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Control analysis of systems with reaction blocks that 'cross-talk'

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Abstract

Practical application of metabolic control analysis has been facilitated by use of the top-down approach, which divides a metabolic system into a small number of reaction blocks, linked by a few key intermediates. Previous papers have stressed that communication between blocks should be only through the explicit intermediates, 'cross-talk' between reaction blocks invalidated the approach. Here we show how the restriction is a result of the use of inhibitors of the blocks, and can be overcome if other system modulations are used. We also show a way to treat the related problem of enzymes that appear in more than one block such as the analysis of glycolytic substrate cycles into ATP consuming and net flux activities.

Keywords: Top-down; Metabolic control analysis; Elasticity; Control coefficient

1. Introduction

Metabolic control analysis [1–3] can give a full system description of the control and regulation of a metabolic system. An analysis can be performed knowing only the stoichiometries and fluxes of the system reactions and their kinetic responses (in the form of elasticities) to the metabolites. The mathematical equations that relate the elasticities and fluxes to the control coefficients have been solved for systems of any complexity [4].

Experimentally, determination of the elasticity of each catalyst to each system intermediate can represent a considerable hurdle to the application of control analysis. One method of elasticity determination is the multiple modulation approach [1,5,6], which uses modulations (such as inhibitions) at various sites of the system in order to arrive at the elasticities. However, the number of variables that must be measured rises exponentially with the complexity of the system. Simplification is possible by considering blocks of reactions, rather than individual processes. This method of simplifying systems has been termed the top-down [7–9] or modular approach [10].

In previous analyses of systems by top-down control analysis there was a fundamental rule that needed to be satisfied for the method to be valid: no metabolic block may influence another except via an explicit intermediate [8,9]. Each intermediate that was measured had to be a unique function of the full set of linking intermediates between blocks. For example, if a metabolite internal to one block of reactions was an allosteric effector of a process in another block then the analysis was invalid if the experimental modulation used altered the unique relationship between the explicit and internal intermediates. Only if the grouping of the system was

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altered and the 'cross-talking' metabolite was measured could a study be performed [11].

In this paper we consider systems that are subdivided in a way that includes such 'cross-talking' metabolites. We show that if the system modulations used in the elasticity determinations do not alter the kinetics of the cross-talking blocks then the control pattern of the system can be solved. The related problem of systems divided into blocks that share common enzymes is analysed with particular reference to substrate cycling within glycolysis.

2. Background theory

Central to metabolic control analysis are elasticities and control coefficients [1–3]. The elasticity is defined as the fractional change in a process (typically the reaction catalysed by an enzyme), i, for a given fractional change in an effector, x:

$$\varepsilon_x^i = \frac{dv_i}{dx}\cdot\frac{x}{v_i} \quad (1)$$

where v_i is the activity of i, and all other effectors are held constant. The effector could be a substrate, product or allosteric activator or inhibitor of the process. The elasticity describes the local kinetics of the process with respect to effector level.

A process, i, can exert control over a system variable, a. The strength of control is given by the control coefficient, defined by:

$$C_i^a = \frac{da}{dv_i}\cdot\frac{v_i}{a}$$

Where a is a system flux the coefficient is termed a flux control coefficient; where a is a metabolite level the coefficient is termed a concentration control coefficient. As the control pattern of the system as a whole is dependent on the individual kinetics of its constituent reactions, then the control coefficients are related to the elasticities by rigorous mathematical relationships [4].

Top-down metabolic control analysis is not concerned with individual enzymes [7–9], but rather with the control and regulation of groups or blocks of reactions. The advantage of grouping reactions of a complex system into blocks is that the number of elasticities that must be experimentally determined is reduced. Only the block elasticities to a small number of linking metabolites need be measured.

The elasticity of a reaction block activity to a linking metabolite, x, is related to the elasticities and control pattern within the block according to the following equation [9,12]:

$$\varepsilon_x^{block} = \sum_{all\ i\ in\ block} C_i^{block}\cdot\varepsilon_x^i \quad (2)$$

where the control over a block by its component processes, i, is given by:

$$C_i^{block} = \frac{C_i^a}{C_{block}^a}$$

(where a is any variable, e.g. the flux through the block).

Previous use of top-down control analysis has been limited to systems in which blocks were only allowed to communicate via explicit linking intermediates [8,9,11]. Where a non-explicit intermediate contained within a second block of reactions has effects on the enzymes of a given block then Eq. 2 no longer holds because x will have extra, non-quantified, interactions with the block through the non-explicit intermediate. However, where such 'cross-talk' arises between blocks it is still possible to measure the elasticities of the blocks to the explicit intermediates, and thus arrive at the control pattern of the system.

