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Modulation of Metabolite Concentrations with no Net Effect on Fluxes

María Luz Cárdenas (cardenas@ibsm.cnrs-mrs.fr)[1], Fernando Ortega (fernando@bst.bq.ub.es)[2], Marta Cascante (marta@sun.bq.ub.es)[2] and Athel Cornish-Bowden (athel@ibsm.cnrs-mrs.fr)[1]

[1]*IBSM – BIP – CNRS, 31 chemin Joseph-Aiguier, B.P. 71, 13402 Marseille Cedex 20, France* and [2]*Departament de Bioquímica i Biologia Molecular, Universitat de Barcelona, Martí i Franqués 1, 08028 Barcelona, Catalunya, Spain*

See important note at the end.

Abstract

The concentration of a metabolite in a metabolic system can be varied without affecting any other concentrations or any fluxes by varying the concentrations of two inhibitors, one a competitive inhibitor of the enzyme that produces the metabolite, the other a competitive inhibitor of the enzyme that consumes it. The two concentrations need to be varied in opposite directions in such a way that the they add up to 100% when each is expressed as a percentage of the concentration that gives the desired flux in the absence of the other. The general approach can be extended to systems in which the inhibited enzymes do not catalyse consecutive reactions.

Key words

Competing inhibitors, competition plot, double modulation, inhibitors, metabolite concentration, multiple modulation

Introduction

The double-modulation method proposed by Kacser and Burns (1979) represented a major step forward in the analysis of metabolic systems, as it offered the hope of analysing pathway segments without knowledge of the intermediate elasticities. It led to a number of related methods for control analysis such as the multiple-modulation method (Giersch, 1994, 1995) and co-response analysis (Hofmeyr, Rohwer and Cornish-Bowden, 1993; Cornish-Bowden and Hofmeyr, 1994; Hofmeyr and Cornish-Bowden, 1996); the double-modulation method itself has been expressed in a matrix formulation applicable to metabolic systems of any structure of size (Acerenza and Cornish-Bowden, 1997). In all of these methods there is no implication that the fluxes or concentrations in the system are constrained in any way: in principle any flux or concentration can change when the enzyme activities are modulated. Here we describe an approach that allows the effects of a double modulation to be restricted to the metabolite concentrations in a specified segment of a system, with no effects on fluxes or on metabolite concentrations elsewhere in the system.

In a naive extension of the competition plot for inhibitors [Appendix] to a multi-enzyme system, one might guess that two competitive inhibitors of different enzymes acting in series in the system would behave like two inhibitors that bind to different sites on the same enzyme in the single-enzyme case: so inhibiting two different enzymes with a mixture of inhibitors in proportions calculated as before from the reference concentrations of pure inhibitors that give the same flux might result in a lower flux than that given by either inhibitor alone. This guess proves to be quite wrong, as we shall show in this

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article, and in fact the flux with two inhibitors mixed in the proportions defined above does not vary with the composition of the mixture. Although this means that a method of this kind cannot be used, as we hoped initially, to recognize whether two metabolic inhibitors act on different enzymes in a pathway, it can be used as a way of modulating a small number of metabolite concentrations without affecting other metabolite concentrations or any fluxes.

Theory

The simplest possible model for inhibitors acting on different enzymes in a pathway is illustrated in Fig. 1. It consists of a two-step transformation of a starting material X_0 into an end product X_2 via a single intermediate S_1, at concentrations x_0, x_2 and s_1 respectively, catalysed by two enzymes E_1, inhibited by a competitive inhibitor I_1 with inhibition constant K_{i1}, and E_2, inhibited by another competitive inhibitor I_2 with inhibition constant K_{i2}. The concentration of E_1 is expressed as e_1, the forward and reverse specificity constants are k_1 and k_{-1} respectively, and forward and reverse Michaelis constants are K_1 and K_{-1} respectively; the parameters for E_2 are expressed in the same way with subscript 1 replaced by 2 throughout.

