Notes on Metabolic Control Analysis

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Abstract

This is an expanded account of a talk on the mathematical aspects of Metabolic Control Analysis which I gave to the Theory Reading Group at the Bauer Centre on 30 September 2002.

Introduction

Metabolism is regarded as rather boring in these days of genomics and proteomics. The principal metabolic pathways have all been worked out; the major challenges dealt with. I thought this meant that control and regulation of metabolism were well understood and that this knowledge might therefore provide useful lessons for studying system issues in the more complex areas of signal transduction and gene expression. That was naive! While a lot is known, I would not characterise it as well understood.

The traditional approach to regulation of metabolism uses the idea of the "rate-limiting step". It is not always clear what this means. The Oxford Dictionary of Biology defines it as the "slowest step in a metabolic pathway". Since pathways are usually studied at steady state and a linear pathway then has the same rate at each step, this definition is puzzling, to say the least. A better definition, which accords with one's intuition, is that it is the step in a pathway which, if its rate were increased, would cause the greatest change in the pathway flux. This at least makes sense. Suppose that it was possible to measure such effects: say, as the percentage change in pathway flux caused by a 1% change in the rate of a particular step. We might call this a "flux control coefficient". By quantifying it, we would be able to make an objective assessment of whether one step was more effective than another in controlling flux. If one of the flux control coefficients was much higher than all the others then this would indicate that the step in question was rate-limiting. If, on the other hand, several steps had roughly the same levels while the others were all much lower then it would indicate that there was no single rate-limiting step and that control was distributed through the pathway. The issue as to whether or not there was a single rate-limiting step would be a testable hypothesis.

Traditional biochemistry did not develop such quantitative measures. Rate-limiting steps were presumed to be single and a variety of *ad-hoc* methods were developed for identifying "it". The resulting confusion, with multiple candidates being deemed to be "it", prompted a cartoon, "The quest for the rate-limiting step", in TIBS in 1986, [16]. Metabolic Control Analysis was developed in Edinburgh in 1973, by Henrik Kacser and Jim Burns, [9], and, independently in Berlin, by Reinhart Heinrich and Tom Rapaport, [6, 5], to provide a rigorous quantitative foundation for defining control coefficients. From this emerges various mathematical relationships between the coefficients (summation theorem, connectivity theorem; see below) and a methodology for calculating

them from empirical data. From a systems perspective, MCA relates local behaviour, the behaviour of a single step considered in isolation, to global behaviour, the behaviour of the step in the context of a pathway.

A different mathematical analysis, with very similar aims, was undertaken, independently and earlier, by Michael Savageau under the name Biochemical Systems Theory, [15]. It would be interesting to compare the two approaches but I will concentrate here on MCA.

Despite these developments and much accumulated evidence since then that metabolic regulation is more subtle than the traditional view, biochemists have seemed reluctant to abandon the rate-limiting step. (For a perceptive analysis of why this might be so, from the viewpoint of someone outside the MCA community, see Louis Hue's essay in [2].) Many current biochemistry textbooks reproduce the traditional view[1]. David Metzler's book, [12] discusses control coefficients and MCA, so perhaps the field is going through a slow transition to a more nuanced view.

Mathematically, MCA is a first order sensitivity analysis in the vicinity of a stable, and structurally stable, fixed point. This conceals many subtleties and the underlying assumptions are often not made explicit in the literature. When I first started reading about MCA, I found I could not derive some of the basic equations on the basis of what was stated and I found myself having to dig back into the literature to fully understand the foundations. What follows is a report on what I found out. It reproduces closely the account given by Reinhart Heinrich and Stefan Schuster in their book, [7], with additional details at certain points.

Enzyme kinetics

Metabolic pathways are composed of individual enzyme-catalysed steps. Before studying the global behaviour of the former, its helpful to recall the local behaviours exhibited by the latter.

Enzymes carry out their functions by binding their substrates, possibly together with co-enzymes and effectors (activators or inhibitors). The details of these molecular mechanisms vary widely and they have correspondingly varied effects at the macroscopic level of enzyme kinetics. For enzymes with multiple substrates the affinity of binding can depend on the order in which the different substrates bind. Enzymes are frequently multimeric, with multiple substrate binding sites. These could be identical or vary in the affinity with which substrate binds. They could be independent of each other or, alternatively, binding at some sites could influence binding at other sites, leading to cooperativity. In addition to substrate and product binding, effectors could bind non-competitively at sites different from the active site (allostery), perhaps on regulatory rather than catalytic domains, and thereby alter the substrate binding affinity. Quite apart from the interactions among homotropic and heterotropic binding sites which affect binding affinities, the catalytic activity of the enzyme may also be affected by the various bound ligands. Finally, the physiological environment—pH, ion concentrations, temperature etc—all influence binding and catalysis and hence also the kinetics of product formation.

The rate, v, of an enzyme is the speed at which it generates its products. It can be regarded as a function of the form $v(S_1, \cdots, S_m)$, where $S_1, \cdots, S_m$ are the concentrations of relevant metabolites. (Throughout this note, I'll freely abuse notation by using the same letter to denote both a chemical species and its concentration.) The relevant metabolites are those which are subject to change, either through the reaction itself (such as substrates and products), or through external action (such as various effectors). In writing such a functional form, we implicitly assume that any other things that might influence the rate—the enzyme concentration, the concentrations of any effectors other than $S_1, \cdots, S_m$, pH, ion concentrations, etc—are all held constant. A substantial body of theoretical and empirical studies has shown that the rate function often takes the form of a fraction in which both the numerator and the denominator are sums of terms of the form $\alpha(S_1)^{a_1}(S_2)^{a_2}\cdots(S_m)^{a_m}$, where

[1] See http://bip.cnrs-mrs.fr/bip10/rls.htm for a comparison of how various biochemistry texts describe flux regulation in glycolysis.

