Minireview

Regulating the cellular economy of supply and demand

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Abstract Cellular metabolism is a molecular economy that is functionally organised into supply and demand blocks linked by metabolic products and cofactor cycles. Supply–demand analysis allows the behaviour, control and regulation of metabolism as a whole to be understood quantitatively in terms of the elasticities of supply and demand, which are experimentally measurable properties of the individual blocks. The kinetic and thermodynamic aspects of regulation are clearly distinguished. One important result is the demonstration that when flux is controlled by one block, the other block determines to which degree the concentration of the linking metabolite is homeostatically maintained.

Key words: Supply–demand analysis; Metabolic regulation; Control analysis; Feedback inhibition

1. Introduction

How highly would one rate an economic analysis of a factory that ignored the consumer demand for its products? Ludicrous as it may sound, this is precisely what most metabolic studies of the past century have been doing. If this seems farfetched, consider for example that we have yet to find a textbook analysis of, say, biosynthetic flux to an amino acid that takes into account the rate of protein synthesis. This state of affairs is perhaps understandable: faced with the huge complexity of the cellular reaction network the only way to proceed was to chop it up into manageable parts and study the parts separately in terms of stoichiometric structure, enzymes and transporters. However, although all these parts are undoubtedly connected, the current view of metabolism and its regulation still seems to be that the parts behave the same whether in isolation or in a cellular context. A telling example is the continued insistence of modern biochemistry textbooks on the purported rate-limiting role of the kinases in glycolysis, despite clear evidence that over-expression of these (and other) glycolytic enzymes either on their own or in combination has no effect on the carbon flux in vivo from glucose to ethanol in yeast [1,2].

Here we outline a quantitative theory called *metabolic supply–demand analysis* that addresses this problem by allowing the integration of the different parts of metabolism with each other and with other intracellular processes. Within this framework the concepts of metabolic regulation and function acquire a clear and quantitative meaning. In addition, a number of concepts central to the classical view of metabolic regulation are shown to be fallacious.

2. Metabolic regulation, organisation and function

We consider metabolic regulation to be inextricably linked to function: to say a system is regulated is to mean that its intrinsic properties have been moulded by evolution to fulfil specific functions [3,4]. Because mass–action is the intrinsic driving force for self-organisation of reaction networks, we broadly define metabolic regulation *as the alteration of reaction properties to augment or counteract the mass–action trend in a network of reactions* [4,5]. A corollary to this definition is that regulatory performance should always be measured in terms of a specified function. Reaction properties can be regulated by altering the concentrations and the catalytic and binding properties of enzymes; a host of such regulatory mechanisms have evolved [4,6]. Enzymes lift the metabolic network from the underlying network of thermodynamically feasible reactions onto a different timescale and therefore act as the primary 'handles' through which evolution can create function.

Central to any understanding of metabolic function is our knowledge of the organisation of the metabolic network. Its core consists of a catabolic block that provides phosphorylation and reducing power plus carbon skeletons, a biosynthetic block that makes building blocks for macromolecular synthesis, and a 'growth' block that makes and maintains the cellular structure and the gene and enzyme machinery. These blocks are coupled by either one common intermediate (e.g. an amino acid or nucleotide) or a pair of common intermediates that form a moiety-conserved cycle in which the sum of the cycle members remains constant (e.g. NAD(P)H–NAD(P), ATP–ADP or, in the presence of adenylate kinase, ATP–ADP–AMP [7]). To remind ourselves that the living process is intrinsically a molecular economy (cf. [8]) we call the producing block in these linkages the *supply* and the consuming block the *demand* (Fig. 1). Although in this article we restrict the discussion to the simplest case (Fig. 1), the same general approach applies with almost no differences to supply–demand systems that involve moiety-conserved cycles [2].

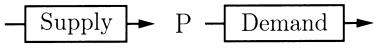

Fig. 1. A metabolic supply–demand system.

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The metabolic network is an open system that can exist in either a transient or a steady state. Although equilibrium is excluded as a possible state for living systems, it is an important reference state: the distance ρ of any reaction or reaction block from equilibrium, defined as Γ/K_{eq}, where Γ is the mass–action ratio and K_{eq} is the equilibrium constant, is an important factor in determining the behaviour of any reaction network.

