c) and (19)]. The graph f_W vs. y is similar to Fig. 3, the only difference being that it is scaled by the value c.

Another relevant quantity is the average number of independent control coefficients per independent variable [$N(C/V)$]. The number of independent control coefficients is given in eqn (18) and the number of independent variables equals r (i.e. $r_I + m_I$). Their ratio is:

$$N(C/V) \equiv \frac{N(C^{W_*}_{v_*})}{r_I + m_I} = m_I^*. \tag{20}$$

Equation (20) is an important result. It indicates that the number of independent control coefficients per independent variable equals the average number of independent metabolite concentrations that affect each rate. As a consequence, to increase the number of independent control coefficients per variable by a certain amount, without affecting the reaction scheme of the network, one would have to increase the number of interactions (e.g. allosteric interactions) by the same amount.

8. How Constrained is Metabolic Control?

In this section we shall give a qualitative answer to the questions: what is the fraction of independent control coefficients in *Escherichia coli*? and, how many independent control coefficients are there per independent variable? To calculate f_W and $N(C/V)$ [eqns (19) and (20)] we must estimate z, y and c.

z is the fraction of independent metabolite concentrations [eqn (12b)]. From a direct inspection of the metabolic map it is clear that only a small proportion of the metabolites are potentially subject to conservation relationships. Most of the cases are molecules playing the role of cofactors that are consumed and regenerated in moiety conserved cycles (e.g. ATP and NADH). In addition, not one of these moieties is strictly conserved. Every organic substance is subject to degradation and must be either taken from the environment or produced by synthesis. The concentration of moiety is therefore affected by the turnover rates. In a short timescale, if the turnover rates are much smaller than the cycling rates, the structure can be treated as a conserved cycle. For longer timescales, the turnover rates must also be considered and as a consequence conservation breaks. Taking into account all these considerations, it is reasonable to accept that $z \approx 1$ (i.e. $m_I \approx m$).

y is the fraction of independent fluxes [eqn (12a)]. Taking into account eqn (7), eqn (11) and $m_I \approx m$ we obtain: $y \approx 1 - (m/r)$. A simple way to estimate m (the number of metabolite concentrations) could be to identify it with the number of different small organic molecules in the cell. It is clear that both numbers are not the same. For instance, many molecules are present in more than one compartment. Each of these substances generate more than one metabolite concentration suggesting that m is greater than the number of small organic molecules. The number of small organic molecules in *E. coli* is $\sim$800 (Alberts *et al.*, 1994). We will round up this amount and consider $m \approx 1000$. To estimate r (the number of rates) we shall assume that each rate is catalysed by a different enzyme (what is in agreement with the fact that most enzymes are highly specific). The problem, therefore, is to estimate the number of enzymes. The number of proteins in *E. coli* is $\sim$3000 and most of them are enzymes (Lehninger, 1978). We will suppose that the number of enzymes is two-thirds of the number of proteins, i.e. $r \approx 2000$. With the values of m and r estimated above (i.e. $m \approx 1000$ and $r \approx 2000$) we obtain $y \approx 1/2$. This value is a qualitative result. At best, one could say that the fraction of independent rates (y) is closer to a half than to the extreme values (zero and one). This result has structural implications. As was discussed in Section 5, $y \approx 1/2$ corresponds, in average, to a structure equivalent to a binary tree. Some portions will be closer to an unbranched chain of steps (e.g. cholesterol biosynthesis) and others to a star structure (e.g. acetyl-CoA transformations).

m_I^* is the mean number of metabolite concentrations that affect the rates (Section 7). To this number contribute the reactants (substrates and products) and the effectors (activators and inhibitors). An estimate of the average number of reactants per rate could be obtained from a list of all metabolic reactions. This was done using a table including $\sim$2000 enzymes known at the end of 1978 (Dixon &

Webb, 1979). The result obtained was ~ 3. This number could be considered a lower limit to m_I^*. The contribution to m_I^* due to the effectors is more difficult to estimate, since a table with all the effectors of each metabolic step is not available. From what is known today one could guess that, an average of 15 effectors per rate could be considered a generous upper limit to the contribution of the effectors to m_I^*. Under these assumptions m_I^* would be between three and 18, and c [eqn (17)] between 0.003 and 0.018.

Introducing the estimates obtained for z, y and c into eqn (19) we conclude that f_W could be in the range of 0.001–0.006. In other words, only a small proportion of the control coefficients (0.1–0.6%) can take arbitrary values. On the other hand, the absolute number of independent control coefficients [eqn (18)] is still quite large, between 6000 and 36000.

Finally, the number of independent control coefficients per independent variable [eqn (20)] is between three and 18.

9. Discussion

The low value (0.001–0.006) estimated for the fraction of independent control coefficients [given in eqn (19)] results from the application of the structural and cybernetic constraints. If we consider the structural constraints only [eqn (14c)] the value would be one-third. The cybernetic constraints are the main factors responsible for the low fraction of independent control coefficients.

The most relevant quantity to answer the question posed in the title of this work is, perhaps, the number of independent control coefficients per independent variable [eqn (20)]. This number indicates by how many rates (on average) an independent variable could be independently controlled. A small number will imply that the control of the independent variables is highly constrained. According to the estimations made in Section 8 this number of rates is between three and 18. On the other hand, if we consider the structural constraints only, the number of rates that could independently control a variable equals the number of dependent rates ($r_D = m_I$), i.e. ~ 1000. It is, therefore, clear that, due to the cybernetic constraints, only a small proportion (0.3–1.8%) of the rates that could have independently controlled the variables, exerts this control. The immediate question is: why do living organisms not evolve to less constrained metabolic responses? This flexibility would, surely, give the organisms some adaptive advantage. There are, at least two explanations for this question.

The first, is that there is an upper limit to the number of sites that an enzyme can accommodate in its surface (active site, allosteric sites, etc). One could make an estimation of this number based on the normal size of the enzymes and their ligands. The estimations based on geometrical considerations (not shown) are closer to the upper limit proposed for m_I^* (i.e. 18) than to the potential value of ~ 1000. Moreover, the estimation does not take into account the structure and physicochemical properties of the interacting molecules and it would be an overestimate to the real value. From another point of view, it could be argued that there are some evolutionary paths that could increase the number of sites, such as the increase in the number of subunits of the enzyme. The fact that many enzymes are known where the active site and the allosteric site are in different subunits is in agreement with these arguments. On the other hand, the increase in the number of subunits has a limit. In practice, proteins with more than 12 subunits are very rare, what could be due to a decrease in the stability of the oligomer with the number of subunits. Finally, the fact that similar effectors can affect the activity of an enzyme through the binding to the same site, could give a bigger number of effectors than sites. This is not an important factor because enzyme sites are, in most cases, highly specific.

The second explanation is associated to the stability of the metabolic system. There is theoretical evidence that, at least in some cases, the larger the number of connections in a dynamical system, the bigger the probability that the steady state is unstable (Gardener & Ashby, 1970; May, 1972, 1974). An increase in m_I^* could, therefore, be disadvantageous for the stability of the metabolic system. This potential detrimental effect on the stability opposes to the beneficial effect on the flexibility of the metabolic responses. From this point of view, the actual value of m_I^* to which the organisms have evolved could be seen as a compromise between stability and control.

The two factors described above, namely, the limit in the number of sites that an enzyme can accommodate in its surface and the relationship between connectance and stability, are not exclusive. Furthermore, both factors could be relevant in explaining why metabolic control is much more constrained than what is imposed by metabolic structure.

I wish to thank Dr. E. Mizraji for useful comments on this work and the European Commission for financial support.

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