$\alpha, a_1, \cdots, a_n \in \mathbb{R}$ are constants, [1, 3]. It is frequently the case that the a_i are natural numbers, $a_i \in \mathbb{N} = (0, 1, 2, \cdots)$, in which case the rate is a rational function of the metabolite concentrations.

Let us briefly review some of this material. The simplest situation is that of Michaelis-Menten kinetics, where, for a reaction of the form

$$S_1 \xrightarrow{E} S_2 \ , \tag{1}$$

in which enzyme E catalyses the transformation of substrate S_1 into product S_2, the rate function takes the form

$$v(S_1, S_2) = \frac{\alpha_1 S_1}{\alpha_2 + S_1} \ .$$

This follows the general rational form described above. The numerator has a term with $\alpha = \alpha_1$ and $a_1 = 1$ and $a_i = 0$ for $i > 1$, while the denominator has two terms, one with $\alpha = \alpha_2$ and $a_i = 0$ for all i and the other with $\alpha = 1$, $a_1 = 1$ and $a_i = 0$ for $i > 1$. The rate does not depend on S_2, for reasons clarified below, and the formula above is a good approximation only when the product concentration is low (see (8) below for a discussion of the more general case). The graph of v against S_1 is a hyperbola, with approaches the maximal value α_1 asymptotically as $S_1 \to \infty$. The constant α_2 is the substrate concentration at which the rate is half-maximal. Although I am sure everyone knows the derivation of Michaelis-Menten, it will be useful for what follows to remind ourselves of the details.

The reaction (1) is assumed to arise through an intermediate complex, C, consisting of the enzyme and substrate bound together, which subsequently breaks down irreversibly to product and enzyme:

$$S_1 + E \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C \overset{k_{+2}}{\to} S_2 + E \ . \tag{2}$$

The rate of this reaction is the net rate of creation of product:

$$v(S_1, S_2) = \frac{dS_2}{dt} = k_{+2} C \ . \tag{3}$$

The irreversibility of the second step in (2) implies that the product concentration does not affect v. To obtain a closed form for v, we make the further assumption that the substrate concentration greatly exceeds that of the enzyme: $S_1 \gg E$. Under these conditions, the concentration of enzyme-substrate complex is expected to rise very rapidly after enzyme and substrate are brought together and to then remain essentially constant. Both empirical studies and further theoretical analysis shows that this is reasonable in the time scale $10^{-1} - 10^3$ seconds, which is predominantly where such enzyme studies are undertaken. Under this *steady state assumption*, $dC/dt = 0$, and so

$$(k_{+2} + k_{-1}) C = k_{+1} S_1 E \ . \tag{4}$$

The steady state assumption, which was introduced by Haldane and Briggs, avoids any further assumptions about the binding between enzyme and substrate. In the original derivation of Michaelis and Menten, it was assumed that the binding step, the first step in (2), was essentially at equilibrium, which is equivalent to assuming that k_{+2} is negligible compared to $k_{\pm 1}$.

The total amount of enzyme in use, E_{tot}, is composed partly of free enzyme and partly of enzyme bound to substrate: $E_{tot} = E + C$. This together with (4) gives two equations for the two quantities E and C. Solving for C, we find that

$$C = \frac{E_{tot} S_1}{K_M + S_1} \ ,$$

where $K_M = (k_{+2} + k_{-1})/k_{+1}$ is the Michaelis-Menten constant. Hence, using (3),

$$v(S_1, S_2) = \frac{k_{+2} E_{tot} S_1}{K_M + S_1} \ . \tag{5}$$

Since C is smaller than E and E is very much smaller than S_1, we can, to a good approximation, identify S_1 with the total amount of substrate present and not just the amount of free substrate. Finally, if we let the amount of substrate get very large, then the rate approaches the value $k_{+2} E_{tot}$, which it is reasonable to treat as the maximum reaction rate, $V_{max} = k_{+2} E_{tot}$. We obtain the usual Michaelis-Menten formula

$$v(S_1, S_2) = \frac{V_{max} S_1}{K_M + S_1} \ . \tag{6}$$

For future reference note that, from (5), the rate of the reaction is proportional to the total amount of enzyme present (provided the substrate concentration is held fixed). The effect of changing the amount of enzyme is to alter, in proportion, the amount of enzyme-substrate complex and thereby to change V_{max}.

In the reversible Michaelis-Menten equation the second step in (2) is no longer assumed to be irreversible:

$$S_1 + E \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} S_2 + E \ . \tag{7}$$

This can be thought of as composed of two irreversible Michaelis-Menten reactions: a forward reaction with $k_{-2} = 0$ and a reverse reaction with $k_{+1} = 0$. Let the constants of the forward reaction be denoted V^F_{max} and K^F_{max} and those of the reverse reaction V^R_{max} and K^R_{max}, so that

$$\begin{array}{lcllcl} V^F_{max} & = & (k_{+2} + k_{-1})/k_{+1} & \qquad V^R_{max} & = & (k_{+2} + k_{-1})/k_{-2} \\ K^F_M & = & k_{+2} E_{tot} & \qquad K^R_M & = & k_{-1} E_{tot} \end{array} \ .$$

Using a similar steady state assumption as above it can be shown after some algebraic manipulation that

$$v(S_1, S_2) = \frac{V^F_{max} S_1 / K^F_{max} - V^R_{max} S_2 / K^R_{max}}{1 + S_1 / K^F_{max} + S_2 / K^R_{max}} \ . \tag{8}$$

Product inhibition acts to slow down the rate through the terms involving S_2 in both the numerator and the denominator. This too has the same general rational form discussed above.