Metabolic function is a multi-level concept. At the lowest level the function of an enzyme is to catalyse a reaction. At the level of the integrated system of coupled enzyme-catalysed reactions its function may be to control a steady state metabolite concentration. Enzymes are regulated to perform these higher level systemic functions, namely: (i) the determination of the steady state itself, (ii) control over the steady state fluxes and intermediate concentrations, (iii) the steady state response to a perturbation in some system parameter or a fluctuation in some intermediate concentration (structural and dynamic stability), (iv) the time of transition from one steady state to another [9] and (v) the dynamic form of the transient or steady states (e.g. point, monotonic, oscillatory, trigger, chaotic [10,11]). Here we only consider the first three functions, although the others are also important in a complete supply–demand analysis.

3. Quantitative analysis of supply–demand systems

We now describe a theory that allows a visual and quantitative analysis of how the properties of the supply and demand blocks determine the behaviour and control of the steady state flux and concentration of P. As our main tools we use rate characteristics [4] and control analysis [12,13].

The graph of combined rate characteristics (Fig. 2) is a powerful tool for visualising how the steady state in a supply–demand system is formed and how the distribution of flux and concentration control depends on the properties of the supply and demand blocks. On the graph, the natural logarithms of the supply and demand rates are plotted as a function of the natural logarithm of the concentration variable that links them. If the supply and demand were catalysed by single enzymes these curves would represent, for example, the familiar Michaelis–Menten or Hill responses of a rate with respect to a product or a substrate. In general, however, the supply and demand are reaction blocks, so that the rate curves actually represent the variation in the local steady state fluxes of the isolated supply and demand blocks as they respond to variation in the concentration of P. The use of logarithmic rather than linear scales has a number of advantages [4], the most important being that it allows direct comparison of the magnitude of steady state responses to perturbations at different positions of the rate and concentration scale.

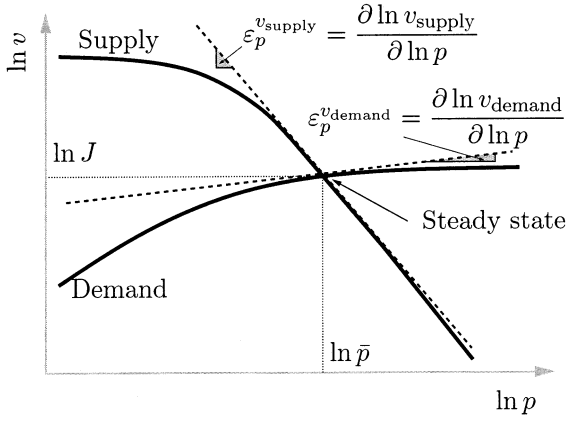

Fig. 2. The rate characteristics of a supply–demand system plotted in double logarithmic space.

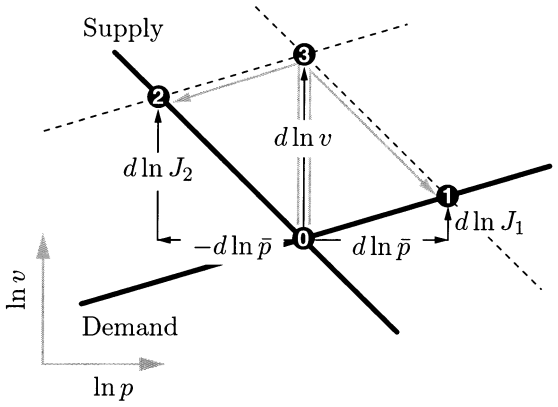

Fig. 3. How the steady state (0) responds to a small increase d ln v in the activities of either supply (leading to a new steady state at 1) or demand (leading to a new steady state at 2) or both supply and demand (leading to a new steady state at 3).

The intersection of the supply and demand rate characteristic represents the steady state, which is characterised by a flux, J, and concentration of P, $\bar{p}$. From the graph it should be clear that the response in the steady state to small perturbations in the activities of supply or demand depend completely on the *elasticity coefficients*, i.e. the slopes of the tangents to the double logarithmic rate characteristics at the steady state point.

