14 An *in vivo* assay for metabolic regulation? 'Control' by a metabolic variable

O.J.G. Somsen and H.V. Westerhoff

Dept. of Molecular Cell Physiology and Mathematical Biochemistry, BioCentrum Amsterdam, Free University, De Boelelaan 1087, NL-1081 HV Amsterdam, Netherlands

Introduction

Regulation is essential for the dynamics of a physiological system. For example, homeostasis is typically caused by negative feedback, while positive feedback may lead to oscillations and bi-stability [Heinrich *et al.*, 1977]. Regulation may be studied *in vitro* for individual enzymes [Torres *et al.*, 1980], but because regulation is a subtle phenomenon, it is also necessary to study regulation phenomena *in vivo*.

A fundamental issue is that to define the extent by which a metabolite regulates other metabolic phenomena, appears to be in conflict with metabolic control analysis [Hofmeyr, 1995]: the concentration of a metabolite is a variable and its value is determined by the parameters. Response coefficients are defined with respect to parameters, not with respect to a variable.

To demonstrate that the above conflict is indeed apparent, and that regulation can be studied *in vivo*, we shall illustrate (i) that it is possible to define unambiguously the effect of one metabolite on another, (ii) that this dependence can, at least in principle, be measured *in vivo*, and (iii) that this dependence provides information on the dynamics of the physiological system.

Theory

In any metabolic system, the time-dependence of the metabolite concentrations $(X_1, \ldots, X_n)$ depends on those concentrations and on a number of parameters $(P_1, \ldots, P_m)$:

$$\begin{aligned} dX_1/dt &= f_1(X_1,\ldots,X_n,P_1,\ldots,P_m) \\ dX_2/dt &= f_2(X_1,\ldots,X_n,P_1,\ldots,P_m) \\ &\vdots \\ dX_n/dt &= f_n(X_1,\ldots,X_n,P_1,\ldots,P_m) \end{aligned} \tag{14.1}$$

To define the regulatory effect of a metabolite (X_i) on the rest of the system one may consider a reduced system obtained by considering all equations 14.1 except that for dX_i/dt. In the reduced system X_i is considered to be a parameter and the steady-state concentrations of the other metabolites (X_j) can be expressed as a function of X_i, i.e., $X_j(X_i)$. Experimentally this might be achieved by clamping the concentration of X_i by adding a large reservoir of the substance at that concentration, such as by permeabilizing the cell membrane in a medium with X_i. We propose that the effect of X_i on the system is defined by the response of the reduced system to modulation of X_i, i.e., by the response coefficients [Hofmeyr, 1995]:

$$R_{Xi}^{Xj} = \frac{d\ln X_j}{d\ln X_i} \tag{14.2}$$

Experimentally it may not always be possible to create a reduced system. It may however be possible to vary a parameter (P_k) and to observe the steady-state concentration $X_i(P_k)$ and $X_j(P_k)$. The co-response coefficient is defined as [Hofmeyr, 1995]:

$$\Omega_{P_k}^{Xj:Xi} = \frac{d\ln X_j}{dP_k} \Big/ \frac{d\ln X_i}{dP_k} \tag{14.3}$$

In general, this co-response coefficient depends on the parameter that is varied, but a special case arises when P_k affects only f_i and does not occur in the other equations in the equation set 14.1. In that case $X_j(P_k)$ is a steady-state of the reduced system at $X_i = X_i(P_k)$ so that $X_j(P_k) = X_j(X_i(P_k))$. In that case the co-response coefficient of eq. 14.3 is equal to the response coefficient of eq.14.2.

We conclude that the regulatory effect of a metabolite in a metabolic network can be defined unambiguously by considering a reduced system, and that it can be measured, at least in principle, by obtaining co-response coefficients *in vivo*. We shall use numerical simulations to illustrate our results and to suggest applications of our definition.

Numerical example

As a basic example of a physiological system we shall consider an unbranched pathway with three intermediates, as illustrated in Fig. 14.1 All reactions are de-

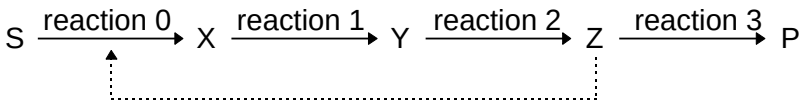

Fig. 14.1 Example of an unbranched pathway of four reactions that convert substrate S to product P through intermediates X, Y and Z. The reaction that produces X is elastic to Z.

scribed by irreversible Michaelis-Menten kinetics with product inhibition. For simplicity the maximal rates of the four reactions are taken equal, as are the eight Michaelis constants. Feedback is included by scaling the rate of reaction 0 with Z^{α}. The elasticity $\alpha = \varepsilon_Z^0$ quantifies the strength of the feedback. Numerical simulations are carried out with the program SCAMP [Sauro, 1993]. The concentrations of S and P are constant and the concentrations of X, Y and Z are determined from the rate equations.