If an enzyme has more than one substrate, as in

$$S_1 + S_2 \overset{E}{\rightarrow} S_3 + S_4 \ ,$$

the kinetic behaviour can depend on the order in which the substrates bind. A similar, albeit more complicated, steady-state analysis shows that the enzyme kinetics has nevertheless the same general

rational form discussed above, [1]. To a first approximation and ignoring the effects of product inhibition,

$$v(S_1, S_2, S_3, S_4) = \frac{V_{max} S_1 S_2}{K_{12} + K_1 S_1 + K_2 S_2 + S_1 S_2} \ . \tag{9}$$

In all these rate formulae, the exponents applying to the metabolite concentrations (denoted a_i in the general fractional form) are all either 0 or 1. The appearance of terms with $a_i \neq 0, 1$ is an indication of co-operative behaviour. The Hill equation

$$v(S_1, S_2) = \frac{\alpha S_1^{a_1}}{\beta + S_1^{a_1}} \ . \tag{10}$$

is often cited as an example of this. If $a_1 > 1$, the graph of v against S_1 is no longer hyperbolic but sigmoidal with the amount of "sigmoid-ality" increasing as a_1 gets larger.

Unlike the formulae (6), (8) and (9) above, which are derived from underlying molecular assumptions, Hill arrived at (10) as an empirical fit for the observed binding behaviour of oxygen to haemoglobin. It is still used to characterise cooperative behaviour in enzyme kinetics. The Hill coefficient, a_1, need not be an integer, although it is frequently taken to be such. (For haemoglobin, which has 4 oxygen binding sites, a_1 values typically lie in the range $2.4 - 2.8$.)

The influence of effectors on enzyme kinetics can sometimes be incorporated into the formulae above in much the same way that the enzyme concentration influences the rate in the Michaelis-Menten formula (6): by assuming that the effectors alter constants like V_{max} or K_M. For example, going back to the irreversible Michaelis-Menten reaction (2), suppose that S_3 is an inhibitor which competes with the substrate S_1 by binding to the active site of the free enzyme. In addition to (2) there is therefore also the reaction $E + S_3 \rightleftharpoons D$, where D is the enzyme-inhibitor complex. Assuming that D is a *dead-end* complex which is not degraded further, this reaction will equilibriate. Let $K_I = S_3 E/D$ be the equilibrium constant. The total enzyme concentration, E_{tot}, is now made up of three parts: free enzyme, enzyme bound to substrate and enzyme bound to inhibitor: $E_{tot} = E + C + D$. This together with $K_I = S_3 E/D$ and (4) gives three equations for E, C and D. Solving first for E, we find that

$$E_{tot} = (1 + S_1/K_M + S_3/K_I)E \ .$$

Hence, using (4),

$$C = \frac{(S_1/K_M)E_{tot}}{1 + S_1/K_M + S_3/K_I}$$

and so, using (3),

$$v(S_1, S_2, S_3) = k_{+2} C = \frac{V_{max} S_1}{K_M(1 + S_3/K_I) + S_1} \ . \tag{11}$$

Comparing this with (6) we see that the effect of competitive inhibition has been to keep V_{max} fixed while increasing the effective Michaelis-Menten constant by the multiplicative factor $(1 + S_3/K_I)$. Other forms of inhibition can have different combinations of effects on V_{max} and K_M, [1].

Effectors can also act allosterically, by binding at sites different from the active site and influencing substrate binding through conformational change. The first examples of such behaviour were observed in bacterial amino-acid synthesis pathways, where a downstream metabolite, quite different

in structure to the initial metabolite, acted via feedback inhibition on the first reaction in a linear pathway. The enzymes involved exhibited sigmoidal kinetics with respect to their substrates and this could not be explained by hyperbolic formulas like (11). Monod, Wyman and Changeux, [13], and, independently, Koshland, Nemethy and Filmer, [10], proposed models of allosteric inhibition which also conformed to the general rational form described above, with higher values of the exponents a_i accounting for the sigmoidal behaviour. The derivation of these results requires more detailed assumptions, beyond that of steady state, about the formation of the various intermediate complexes caused by ligand binding, [1, 3].

Pathways

Metabolic pathways are sometimes linear, as in the simple pathway

$$P_1 \stackrel{E_1}{\rightarrow} S_1 \stackrel{E_2}{\rightarrow} P_2 \tag{12}$$

and it may help to keep something like (12) in mind as a concrete example of what we shall be studying. However, pathways may also be branched, either forwards or backwards, or may contain cycles, or other complex forms of interaction between individual enzymatic reactions. This is particularly the case when the reactions have multiple substrates or products.

In the linear pathway (12), metabolite P_1, which is only a substrate, and metabolite P_2, which is only a product, play a special role. These metabolites are on the edge of the pathway and represent its interface with the environment in which the pathway is operating. The concentrations of P_1 and P_2 are therefore regarded as being kept constant. (To achieve this in practice may require some deft experimental technique but our concern here is with getting the mathematics straight.) As far as the mathematical analysis of the pathway is concerned, there is only one metabolite, S_1, whose concentration is being affected by the action of the pathway. All pathways have edge effects like this and they must be taken into account in order to treat the pathway as a closed system. The concentrations of edge metabolites can be treated as *parameters* of the system (hence the use of the letter P) and we will discuss these later.

Suppose that a pathway consists of r reactions among m metabolites. Let us label the reactions $1, \cdots, r$ and the metabolites $1, \cdots, m$ in some arbitrary order. (Following the remarks above, we see that the pathway (12) has $r = 2$ and $m = 1$.) Each of the metabolites will have a concentration, denoted S_i for $1 \leq i \leq m$, and each of the reactions will have a rate, denoted v_i for $1 \leq i \leq r$. Each v_i will be regarded as a function of all the S_i: $v_i = v_i(S_1, \cdots, S_m)$. (Only those metabolites which participate in reaction i or are effectors of that reaction will actually have an effect on v_i but it is notationally easier not to keep track of this.) It may help in what follows to assume that the functional form of v_i is one of those, like (6), (8), (9) or (11), discussed in the previous section.

The various rates can be collected together into a vector, $\mathbf{v}$, which is itself a function of the vector of metabolite concentrations, $\mathbf{v} = \mathbf{v}(\mathbf{S})$. These should be thought of as column vectors, to fit in with the matrix notation used below: $\mathbf{v}$ is thus an a $r \times 1$ matrix while $\mathbf{S}$ is an $m \times 1$ matrix.