Fig. 3 shows how flux and concentration control can be quantified [14]. Consider a small increase d ln v in the activity of the supply, caused by, say, an increase in the concentrations of the supply enzymes. The system moves from the original steady state 0 to a new steady state 1; flux increases by d ln J_1 and $\bar{p}$ by d ln $\bar{p}$. Similarly, if the demand activity is increased by d ln v the system moves from steady state 0 to 2 with a flux increase of d ln J_2 and a decrease in $\bar{p}$ of d ln $\bar{p}$. The degrees to which supply and demand control J and $\bar{p}$ are given by the *flux-control coefficients*:

$$C^J_{supply} = \frac{d\ln J_1}{d\ln v_{supply}},\quad C^J_{demand} = \frac{d\ln J_2}{d\ln v_{demand}} \tag{1}$$

and the *concentration-control coefficients*:

$$C^p_{supply} = \frac{d\ln p}{d\ln v_{supply}},\quad C^p_{demand} = \frac{-d\ln p}{d\ln v_{demand}} \tag{2}$$

If both supply and demand are both increased by d ln v the system moves to steady state 3 in which the flux has increased by d ln J_2+d ln J_2 = d ln v while $\bar{p}$ remains unchanged. Using the definition of control coefficients given in Eqs. 1 and 2 it follows that:

$$C^J_{supply} + C^J_{demand} = 1 \tag{3}$$

$$C^p_{supply} + C^p_{demand} = 0 \tag{4}$$

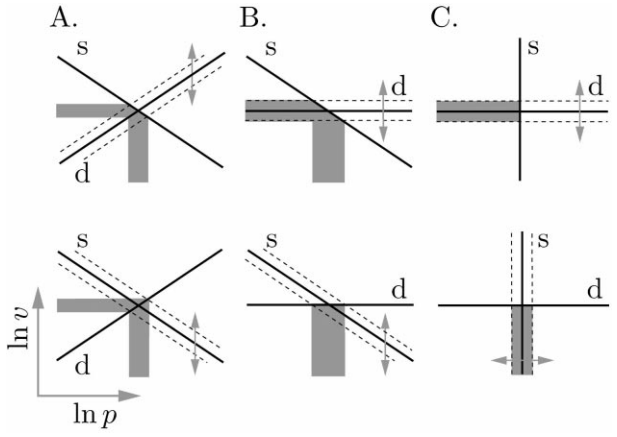

Fig. 4. The effect on the steady state of varying the demand (upper half of the figure) or supply (lower half of the figure). The slope of each line is an elasticity of either supply (s) or demand (d) at the steady state. The dotted lines show a set percentage increase or decrease in activity. The shaded regions show the magnitude of the response in the steady state flux (horizontal) and concentration of P (vertical).

These are specific cases of the so-called *summation theorems* of control analysis [12].

Furthermore, using the definitions of the elasticities of supply and demand given in Fig. 2 the *connectivity theorems* [12] can also be derived:

$$C^J_{\text{supply}}\,\varepsilon_p^{v_{\text{supply}}} + C^J_{\text{demand}}\,\varepsilon_p^{v_{\text{demand}}} = 0 \tag{5}$$

$$C^p_{\text{supply}}\,\varepsilon_p^{v_{\text{supply}}} + C^p_{\text{demand}}\,\varepsilon_p^{v_{\text{demand}}} = -1 \tag{6}$$

The summation and connectivity theorems provide enough information to express the control coefficients in terms of elasticities of supply and demand [3]. The flux-control coefficients are:

$$C^J_{\text{supply}} = \frac{\varepsilon_p^{v_{\text{demand}}}}{\varepsilon_p^{v_{\text{demand}}} - \varepsilon_p^{v_{\text{supply}}}} \tag{7}$$

and

$$C^J_{\text{demand}} = \frac{-\varepsilon_p^{v_{\text{supply}}}}{\varepsilon_p^{v_{\text{demand}}} - \varepsilon_p^{v_{\text{supply}}}} \tag{8}$$

and the concentration-control coefficients are:

$$C^p_{\text{supply}} = -C^p_{\text{demand}} = \frac{1}{\varepsilon_p^{v_{\text{demand}}} - \varepsilon_p^{v_{\text{supply}}}} \tag{9}$$

