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Control of Dynamics and Steady State: Applications to Multidrug Resistance

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1. Introduction

The Control Theory of steady states in "ideal" cells is largely complete. We shall discuss some recent advances in the development of control theory for a more realistic cellular physiology, which includes channelling of metabolites, group relay and signal transduction pathways, as well as hierarchical control systems involving regulated gene expression. With respect to dynamic systems we shall introduce some definitions relevant for the characterization of the control of dynamic systems such as glycolytic oscillations in yeast. These include a control coefficient quantifying the control of the distance from the Hopf bifurcation point, as well as the control exerted by parameters on the amplitudes of the various Fourier components of the sustained oscillations we observe experimentally.

We also apply control analysis to the control of the drug resistance in cells with amplified P-glycoprotein.

2. Group transfer

In ideal metabolic pathways, there is no direct interaction between enzymes and enzymes do not significantly reduce the total concentration of coenzymes. In realistic pathways of metabolism, gene expression and signal transduction direct, enzyme-enzyme interactions may occur and in fact be important. An example is any relay (or group-transfer) pathway. Here a phosphoryl group is transferred sequentially between a set of enzymes. Enzyme-enzyme interactions are essential for the transfer of the phosphoryl group.

In ideal metabolic pathways, the sum of the control exerted by all the enzymes on any flux is 100%. In nonideal pathways, the enzymes have extra control because they participate in more than one process (Westerhoff and Kell, 1988). Recently, exact expressions for the total control have been derived both for group-transfer pathways (Van Dam *et al.*, 1993) and for metabolite channelling (Kholodenko and Westerhoff, 1993, 1994).

3. Sustained metabolic oscillations

Control analysis of time dependent systems is a more recent development. We focus on the control of metabolite systems that exhibit transient or sustained oscillations. Intact yeast cells is a long-known experimental system exhibiting transient oscillations of glycolysis under some conditions. Only recently, we have been able to define the conditions that lead to sustained oscillations (Richard *et al.*, 1993, 1994).

Standard metabolic control analysis focuses on the control of steady-state fluxes and concentrations by system parameters such as enzyme activities (Kacser and Burns, 1973; Heinrich and Rapoport, 1974; Fell, 1992). Of which properties should one study the control, in the case of oscillations? The properties that have come to mind include the frequency and amplitude of the oscillations. One may, however, also ask what determines whether the system exhibits steady state, transient oscillations or sustained oscillations.

For systems close to the Hopf bifurcation, and probably for other systems as well, it may be useful to inspect how the eigenvalues of the Jacobian at the fixed point, are controlled by the activities of the enzymes in the system. For a two variable system the control of the frequency of the oscillations becomes equal to the control exerted on the imaginary part of the eigenvalues:

$$C_{e_i}^{\omega} = C_{e_i}^{Im\lambda} = \frac{d \ln(Im\lambda)}{d \ln e_i} \quad (1)$$

The control of the real part of these eigenvalues describes the extent to which an enzyme determines the distance of the fixed point from the Hopf bifurcation, and has been defined by:

$$C_{e_i}^{Re\lambda} = \frac{d \ln(Re\lambda)}{d \ln e_i} \quad (2)$$

At the Hopf bifurcation point itself, this control coefficient becomes infinite.

4. Multidrug resistance

These more elaborate aspects of metabolic control theory, but also its standard aspects, may be applied to various experimental systems of interest. One of the latter is the multidrug resistance that often develops in human tumors treated with anticancer drugs (Gottesman, 1993). Here the concentration of a plasma membrane protein, such as P-glycoprotein, may increase. The P-glycoprotein extrudes many hydrophobic molecules rather indiscriminately from the cells. Some inhibitors of the P-glycoprotein exist.

The intracellular drug concentration (X in Fig. 1) is a function of the plasma membrane permeability for that drug, as well as of the activity of the pump. One may ask what is the limiting step for drug toxicity.

Metabolic Control Analysis of various systems has led to the recognition that asking for "the limiting step" may not always be the right thing to do; control may be distributed over various steps (Groen *et al.*, 1982). For P-glycoprotein mediated multidrug resistance

the following quantitative model has been shown to be in accordance with experimental data (Spoelstra *et al.*, 1992, 1994) (we here simplify the model somewhat, see Fig. 1):

$$v_p = \frac{V \cdot \left(\frac{X}{K_X}\right)^h}{1 + \left(\frac{X}{K_X}\right)^h} \quad (3)$$

$$v_l = k \cdot (X - D) \quad (4)$$

Here v_p and v_l are the pump and leak rates respectively. D and X are the extracellular and intracellular drug concentrations, respectively. h is the Hill coefficient measuring the positive cooperativity between substrate molecules (Spoelstra *et al.*, 1992, 1994; Guiral *et al.*, 1994). k is proportional to the passive membrane permeability for the drug. V is the maximum rate of the pump. The complete model (Spoelstra *et al.*, 1994) describes the pump as reversible, but in practice the reverse rates seem negligible.