The metabolite concentrations change with time, as the various reactions consume substrates and produce products. We will not show the time dependency explicitly, in part because we shall be mostly concerned with fixed points, where the concentration does not change in time. The rate at which a given metabolite concentration changes is the net result of those reactions in which it is generated as a product and those reactions in which it is consumed as a substrate. We just need to keep track of the possibility that a reaction may produce or consume more than one molecule of a given metabolite.

To do so we introduce the stoichiometric matrix, $\mathbf{N}$, an $m \times r$ matrix with integer entries. If k molecules of metabolite i appear as a product in reaction j, then $N_{ij} = k$, while if k molecules of

metabolite i appear as a substrate in reaction j, then $N_{ij} = -k$. Suppose, for instance, that reaction 1 looks like this:

$$2S_1 + S_2 \rightarrow 3S_3 + 2S_4 \ ,$$

then $N_{11} = -2$, $N_{21} = -1$, $N_{31} = 3$ and $N_{41} = 2$. For the linear pathway (12), the stoichiometric matrix is given by $\mathbf{N} = (+1, -1)$. The signs in the stoichiometric matrix have been chosen to make it easy to work out the net rate of change of each metabolite. The contribution that reaction j makes to the rate of generation of metabolite i is given simply by $N_{ij}v_j$. It follows that

$$\frac{d\mathbf{S}}{dt} = \mathbf{N}\mathbf{v}(\mathbf{S}) \ . \tag{13}$$

(Since $\mathbf{N}$ is $m \times r$ and $\mathbf{v}$ is $r \times 1$, their product is $m \times 1$, which is also the size of $d\mathbf{S}/dt$.) This nonlinear vector equation governs the time dynamics of the pathway.

Fixed points and stability

MCA looks at the behaviour of a pathway in the vicinity of a *fixed point*. It assumes that, under physiological conditions and with the environment maintained in a constant state, the metabolite concentrations, perhaps after some initial transient phase, settle down at constant values. This appears to be a reasonable assumption within the time scale on which enzymes and pathways are typically studied and is consistent with the assumptions made above in studying enzyme kinetics. If indeed metabolite concentrations were varying substantially, it would be hard to get repeatable measurements on the pathway. However, there is no mathematical reason why an equation like (13) should have a fixed point. It might exhibit periodic oscillation or some more complex form of dynamical behaviour. Oscillations in the glycolysis pathway were observed in the 1950s and have been extensively studied since then, although their physiological significance remains unclear, [4, Chapter 2]. It is also worth noting that had we not insisted that edge metabolites, such as P_1 and P_2 in (12), were held at constant concentration, the pathway would simply consume its substrates until it came to equilibrium with zero flux through it. This is not a very interesting fixed point! It is only by holding the pathway away from equilibrium that interesting fixed points can appear. Be that as it may, MCA can be applied only when a pathway is at some fixed point. Let $\mathbf{S^0}$ denote the vector of concentrations at the fixed point.

The condition for a fixed point is that $d\mathbf{S}/dt = 0$ so we deduce from (13) that

$$\mathbf{N}\mathbf{v}(\mathbf{S^0}) = 0 \ . \tag{14}$$

The argument given above, which suggests that a fixed point is a reasonable assumption, actually says rather more than that. If we imagine repeating the same experiment on a pathway many times, perhaps with different cells or cell extracts, it is very likely that the initial concentrations of the various metabolites will be different each time. If, nevertheless, the pathway settles down at the same fixed point then it would appear that this fixed point is *stable*. That is, if the pathway is at the fixed point and a small change is made to some of the concentrations, then, after some transient behaviour, the pathway settles back to the same fixed point. In an unstable fixed point, a small change could cause the system to change radically and move far away from its original position. (A pencil balanced on its tip is at an unstable fixed point, while a marble placed in the bottom of a bowl is at a stable fixed point.)

There is a mathematical condition for a stable fixed point which we shall need to use. To explain it, let us consider the simple case of a system with just one metabolite, s, and one reaction, so that we can rewrite the matrix equation (13) as

$$\frac{ds}{dt} = f(s) \ . \tag{15}$$

Suppose that the fixed point occurs at $s = 0$, so that the graph of f against s goes through the origin: $f(0) = 0$. Suppose further that the derivative of f with respect to s is negative at 0: $(df/ds)_{s=0} < 0$. In this case, the tangent to the graph of f at the origin slopes downwards from left to right and, under very reasonable conditions on f, we can use the Mean Value Theorem to show that there must be a small interval around the origin, say $(-\epsilon, +\epsilon)$, in which f is decreasing: if $-\epsilon < u < v < +\epsilon$, then $f(u) > f(v)$. Suppose that we now displace the system from its fixed point at the origin by an amount which is small enough to fall within this interval, say to u where $-\epsilon < u < 0$. Then $f(u) > f(0) = 0$ and we see from (15) that $(ds/dt)_{s=u} > 0$. Since the time derivative of s is positive, s must increase. Hence the system moves from u back towards the origin. Similarly, if we displace the system to v, where v is on the other side of the origin, $0 < v < +\epsilon$, then by the same argument, $(ds/dt)_{s=v} < 0$, and so s must decrease, bringing the system again back towards the origin. We have shown that the condition $(df/ds)_{s=0} < 0$ is sufficient for the fixed point to be stable.

In the case when $(df/ds)_{s=0} > 0$, a similar analysis shows that the origin is an unstable fixed point: any small displacement gets increased. The case when $(df/ds)_{s=0} = 0$ is intermediate and could result in stability or instability or neither.