Assay: 'Control' by the concentration of Z

In Figs. 14.2A–C the steady-state values of X and Z are plotted as a function of different parameters. In each case the value of only one parameter is varied. The result is different for each scan, because different parameters and different reactions are perturbed in each case. However, when X is plotted against Z in Fig. 14.2D the first two scans produce identical curves. As was established in the theory section, this is exactly the curve that would have been obtained if Z had been taken as a parameter and scanned, and the steady-state value of X had been plotted, because the parameters that were perturbed (i.e., V_{max} and K_M of reaction 3) directly affected only the time-dependence of Z. The slope of the curve reflects what we here define as the response coefficient that determines the effect of Z on X (cf. eq. 14.2). The positive slope of the curve indicates that the positive feedback by Z is stronger than the negative effect of product inhibition through the chain of pathway reactions.

The curve, in Fig. 14.2D, that is produced with the third scan does not overlay with the other two and erroneously suggests a negative feedback. This curve does not reflect the effect of Z on X, because here the V_{max} of reaction 2 is the modulated parameter and this parameter directly stimulates the removal of Y as well as the production of Z. Our numerical results confirm that one can, in principle, measure, hence define the effect of one metabolite on another, provided that one perturbs the differential equation of the former only:

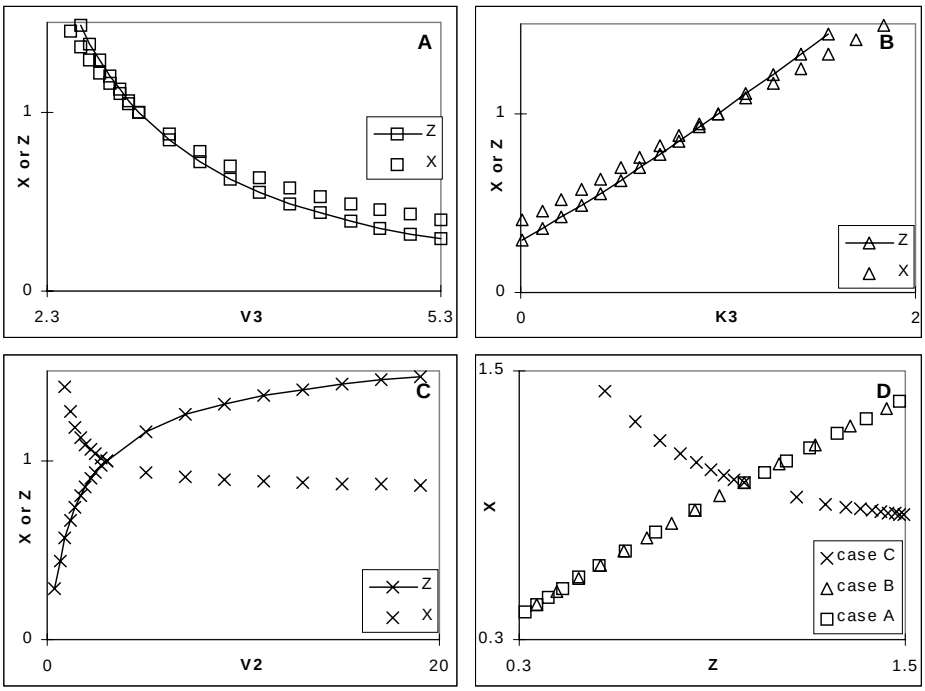

Fig. 14.2 Steady-state values of Z (joined markers) and X (unjoined markers) as a function of the maximal rate (panel A) and Michaelis constant (panel B) of reaction 3 and the maximal rate of reaction 2 (panel C). In panel D, X is plotted against Z for all three cases. The maximal rates and Michaelis constants of all other reactions and the concentrations of S and P were set to unity. Feedback parameter: $\alpha = 0.5$.

Application: Detecting a bifurcation

In Figs. 14.2A and B, the dependence of X on Z was determined by perturbing reaction 3. By perturbing reaction 0, the dependence of Z on X can also be determined. The two curves, shown together in Fig. 14.3A, are reminiscent of so called null-clines in a two-variable system [Segel, 1980]. In particular the intersection of the two curves forms the steady-state of the unperturbed system, and when the stable steady-state disappears through a saddle-node bifurcation (i.e., when an eigenvalue of the Jacobian of the system becomes positive), the curves become tangent to each other.