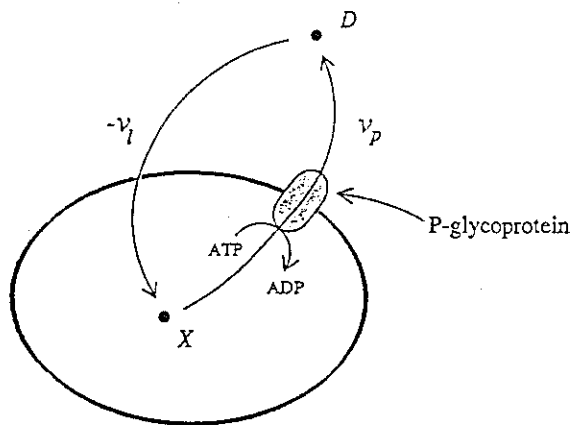


Figure 1 Scheme of passive and active drug fluxes in a multidrug resistant cell.

Accordingly, Eq. 3 assumes Michaelis-Menten kinetics for an irreversible, and even product uninhibited pump reaction. Eq. 4 assumes a parallel passive membrane permeation for the drug. We shall make a simplification that is realistic for most drugs (Spoelstra *et al.*, 1992): the intracellular drug concentration is far below its Michaelis constant (i.e., $X \ll K_X$). Pending the steady state requirement that leak rate plus pump rate must equal zero, D then depends on X by:

$$D = X + \frac{V}{k} \cdot \left(\frac{X}{K_X} \right)^h \quad (5)$$

In our models the toxicity of the drug is determined by its intracellular concentration. One may now compare the extracellular drug doses that are equal ("iso-") toxic for two cell lines by requiring that their intracellular drug concentrations be equal (Westerhoff *et al.*, in preparation). The question what limits drug toxicity then translates into: What controls the extracellular isotoxic drug concentration? The control coefficient for P-glycoprotein activity with respect to the isotoxic drug dose is defined by:

$$C_{P-gp}^d = \frac{d \ln D}{d \ln [P-gp]} \quad (6)$$

where [P-gp] represents the concentration of P-glycoprotein. Since V is proportional to [P-gp] one finds by differentiation of Eq. 5:

$$\frac{C_{P-gp}^D}{C_{P-gp}^D} = \frac{1}{\left(\frac{d \ln D}{d \ln V} \right)_X} = 1 + \frac{K_X \cdot k}{V} \cdot \left(\frac{K_X}{X} \right)^{h-1} \quad (7)$$

In line with the summation theorem for concentration control coefficients (Heinrich and Rapoport, 1974), the control by the passive drug permeability is negative and precisely the opposite of the control by the drug efflux pump activity.

For the control by the passive permeability, k, one finds:

$$\frac{1}{C_k^d} = - \left(1 + \frac{K_X \cdot k}{V} \cdot \left(\frac{K_X}{X} \right)^{h-1} \right) \quad (8)$$

Using data for 2780^{AD} cells from Spoelstra *et al.*, (1994) and Jongsma *et al.*, in preparation ($V = 10\text{-}15 \text{ mol min}^{-1}\text{cell}^{-1}$; $K_X = 1 \text{ } \mu\text{M}$; $h = 1.5$; $X = 3 \text{ nM}$; $k = 10^{-11} \text{ min}^{-1}\text{cell}^{-1}$), the above equation predicts that the coefficient for the control by the pump activity of the isotoxic drug dose should amount to 0.82. The passive permeability should control the isotoxic drug concentration for minus 85%. If the Hill coefficient amounts to 2, then the control by pump and leak are estimated at +23 and -23% respectively. These estimations suggest that there is no single limiting step for the toxic drug dose. Even in this simple model, control is necessarily distributed among pump and leak. Most likely, the control of either is significant.

In this paper we have discussed extensions to the theory of metabolic control. Importantly, these extensions allow metabolic control analysis to proceed beyond the limits of ideal, text book metabolic chemistry, into the realms of signal transduction and metabolic dynamics. These limits removed, much of the interest may shift to experimental applications. The application to multidrug resistance of tumor cells, initiated here, may ultimately help in assessing optimum strategies of its management.

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