Note that $\varepsilon_p^{v_{\text{supply}}}$ is typically a negative quantity, i.e. product inhibits supply. The ratio of elasticities determines the distribution of flux-control between supply and demand (if $|\varepsilon_p^{v_{\text{supply}}}/\varepsilon_p^{v_{\text{demand}}}|>1$ the demand has more control over the flux than the supply; if $|\varepsilon_p^{v_{\text{supply}}}/\varepsilon_p^{v_{\text{demand}}}|<1$ the demand has less control over the flux than the supply). With regard to $\overline{p}$, it is not the distribution of $\overline{p}$-control that is of interest (C^p_{supply} always being equal to $-C^p_{\text{demand}}$ no matter what the values of the elasticities), but what determines the magnitude of the variation in $\overline{p}$ (and, therefore, its homeostatic maintenance): the larger $\varepsilon_p^{v_{\text{demand}}} - \varepsilon_p^{v_{\text{supply}}}$, the smaller the absolute values of both C^p_{supply} and C^p_{demand}. This algebraic analysis is clearly illustrated by the different configuration of rate characteristics around the steady state shown in Fig. 4.

Fig. 4A shows a situation where the elasticities of supply and demand are equal, so that the functions of flux and concentration control are equally distributed: the same percentage change in the activity of either supply or demand causes the same change in the flux ($C^J_{\text{supply}} = C^J_{\text{demand}} = 0.5$). The magnitude of the variation in $\overline{p}$ is determined to the same degree by supply and demand.

In Fig. 4B the elasticity of demand is decreased to zero (the demand becomes saturated with P): it is clear that the demand now has complete control over the flux, while the supply has none. However, the elasticity of supply now completely determines the magnitude of the variation in $\overline{p}$ ($C^p_{\text{supply}} = -C^p_{\text{demand}} = -1/\varepsilon_p^{v_{\text{supply}}}$). The steeper the slope of the supply characteristic, the narrower the band of variation in $\overline{p}$ and, therefore, the better the homeostatic maintenance of $\overline{p}$. The opposite would be obtained if the supply elasticity was zero whereas the demand elasticity remained finite: supply would completely control flux and the elasticity of demand would completely determine the magnitude of variation in $\overline{p}$.

In Fig. 4C not only is the elasticity of demand zero (as in Fig. 4B) but, in addition, that of supply is $-\infty$. The homeostatic maintenance of $\overline{p}$ in the face of changes in the maximal activity of either supply or demand is now perfect; the only way in which $\overline{p}$ can change is if the half-limiting concentration $p_{0.5}$ of the supply block changes, as in the bottom half of Fig. 4C.

Supply–demand analysis therefore shows that the functions of flux and concentration control are mutually exclusive in the sense that if one block controls the flux it loses any influence over the magnitude of variation in the linking product $\overline{p}$: this becomes the sole function of the other block. This finding has profound consequences for any view of metabolic regulation.

Up to now the analysis has been limited to the response of the steady state to small variations in the activity of supply or demand without considering either the form of the full rate characteristics or the position of the steady state in relation to equilibrium. We now expand the picture to obtain an overall

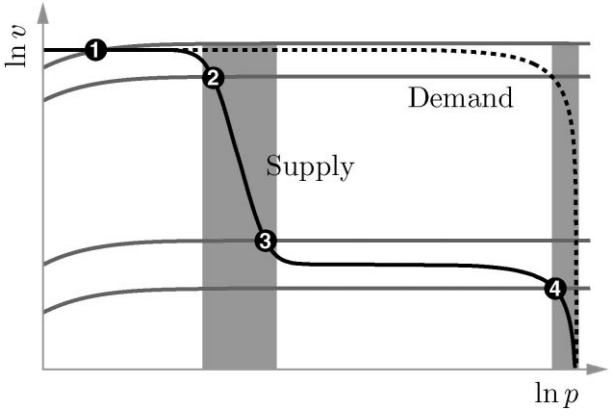

Fig. 5. The steady state behaviour of a supply–demand system with (solid) and without (dashed) inhibition of supply by its product P. The grey lines represent different demand activities. The four marked steady states are discussed in the text. The rate characteristics were generated with `Gepasi` [15] for the supply–demand system described in [3] using the reversible Hill [16] and reversible Michaelis–Menten rate equations with realistic parameter values.

view of the limits within which the system can fulfil its functions.