To illustrate the above point, we increased the feedback in Fig. 14.3B-D. Indeed, the angle decreased until the curves became virtually tangent to one another in Fig. 14.3D. It can be shown that the system does indeed approach a saddle-node bifurcation in Fig. 14.3D. The system can not be followed beyond the bifurcation. In fact, the curves could not be scanned completely in Fig. 14.3C and D, because the stability of the steady states was lost. Nevertheless, our results indicate that an approaching bifurcation may be detected by measuring the mutual dependence of two variables.

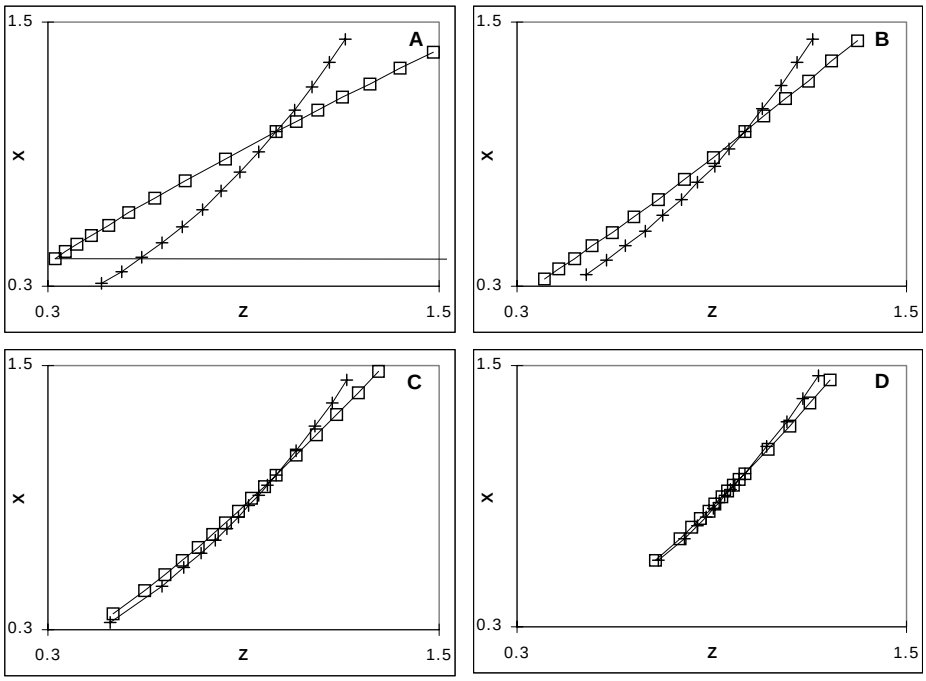

Fig. 14.3 Steady-state value of X as function of Z (squares) and steady-state value of Z as a function to X (plus) with increasing positive feedback. Feedback parameter: $\alpha = 0.5$ (A), 0.8 (B), 1.0 (C) or 1.1 (D). Other parameters as in Fig. 14.2.

Discussion

In this paper, we have studied the possibility to measure metabolic regulation *in vivo*. We illustrated that it is both possible and relevant to determine the extent by which a metabolite regulates other metabolic phenomena. First, we illustrated that the effect of one metabolite on the steady-state concentration of another can be defined unambiguously, and that this can be measured by perturbing a reaction. The result is independent of the type of perturbation that is used, provided that the kinetics of only one variable are perturbed. In practice, this condition is not so restrictive. For example one may add a dummy reaction that removes or inactivates a metabolite, for as long as the products of this dummy reaction do not affect any other process. The approach can also be applied to metabolites that are cycled between two forms, such as NAD(H) or signal transduction proteins. Due to moiety conservation the two forms are described by a single variable and a parameter that perturbs their interconversion. This satisfies the above condition.

Secondly, we illustrated that the aforementioned effects do indeed provide information on the dynamics of the physiological system. In particular, the curves that define the mutual dependence of two variables become tangent when the system approaches a saddle-node bifurcation. In addition they provide insight into the homeostatic regulation of the system [cf. Hofmeyr, 1995].

In an earlier study Sauro proposed to partition the control of a flux into regulatory effects of different metabolites [Sauro, 1990]. Our results differ in that they describe the total of direct (e.g., feedback) and indirect (e.g., product inhibition in a chain) regulatory effects of an internal variable, rather than to differentiate between them and set the total to an arbitrary 100%. Our results also demonstrate that although one can only study a physiological system in a mechanistic sense by entering it, and that although this changes the properties of the physiological system, it is nevertheless possible to obtain properties of the unperturbed system in this way. In this sense it shows the way into the living cell.

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