In the general case, with r reactions and m metabolites, the function f in (15) is now a vector function: $\mathbf{N}\mathbf{v}(\mathbf{S})$. As we checked above, this is an m vector. The derivative with respect to s has to be replaced by the Jacobian, the $m \times m$ matrix of first partial derivatives

$$\frac{\partial}{\partial \mathbf{S}}(\mathbf{N}\mathbf{v}(\mathbf{S})) \ .$$

Because $\mathbf{N}$ is a constant matrix this can be simplified to

$$\mathbf{N}\frac{\partial \mathbf{v}(\mathbf{S})}{\partial \mathbf{S}} \ . \tag{16}$$

It may be less clear what replaces the condition $(df/ds)_{s=0} < 0$. Every $m \times m$ square matrix, $\mathbf{A}$, has m *eigenvalues*. These are the values λ for which the vector equation $\mathbf{A}\mathbf{x} = \lambda\mathbf{x}$ has a non-zero solution, $\mathbf{x} \neq 0$. It is equivalent to say that the matrix $(\mathbf{A} - \lambda\mathbf{I})$ is singular, where $\mathbf{I}$ is the $m \times m$ identity matrix, or that $det(\mathbf{A} - \lambda\mathbf{I}) = 0$. The determinant gives an equation for λ which is a polynomial of degree m. It has m solutions, although we have to allow for the possibility that some of them may be complex numbers and that some of them may be repeated. These solutions are the m eigenvalues of A. We can now reinterpret the condition $(df/ds)_{s=0} < 0$ in matrix terms by asking that all the eigenvalues of the matrix of partial derivatives at the fixed point satisfy the condition $Re(\lambda) < 0$. (We need to take real parts to allow for the possibility of complex eigenvalues.) If that is the case, then an argument along similar lines to the one given above in dimension 1, shows that the fixed point is stable. Hence, we shall assume that

$$Re(\lambda) < 0 \ \text{ for all eigenvalues, } \lambda, \tag{17}$$

of (16). It is also the case, in a similar way to dimension 1, that if any of the eigenvalues satisfies $Re(\lambda) > 0$ then the system is unstable. This is helpful to know from the point of view of the justification given below.

For the mathematical analysis, it is not the condition for stability that we will directly use, but a consequence of it: if the matrix A is such that all its eigenvalues satisfy $Re(\lambda) < 0$ then it must be invertible. If the eigenvalue condition is satisfied, it is certainly not the case that any of the eigenvalues of A are 0. Since the determinant of A is given by the product of its eigenvalues,

$det(A) \neq 0$, and so A is non-singular and hence invertible. We shall write A^{-1} for the inverse matrix to A. In particular, assuming condition (17), we see that the Jacobian matrix (16) has an inverse

$$\left(\mathbf{N}\frac{\partial \mathbf{v}(\mathbf{S^0})}{\partial \mathbf{S}}\right)^{-1} . \tag{18}$$

This is one of the two main results that we need for MCA.

Although it seems reasonable to assume that the fixed point $\mathbf{S^0}$ is stable, we actually require somewhat more than that, since we need the sufficient condition (17). Is this reasonable? As we saw above, if one of the eigenvalues of (16) satisfies $Re(\lambda) > 0$, then the system would be unstable. The only thing we have to worry about is whether one of the eigenvalues satisfies $Re(\lambda) = 0$. As we saw above, this case is indeterminate and it is possible for the system to be stable and still have $Re(\lambda) = 0$. The matrix of partial derivatives, $\partial \mathbf{v}(\mathbf{S^0})/\partial \mathbf{S}$, contains a mixture of algebraic and empirical data and it seems unlikely that this would give something exactly 0. The stoichiometric matrix $\mathbf{N}$, however, is another matter. If the system (13) has some conserved moiety (for instance, a phosphate group) then that would imply a linear relationship among the rows of the stoichiometric matrix $\mathbf{N}$. In other words, there would exist some m vector $\mathbf{x} \neq 0$ such that $\mathbf{xN} = 0$. But then $\mathbf{x}$ is in the null space of the Jacobian, $\mathbf{x}(\mathbf{N}\partial \mathbf{v}(\mathbf{S^0})/\partial \mathbf{S}) = 0$, which is therefore not invertible and must have at least one of its eigenvalue at 0. In this case it is possible to reduce the effective dimension of the system by "taking out" the conservation laws and the analysis can proceed along the same lines as below; see [7, §3.1.1] for more details. Let us keep life simple and assume that the system (13) does not have any conservation laws. In that case, it does appear to be reasonable to assume that (17) holds.

Introducing parameters: from local to global behaviour

MCA tries to quantify what happens to a pathway in the vicinity of a fixed point. If some aspect of the pathway is changed, what quantitative effect will this have on the various metabolite concentrations or reaction rates? To formalise the "aspects of the pathway that are changed", we explicitly introduce *parameters* into the equations above. Many papers on MCA do not use parameters explicitly but I believe that the mathematical analysis becomes both clearer and more general if we do so. As we shall see, it is then very easy to reduce to the special cases considered in the literature.

The parameters can be any variables whose values affect the rates of the various reactions in the pathway: enzyme concentrations, "constant" concentrations of edge metabolites such as P_1 and P_2 in (12), effector concentrations which are not included among the metabolites S_i, V_{max} values, K_M values, pH, temperature, etc. Let us assume that there are n parameters, denoted $P_1, \cdots, P_n$ and that the rates of the various reactions are now functions of these parameters as well as of the metabolite concentrations:

$$\mathbf{v} = \mathbf{v}(S_1, \cdots, S_m, P_1, \cdots, P_n) = \mathbf{v}(\mathbf{S}, \mathbf{P}) . \tag{19}$$

Let us also assume that the parameter values at the fixed point $\mathbf{S^0}$ are given by $\mathbf{P^0}$. What happens if one of the parameter values is changed slightly around $\mathbf{P^0}$? The change will cause various affects on the reactions in which the parameter plays a role. These changes will affect the metabolite concentrations in those reactions and hence affect the rate of other reactions in which those metabolites appear. The changes will thus propagate through the system. What happens then depends on making another assumption about the behaviour of the system. We shall assume that it is *structurally stable*. That is, after a small perturbation of any parameter, the system returns to a fixed point. For much the same reasons as discussed above for stability of fixed points, structural stability is also a reasonable assumption. Note, however, that there is an important difference between fixed point stability and structural stability. For the former, if the concentrations are altered slightly around

the fixed point, $\mathbf{S^0}$, and all the parameters are kept fixed, the system returns back to the same fixed point, $\mathbf{S^0}$. For the latter, if the parameters are altered in the vicinity of $\mathbf{P^0}$, the system could settle back to a different fixed point, which would be close to $\mathbf{S^0}$ but need not be exactly $\mathbf{S^0}$.