3. Results

3.1. A simple system with feedback inhibition

Consider the system shown in Fig. 1a. This has two metabolites, x and y linked by three reactions catalysed by the enzymes *E1*, *E2* and *E3*. In addition there is an allosteric inhibition of *E1* by y. There are two possible ways that a top-down analysis could group this system, either around y (Fig. 1b) or around x (Fig. 1c). A conventional approach would state that the grouping around x is invalid due to 'cross-talk' by y between the metabolic blocks.

This view is correct only when inhibitions are used to modulate the system. The experimental method of determining the elasticities of the blocks to the intermediates often involves inhibiting one block (any-

where in the block) and measuring the response of the other blocks to the change in the levels of the linking intermediates [8,9].

If we consider the grouping around x (Fig. 1c) then the classic method of determining the elasticity to x of the block containing *E1* (block 1) would be an inhibition of the other group of reactions (i.e. inhibition of *E2* or *E3*). A problem arises because inhibition of *E2* would tend to lower y, whereas inhibition of *E3* would tend to raise y. The effect on the activity of block 1 would be different depending on the inhibitor of the other block that was used. In neither case would the calculated elasticity of block 1 reflect the kinetics of the reaction purely to a change in x. What is needed for the analysis to work is a means of altering x without altering the kinetics of either block. One way in which to do this is the introduction of a branch feeding in or out at x.

Eq. 2 holds when there is no cross-talk between blocks. However an additional relationship is needed to describe the derivation of the elasticity when 'cross-talk' is taking place between the blocks. If we concentrate on the case in Fig. 1c then when we group around x the appropriate elasticity of block 1 must take into account not only the elasticity of *E1* to x but also the elasticity of *E1* to y.

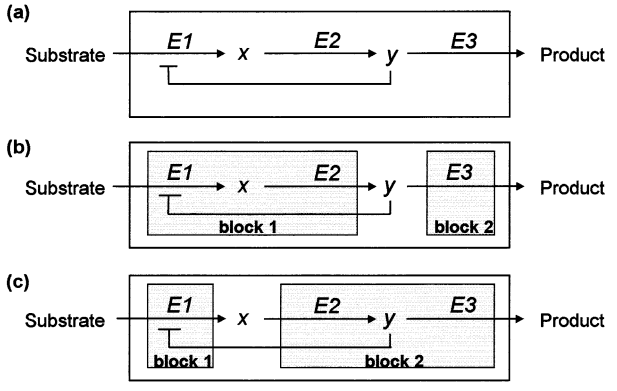

Fig. 1. Different ways of grouping a simple system. (a) The simple system containing feedback inhibition. (b) Top-down grouping around intermediate y. (c) Top-down grouping around intermediate x. In this simple three-reaction system there is a feedback inhibition on *E1* by y. The system may be grouped around either intermediate, as in (b) and (c). The grouping in (b) has a non-explicit intermediate within block 1 that does not interact with any reaction outside block 1; this grouping is allowed in conventional top-down analysis. The grouping shown in (c) contains cross-talk between an internal intermediate of one block (y) and another block; this grouping is forbidden in a conventional top-down analysis.

The fractional change in y for a fractional change in x due to the introduction of the branch is approximated by:

$$\frac{\delta y}{y} \approx \left(\frac{\frac{dy}{y}}{\frac{dx}{x}} \right) \cdot \frac{\delta x}{x} \tag{3}$$

The fractional change in the activity of block 1 upon modulation by the branch is given by:

$$\frac{\delta v_{block1}}{v_{block1}} \approx \left(\frac{\frac{dv_{E1}}{v_{E1}}}{\frac{dx}{x}} \right) \cdot \frac{\delta x}{x} + \left(\frac{\frac{dv_{E1}}{v_{E1}}}{\frac{dy}{y}} \right) \cdot \frac{\delta y}{y} \tag{4}$$

where $v_{block\ 1}$ and v_{E1} are the activities of block 1 and enzyme *E1*, respectively.

From the definition of elasticities, Eq. 2, combined with Eqs. 3 and 4 gives:

$$\varepsilon_x^{block1} = \varepsilon_x^{E1} + \varepsilon_y^{E1} \cdot \frac{\frac{dy}{y}}{\frac{dx}{x}} \tag{5}$$

From this point on we change our view of x. Instead of being a single intermediate it now becomes a representative of all the intermediates of the system that are a unique function of the concentration of x. This is a common ploy in top-down control analysis, for example when mitochondrial membrane potential is used as a representative for proton motive force [13,14).