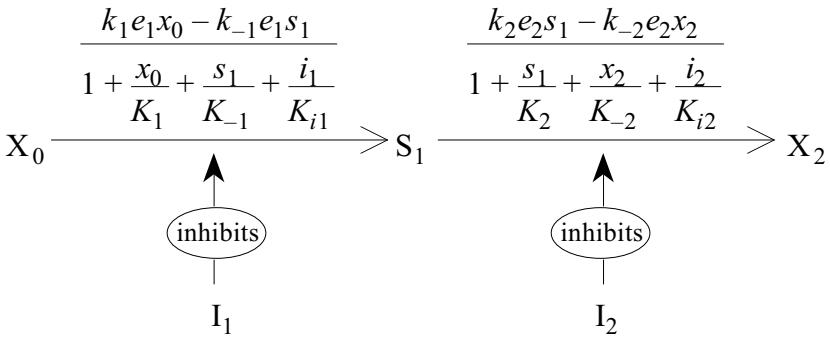

Fig. 1. System of two reactions inhibited by two different inhibitors.

As both enzymes obey reversible Michaelis–Menten kinetics with competitive inhibition the rates are given by the equations shown above the reactions in the Figure. These can be rearranged to provide expressions for the intermediate concentration that arises at different rates and inhibitor concentrations:

$$s_1 = \frac{k_1e_1x_0 - v_1\left(1 + \frac{x_0}{K_1} + \frac{i_1}{K_{i1}}\right)}{\frac{v_1}{K_{-1}} + k_{-1}e_1} \qquad s_1 = \frac{v_2\left(1 + \frac{x_2}{K_{-2}} + \frac{i_2}{K_{i2}}\right) + k_{-2}e_2x_2}{k_2e_2 - \frac{v_2}{K_2}}$$

At a constant value of v_1 the left-hand equation expresses s_1 as a linear function of i_1, and as S_1 is the product of the reaction s_1 decreases as i_1 increases. Similarly, the right-hand equation expresses s_1 as a linear function of i_2 at a constant value of v_2, but as S_1 is now the substrate of the reaction s_1 now increases as i_2 increases (Fig. 2). Suppose now that $s_1 = s_{11}$ when $v_1 = J$, where J is a constant, and $i_1 = 0$, and that $s_1 = s_{12}$ when $v_1 = J$ and $i_1 = i_{10}$ (the same constant J in both cases). Considering the same rate for E_2, and the same pair of intermediate concentrations, we further suppose that $s_1 = s_{12}$ at $i_2 = 0$, and that $s_1 = s_{11}$ at $i_2 = i_{20}$, in both cases when $v_2 = J$. As both dependences on inhibitor concentration are linear at constant rate, the behaviour at intermediate inhibitor concentrations must be as illustrated in Fig. 2.

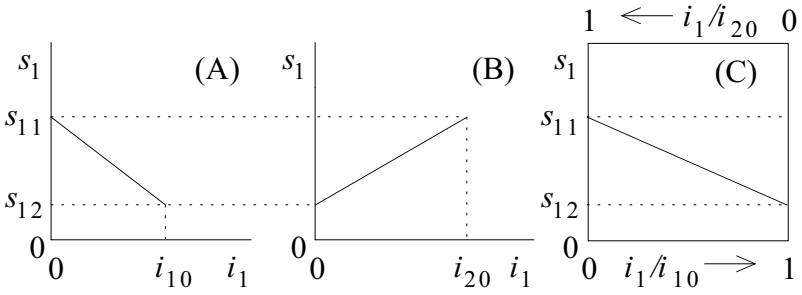

Fig. 2. Coordinate variation of (A) product and inhibitor concentration for E_1 and (B) substrate and inhibitor concentration for E_2 such that the rate remains unchanged. (C) The right-hand graph is the result of combining the other two into a single one.

To this point we have considered the two enzymes independently, ignoring the fact that they belong to the same pathway. In the steady state, however, the two rates v_1 and v_2 must be equal to J, the net flux from X_0 to X_2, and the common intermediate S_1 must have the same concentration for both enzymes. For a system in steady state, therefore, the two graphs in Fig. 2A–B must be interpreted as two aspects of a single graph, so that variation of s_1 at constant J implies varying the two inhibitor concentrations in a coordinated fashion. As the abscissa scales in the two graphs are unrelated to one another we must first express the two inhibitor concentrations on a common scale, which can most easily be done by making them dimensionless, dividing each by the concentration that gives the reference rate J in the absence of the other inhibitor, i.e. replacing i_1 by i_1/i_{10} and i_2 by i_2/i_{20}. The resulting combined graph is illustrated in Fig. 2C.