In view of structural stability, we can regard the metabolite concentrations $\mathbf{S}$ as functions of the parameter values (at least in the immediate vicinity of $\mathbf{P^0}$). We shall indicate this by writing $\mathbf{S}(\mathbf{P})$, so that at the fixed point, $\mathbf{S^0} = \mathbf{S}(\mathbf{P^0})$. It is important to keep in mind that this functional relationship is not known explicitly through some formula but arises instead through the mediation of equation (13). If the parameter values are changed from the fixed point $\mathbf{P^0}$ to, say, $\mathbf{P^1}$, the equation (13) comes into play, the metabolite concentrations change according to (13) and eventually settle to a new stable fixed point at, say, $\mathbf{S^1}$. This is the new value of $\mathbf{S}$: $\mathbf{S^1} = \mathbf{S}(\mathbf{P^1})$.

Not only will the metabolite concentrations change but also the rates of the various reactions. The result is given by $\mathbf{v}(\mathbf{S}(\mathbf{P}), \mathbf{P})$ and it will be convenient to use the notation $\mathbf{J}(\mathbf{P})$ for this:

$$\mathbf{J}(\mathbf{P}) = \mathbf{v}(\mathbf{S}(\mathbf{P}), \mathbf{P}) \tag{20}$$

The different notation may help to keep in mind the distinction between local effects and global effects. The rate vector, $\mathbf{v}$, is a function of metabolite concentrations and parameter values as in (19) above. We can study local changes to the rate by changing either a metabolite concentration or a parameter value, while keeping all other variables fixed. The rates of these changes are quantified by the partial derivatives $\partial\mathbf{v}/\partial\mathbf{S}$ and $\partial\mathbf{v}/\partial\mathbf{P}$. If we have formulae like (6), (8) or (9) for each of the individual reactions, it is an easy matter to work these out. If not, they could be determined empirically by studying the reactions whose rates are affected in isolation from the other reactions. They are local quantities and do not take into account any changes at the system level. But the system changes are what we are really interested in. To quantify these, we have to work out the quantities $\partial\mathbf{S}/\partial\mathbf{P}$ and $\partial\mathbf{J}/\partial\mathbf{P}$. These are global quantities, in which we have (implicitly) taken into account the effect of changes on the whole system. MCA shows us how to obtain the global quantities in terms of the local quantities.

Before doing this, we need to draw one more conclusion from the assumption of structural stability. When the parameter values are changed in the immediate vicinity of the fixed point $\mathbf{P^0}$, the system settles back to a new fixed point. It follows that, by the same reasoning used to deduce (14),

$$\mathbf{N}\mathbf{v}(\mathbf{S}(\mathbf{P}), \mathbf{P}) = 0\ . \tag{21}$$

for all $\mathbf{P}$ in the immediate vicinity of $\mathbf{P^0}$. This is the other main result needed for MCA.

The basic results

We now have all the pieces in place. We want to work out the global quantities $\partial\mathbf{S}/\partial\mathbf{P}$ and $\partial\mathbf{J}/\partial\mathbf{P}$ in terms of the local quantities $\partial\mathbf{v}/\partial\mathbf{S}$ and $\partial\mathbf{v}/\partial\mathbf{P}$. Because equation (21) is valid in a neighbourhood of $\mathbf{P^0}$, we are at liberty to differentiate it at that point with respect to the parameter values. We find, evaluating all quantities at $\mathbf{P} = \mathbf{P^0}$, that

$$\frac{\partial}{\partial\mathbf{P}}\left(\mathbf{N}\mathbf{v}(\mathbf{S}(\mathbf{P}), \mathbf{P})\right) = 0\ .$$

Simplifying this using the chain rule for derivatives, we find that

$$\mathbf{N}\frac{\partial\mathbf{v}}{\partial\mathbf{P}} + \mathbf{N}\frac{\partial\mathbf{v}}{\partial\mathbf{S}}\frac{\partial\mathbf{S}}{\partial\mathbf{P}} = 0\ . \tag{22}$$

(It is always a good idea to check the dimensions in matrix equations like this to make sure we are not doing anything stupid. The first term is the product of an $m \times r$ matrix with an $r \times n$ matrix and is thus $m \times n$. The second term is a product of an $m \times r$, an $r \times m$ and an $m \times n$ matrix and so is also $m \times n$. At least we are consistent!) We see embedded inside (22) the Jacobian matrix, which we know to be invertible by (18). Hence we can solve for at least one of the global quantities we are looking for:

$$\frac{\partial \mathbf{S}}{\partial \mathbf{P}} = -\left(\mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{S}}\right)^{-1}\mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{P}} \,. \tag{23}$$

As for the other one, we can now obtain it by directly differentiating (20), again at the point $\mathbf{P^0}$. This is almost the same as what we have just done, except that we do not carry along the stoichiometric matrix $\mathbf{N}$:

$$\frac{\partial \mathbf{J}}{\partial \mathbf{P}} = \frac{\partial \mathbf{v}}{\partial \mathbf{P}} + \frac{\partial \mathbf{v}}{\partial \mathbf{S}}\frac{\partial \mathbf{S}}{\partial \mathbf{P}} \,.$$

Substituting the expression (23), we find that

$$\frac{\partial \mathbf{J}}{\partial \mathbf{P}} = \left(\mathbf{I} - \frac{\partial \mathbf{v}}{\partial \mathbf{S}}\left(\mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{S}}\right)^{-1}\mathbf{N}\right)\frac{\partial \mathbf{v}}{\partial \mathbf{P}} \,, \tag{24}$$

where $\mathbf{I}$ has to be an $r \times r$ identity matrix for this to make sense.