Like any factory, a supply pathway must be able to fulfil two primary functions: to meet increasing demand for its product at least up to some limit and to cope with low demand in such a way that its product and intermediate metabolite concentrations do not tend towards their equilibrium concentrations (most biosynthetic pathways have huge equilibrium constants so that near-equilibrium conditions would cause a fatally high accumulation of supply pathway intermediates and product [7]). Textbook wisdom has it that allosteric feedback inhibition of supply by its product is responsible for satisfying demand, while it has little to say about low demand. What can supply–demand analysis teach us?

Fig. 5 gives a bird's-eye view of a hypothetical set of supply–demand rate characteristics spanning the full range of p to its equilibrium value (assuming that the substrate for the supply pathway is buffered and therefore constant). For the supply to be able to meet a specific range of variation in demand activity it cannot have any flux control in that range. Focussing for the moment on the solid supply curve, it is clear that only in the shaded band between steady states 2 and 3 will the supply be able to meet the variation in demand while keeping $\overline{p}$ reasonably constant. When demand becomes higher that 2 it loses control over the flux (steady state 1) with a concomitant sharp decrease in $\overline{p}$. An increase in the maximal activity of the supply (the plateau at 1) would extend the range in which the supply can meet the demand. However, it is also clear that the presence of allosteric feedback inhibition is not a prerequisite for flux control by demand: in the shaded band on the right, demand also controls the flux in the absence of allosteric feedback (the dashed supply characteristic) and the supply is equally effective in keeping $\overline{p}$ homeostatic. The dramatic difference between the two situations is the concentration at which P is homeostatically maintained: without feedback inhibition it can only be near equilibrium (with all the accompanying disadvantages), whereas with feedback inhibition it can be maintained orders of magnitude away from equilibrium (at a concentration around the $p_{0.5}$ of the allosteric enzyme). Clearly, therefore, when demand controls flux the functional role of feedback inhibition is homeostatic maintenance of $\overline{p}$ at a concentration far from equilibrium.

In general, each elasticity coefficient is the sum of a thermodynamic term that depends only on Γ/K_{eq} and a kinetic term that is determined by the binding properties of the enzyme. The thermodynamic term in the supply elasticity approaches 0 at conditions far from equilibrium and $-\infty$ near equilibrium, where it completely swamps the kinetic term which typically varies between 0 and the Hill coefficient [4]. Kinetic effects such as allosteric feedback inhibition can therefore only play a regulatory role far from equilibrium where the thermodynamic term is negligible. This is also shown by the solid curve in Fig. 5: there is a lower limit (around 3) to the range in which $\overline{p}$ can be kinetically regulated; below this limit $\overline{p}$ jumps to the region where the thermodynamic term dominates the supply elasticity.

4. Discussion

The central regulatory problem of metabolism is to be able to satisfy a varying demand for its products from low to high values while maintaining these products within narrow concentration ranges far from equilibrium. Supply–demand analysis shows that these two functions are inextricably linked: the more control either block has over flux, the less it determines the degree of homeostasis and the distance from equilibrium where homeostasis is maintained, which becomes the function of the other block. A common solution to this design problem in living cells is that the flux is largely controlled by the demand block, whereas the supply block determines homeostasis of the linking metabolite. Direct experimental evidence for control by demand exists (see, for example, [1,17–20]), while it can be deduced for many systems on the basis of known kinetics (in general, for example, aminoacyl-tRNA transferases have K_m-values for their amino acid substrates at least an order of magnitude lower than the intracellular concentrations of amino acids, thereby ensuring that protein synthetic demand is saturated, giving a demand elasticity of zero [21]). By identifying the elasticities of supply and demand as the keys to a quantitative understanding of the integrated cellular process, supply–demand analysis provides a framework for further experimentation. A number of experimental strategies for measuring block elasticities are already available [22,23].

Supply–demand analysis also has major implications for biotechnology [2,24], biomedicine and drug design [25,26] because it shows that what were thought to be 'rate-limiting' steps catalysed by allosteric enzymes actually have nothing to do with flux control, but are responsible for the homeostasis of metabolites. It opens a new window on our understanding of metabolic design and regulation [27].

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