It follows from this analysis that if the two concentrations are varied simultaneously such that $i_1 = pi_{10}$ and $i_2 = (1 - p)i_{20}$ then the flux through the system of two enzymes will remain constant. The concentration s_1 of intermediate does not remain constant, however. Note that the analysis depends on the fact that in each rate equation there is a linear relationship between s_1 and i_1 or i_2, a consequence of assuming that the inhibition is competitive in both cases. If either inhibition has any uncompetitive character the flux will not remain constant when the two inhibitor concentrations are varied.

We discuss the extension of this method to other cases and its relationship to the connectivity theorem in the Appendix.

References

Acerenza, L. and Cornish-Bowden, A., Biochem. J., 327 (1997), 217.
Chevillard, C., Cárdenas, M. L. and Cornish-Bowden, A., Biochem. J., 289 (1993), 599.
Cárdenas, M. L., Methods, 24 (2001), 175.
Cornish-Bowden, A. and Hofmeyr, J.-H. S., Biochem. J., 298 (1994), 367.
Giersch, C., J. Theor. Biol., 169 (1994), 88.
Giersch, C., Eur. J. Biochem., 227 (1995), 194.
Hofmeyr, J.-H. S. and Cornish-Bowden, A., J. Theor. Biol., 182. (1996), 371.
Hofmeyr, J.-H. S., Rohwer, J. and Cornish-Bowden, A., Eur. J. Biochem., 212 (1993), 833.
Kacser, H. and Burns, J. A., Symp. Soc. Exp. Biol., 27 (1973), 65.
Kacser, H. and Burns, J. A., Biochem. Soc. Trans., 7 (1979), 1149.

Appendix

Background information on the competition plot. The starting point for this method was a failed attempt to generalize to multienzyme systems a result for the kinetic behaviour of individual enzymes. The competition plot (Chevillard, Cárdenas and Cornish-Bowden, 1993; Cárdenas, 2001) is a method for determining whether alternative substrates for the same enzyme react at the same site on the enzyme. It depends on finding reference concentrations of two substrates that each give the same rate in the absence of the other. If the two substrates are mixed in proportion to these reference concentrations in such a way that the sum of their concentrations is 100% of the reference value, then the behaviour depends on whether or not the substrates compete for the same site on the enzyme. If they do, then the rate for all mixtures prepared as described is the same as the rate for pure substrates. It is easy to show that the same behaviour applies to two different inhibitors of the same enzyme, and indeed the analysis is simpler than for substrates because some of the complicating factors for substrates do not arise with inhibitors.

To illustrate this idea, suppose that one has determined that a concentration of 1 mM of an inhibitor produces exactly the same measured rate as a concentration of 3 mM of another inhibitor (for all other conditions unchanged), then if they both compete for the same site on the enzyme the rate will be exactly the same for a mixture of the two inhibitors at concentrations 0.5 mM of the first and 1.5 mM of the other (i.e. 50% of the reference concentration in each case), or will be smaller if it is possible for both inhibitors to be bound simultaneously.

Extension to other cases. Essentially the same analysis applies to the case where the two enzymes of Fig. 1 do not constitute an entire system but are embedded as two consecutive enzymes in a larger system. Even though X_0 and X_2 are now S_0 and S_2, i.e. intermediates rather than reservoirs, with concentrations that can in principle vary, they do not in fact vary when the combined modulation is made by varying *p,* because any variation in their concentrations would inevitably be communicated to the rest of the system, because they would cause the flux or fluxes to vary. Thus modulation with inhibitors (or activators) of the activities of two consecutive enzymes in a system provides a way of varying the concentration of the common intermediate without producing any other changes in the system.

Although we have only considered here the case of two consecutive enzymes with a single intermediate metabolite, the approach can also be extended to the more general case of two enzymes that catalyse reactions that are in series but are not consecutive: in this case the concentrations of several metabolites (those located between the two inhibited enzymes) will vary. An extension of the method would also allow the concentrations of branch-point metabolites to be modulated, but this is more complicated, and for the present we have not examined this possibility in detail.

Relationship to the connectivity property. There is a close relationship between the method we discuss in this paper and the original presentation of the connectivity theorem by Kacser and Burns (1973). In their thought experiment to explain it they considered modulations of enzyme activities that would change nothing except the concentration of a metabolite. Our analysis thus provides a very simple way of achieving such modulations in practice. The connectivity equation contains elasticities and flux control coefficients for as many enzymes as have non-zero elasticities for the modulated metabolite, i.e. for more than just the two for which it is product or substrate if it interacts with any others. Our method is likewise restricted to cases where the modulated metabolite does not inhibit or activate another enzyme in the system.

NOTE

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