It is important to remember that these equations are all evaluated at the fixed point $\mathbf{P^0}$. They are numerical, not functional relationships.

Equations (23) and (24) are the two basic equations of MCA, which express the global quantities in terms of the local quantities. As we shall see, all the other formulas of MCA follow easily from them. The two basic facts used to deduce (23) and (24) were structural stability, which we needed to ensure that (14) held in a neighbourhood of the fixed point (in other words (21)), and the existence of a stable fixed point, which we needed for the invertibility of the Jacobian, (18).

Elasticities and control coefficients

We can now identify the main quantitative ingredients of MCA. Equations (23) and (24) have a similar structure. Both consist of matrices which multiply the local quantity $\partial \mathbf{v}/\partial \mathbf{P}$ on the left to yield each of the two required global quantities, $\partial \mathbf{S}/\partial \mathbf{P}$ and $\partial \mathbf{J}/\partial \mathbf{P}$. These matrices are the *control matrices* of MCA. The $m \times r$ matrix *concentration control matrix*, denoted $\mathbf{C^S}$, is given by:

$$\mathbf{C^S} = -\left(\mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{S}}\right)^{-1}\mathbf{N} \,, \tag{25}$$

while the $r \times r$ *flux control matrix*, denoted $\mathbf{C^J}$, is given by

$$\mathbf{C^J} = \mathbf{I} - \frac{\partial \mathbf{v}}{\partial \mathbf{S}}\left(\mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{S}}\right)^{-1}\mathbf{N} \,. \tag{26}$$

We can now rewrite (23) and (24) more compactly as

$$\frac{\partial \mathbf{S}}{\partial \mathbf{P}} = \mathbf{C^S}\frac{\partial \mathbf{v}}{\partial \mathbf{P}} \qquad\qquad \frac{\partial \mathbf{J}}{\partial \mathbf{P}} = \mathbf{C^J}\frac{\partial \mathbf{v}}{\partial \mathbf{P}} \; . \tag{27}$$

The entries in $\mathbf{C^S}$ and $\mathbf{C^J}$ are referred to as *coefficients*, so that we have mr concentration control coefficients and r^2 flux control coefficients. Be warned that the literature on MCA mostly uses *scaled* control coefficients, which we will consider shortly. I think it is easier to work with unscaled coefficients to derive the main results and to then incorporate scaling as needed.

The control matrices are built up out of the stoichiometric matrix, $\mathbf{N}$, and the other local quantity, the $r \times m$ matrix of partial derivatives $\partial \mathbf{v}/\partial \mathbf{S}$. The latter is referred to as the *elasticity matrix* and denoted ϵ:

$$\epsilon = \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \; . \tag{28}$$

The main theorems

Having done all the hard work of assembling the mathematical infrastructure of the previous sections and identifying the control matrices and the elasticities, it is now very simple to deduce the main results of MCA. First, note that both control matrices are products which end with the stoichiometric matrix $\mathbf{N}$. Let us use the notation $\mathbf{v^0}$ to denote the reaction rates at the fixed point:

$$\mathbf{v^0} = \mathbf{v}(\mathbf{S^0}, \mathbf{P^0}) = \mathbf{J}(\mathbf{P^0}) \; .$$

Using the fixed point equation (14) we immediately deduce the *summation theorems* of MCA:

$$\mathbf{C^S v^0} = 0 \qquad\qquad \mathbf{C^J v^0} = \mathbf{v^0} \; . \tag{29}$$

Second, it is also easy to work out what happens if we multiply the control matrices on the right by the elasticity matrix (28). This gives the *connectivity theorems* of MCA:

$$\mathbf{C^S}\epsilon = -\mathbf{I} \qquad\qquad \mathbf{C^J}\epsilon = 0 \; , \tag{30}$$

(Here $\mathbf{I}$ must be the $m \times m$ identity matrix!) These are the main theorems of MCA. While we are on the subject, let us just note one other result, which is not so widely used but sheds an interesting light on the flux control matrix. It is easy to see by working out the multiplications that

$$\mathbf{C^J C^J} = \mathbf{C^J} \tag{31}$$

so that the flux control matrix is a *projection matrix*. This suggests a geometric interpretation of the flux control coefficients, more details of which can be found in [11] or [7, §5.3.4].

As we have stated them, the summation and connectivity theorems, (29) and (30), are not in their most general forms. For instance, it is easy to see that any vector $\mathbf{x}$, which is in the null space of the stoichiometric matrix, as is $\mathbf{v^0}$ in view of (14), would satisfy (29) with $\mathbf{x}$ in place of $\mathbf{v^0}$. However, (29) and (30) are the forms most commonly found in the literature. The more general versions are discussed in [14] or [7, §5.3].

Scaling and other customary simplifications

Most papers on MCA use *scaled* control coefficients. The purpose of scaling is to make the coefficients reflect rates of change in proportion to the concentrations, $\mathbf{S^0}$, and reaction rates, $\mathbf{v^0}$, at the fixed point. It also makes the control coefficients dimensionless, so that we no longer have to worry about the units in which they are measured.

If $\mathbf{x}$ is any vector, let $(\nabla\mathbf{x})$ denote the diagonal matrix whose diagonal entries are given by $\mathbf{x}$:

$$(\nabla\mathbf{x})_{ij} = \begin{cases} 0 & \text{if } i \neq j \\ x_i & \text{if } i = j \end{cases} .$$

Let us denote scaled quantities with overlines. The scaled elasticity matrix is given by

$$\overline{\epsilon} = (\nabla\mathbf{v^0})^{-1}\epsilon(\nabla\mathbf{S^0}) , \tag{32}$$

while the scaled control matrices are given by

$$\overline{\mathbf{C}}^{\mathbf{S}} = (\nabla\mathbf{S^0})^{-1}\mathbf{C^S}(\nabla\mathbf{v^0}) \qquad\qquad \overline{\mathbf{C}}^{\mathbf{J}} = (\nabla\mathbf{v^0})^{-1}\mathbf{C^J}(\nabla\mathbf{v^0}) . \tag{33}$$

We are making the reasonable assumption here that none of the entries in $\mathbf{v^0}$ or $\mathbf{S^0}$ are 0. We can now rewrite the summation and connectivity theorems in their scaled forms. Let $\mathbf{1}$ denote the vector, of appropriate size, all of whose entries are 1. Note that $(\nabla\mathbf{x})\mathbf{1} = \mathbf{x}$. The scaled summation theorems follow easily from (29):

$$\overline{\mathbf{C}}^{\mathbf{S}}\mathbf{1} = 0 \qquad\qquad \overline{\mathbf{C}}^{\mathbf{J}}\mathbf{1} = \mathbf{1} , \tag{34}$$

while the scaled connectivity theorems are easily seen to be identical in form to the unscaled versions:

$$\overline{\mathbf{C}}^{\mathbf{S}}\overline{\epsilon} = -\mathbf{I} \qquad\qquad \overline{\mathbf{C}}^{\mathbf{J}}\overline{\epsilon} = 0 . \tag{35}$$

The summation theorem for flux control coefficients has played an important role in MCA. Consider a linear pathway with r reactions:

$$P_1 \overset{E_1}{\to} S_1 \overset{E_2}{\to} \cdots \overset{E_{r-1}}{\to} S_{r-1} \overset{E_r}{\to} P_r . \tag{36}$$

We deduce from the scaled summation theorem (34) that

$$\overline{\mathbf{C}}^{\mathbf{J}}_{11} + \overline{\mathbf{C}}^{\mathbf{J}}_{12} + \cdots + \overline{\mathbf{C}}^{\mathbf{J}}_{1r} = 1 . \tag{37}$$

(When a linear pathway like (36) is at steady state it is obvious that each reaction must have the same rate: $v_1(\mathbf{S^0}, \mathbf{P^0}) = \cdots = v_r(\mathbf{S^0}, \mathbf{P^0})$. It follows that there are only r different flux control coefficients, rather than r^2 and equation (37) is the only consequence of (34).) Flux control coefficients can sometimes be negative, for instance in the case of a branched pathway, but for a linear pathway an increase in the rate of a reaction cannot lead to a decrease in the pathway flux. Hence the r flux control coefficients of (36) are forced by (37) to lie between 0 and 1. If one of them is high, the others must all be low to make the sum add up to 1. The single high value would indicate a rate-limiting step. Empirical measurements of flux control coefficients have revealed extremely few with high values like 0.8. Kacser estimates that of those that have been measured the average

value lies between 0 and 0.1, [8]. Most flux control coefficients are small and most flux control is distributed at several points in a pathway, [3].

The scaled connectivity theorem gives further equations for the flux control coefficients. If S_i is any metabolite, where $1 \leq i \leq r-1$, then (35) implies that

$$\overline{\mathbf{C}}^{\mathbf{J}}_{1i}\epsilon_{ii} + \overline{\mathbf{C}}^{\mathbf{J}}_{1(i+1)}\epsilon_{(i+1)i} = 0 \,, \tag{38}$$

where we have taken advantage of the fact that metabolite i only influences reaction i, of which it is a product, and reaction $i+1$, of which it is a substrate. Hence, $\epsilon_{ki} = 0$ for $k \neq i, i+1$. The $r-1$ equations of the form (38), together with (37), provide r linear equations for the r flux control coefficients, which can hence be recovered from the elasticities. This has been used in practice to determine the flux control coefficients [3, §6.3]. Similar methods can be applied to more general pathways than linear ones but require the generalised forms of the summation and connectivity theorems mentioned in the previous section.

One final point: it is worth noting that the control matrices are not dependent on the parameters $\mathbf{P}$. This is evident from (25) and (26). If we used a completely different parametrization but the same fixed point, $\mathbf{S^0}$, we would get exactly the same control coefficients. This is perhaps why many authors do not use parameters in defining the control coefficients. Consider the situation when the parameters are chosen in such a way that parameter P_i affects only reaction i. For instance, P_i could be the concentration of the enzyme that catalyses reaction i. In this case, the matrix $\partial\mathbf{v}/\partial\mathbf{P}$ simplifies to a diagonal matrix and, making the reasonable assumption that none of its entries are 0, we can invert it. The scaled flux control coefficients can then be written

$$\overline{\mathbf{C}}^{\mathbf{J}}_{ij} = \frac{\mathbf{v}^{\mathbf{0}}_j}{\mathbf{v}^{\mathbf{0}}_i}\frac{\partial \mathbf{J}_i}{\partial \mathbf{P}_j}\left(\frac{\partial \mathbf{v}_j}{\partial \mathbf{P}_j}\right)^{-1} \,.$$

which is sometimes how they are defined. If we assume that there is a functional relationship between the local rates, $\mathbf{v}$, and the steady-state pathway fluxes $\mathbf{J}$, then we could also, using the chain rule, "cancel" the differentials to get

$$\overline{\mathbf{C}}^{\mathbf{J}}_{ij} = \frac{\mathbf{v}^{\mathbf{0}}_j}{\mathbf{v}^{\mathbf{0}}_i}\frac{\partial \mathbf{J}_i}{\partial \mathbf{v}_j} \,,$$

and this is also frequently used as a definition. It captures the idea that the control coefficients are intrinsic numbers associated with the pathway and not with any particular parametrisation of it. Although this makes for an easy definition of the control coefficients, I think it cause enormous headaches in understanding how to derive anything from the definitions and I strongly recommend using parameters as we have done here.

I think you should now be in a position to read the literature with some understanding of how the mathematics works.

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