Eq. 5 is a special case in that the block contains only one enzyme. In the general case each interaction on enzymes within a block must be scaled by the control that enzyme has over block activity:

$$\varepsilon_{x_e}^{block} = \sum_{all\ E\ in\ block} C_E^{block} \cdot \left(\varepsilon_{x_e}^{E} + \sum_{all\ x_c} \varepsilon_{x_c}^{E} \cdot \frac{\frac{dx_c}{x_c}}{\frac{dx_e}{x_e}} \right) \tag{6}$$

where x_e is an explicit intermediate and x_c are the 'cross-talking' intermediates to the block. Note that if their are no 'cross-talking' intermediates, then Eq. 6 reduces to Eq. 2.

For the grouping shown in Fig. 1c, when a branch

is used to modulate the level of x it will also result in a modulation of the level of y. The new level of y is a function of the level of x, defined by the kinetics of the enzymes *E2* and *E3*. The level of inhibition of *E1* by y will thus be a function of the level of x. The function is represented in Eq. 5 as the fractional change in y for a fractional change in x scaled for the elasticity of *E1* to y.

The measured strength of the response of a block to the explicit intermediate will take into account the regulatory loop acting via the cross-talking intermediate. Consequently, the elasticity is a true representation of the response of a block to a change in the level of the explicit intermediate and the other intermediates it uniquely represents, and as such can be used in the determination control coefficients. However, the measured elasticity will not have the same value as the conventional elasticity to x alone. (The situation is similar to using a singular function of the elements of a moiety conserved cycle as a theoretical link between two processes [14].)

Inhibition of a block results in altered kinetics of that block. As such the response of the internal intermediates of an inhibited block to the explicit intermediates will differ from the response in the reference state. Where an internal intermediate cross-talks to another block the inhibitor will alter the response of the cross-talking intermediate to the explicit intermediates, and will alter the strength of the cross-talk interaction so that y is no longer the same unique function of x as in the reference state. Consequently, inhibitors of blocks that contain cross-talking intermediates cannot be used to determine the elasticities of the target blocks. For example, in Fig. 1c inhibition of either enzyme *E2* or *E3* cannot be used to determine of the elasticities of block 1, since the inhibition alters the unique relationship between y and x. Hence, the fractional change in activity in block 1 divided by the fractional change in x does not give a value of for the elasticity that represents the kinetics of the system in the reference state.

3.2. Analysis of blocks which share common elements

A related problem concerns the division of a system so that a single enzyme pool appears in more than one block. Consider the system in Fig. 2. There are two pathways that produce a common product which is then consumed. A biological example of such a system is the oxidation of reducing equivalents from NADH-linked and FADH-linked substrates by the mitochondrial respiratory chain. *E1* and *E2* would be NADH and FADH reducing reactions, the intermediates x and y would be the NADH/NAD and FADH/FAD redox potentials. The electrons from both sources converge on the quinone pool (intermediate z), and then are used to reduce oxygen by complexes III and IV of the respiratory chain (*E5*).

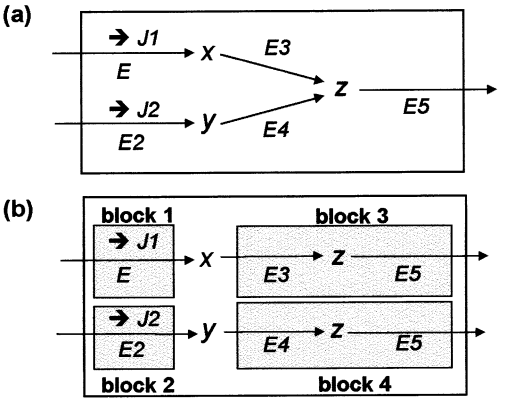

Fig. 2. Division of a system with a reaction shared between two blocks. (a) The simple system containing two branches converging on a single intermediate (z) followed by a common pathway. (b) Top-down grouping with z and *E5* appearing in both block 3 and block 4.

Fig. 2a shows a pathway that has an intermediate, z, formed by two independent pathways, and consumed by a single reaction. This pathway is divided into four blocks in Fig. 2b with z and *E5* internal to both block 3 and block 4. The flux through *E1* is termed *J1* and the flux through *E2* is termed *J2*.

The reaction catalysed by *E5* can be divided into the part that catalyses the removal of z formed via x and the remainder that catalyses the removal of z formed via y. As z appears in both block 3 and block 4 and has not been made an explicit intermediate there will be cross-talk between the two pools of *E5*.

The kinetics of block 3 and block 4 are linked by the level of z and by the activity of *E5*. If an inhibitor of *E3* were added, then this would change the level of z, and consequently alter the kinetics of block 4. Thus, the cross-talk means that any modulation of *E3* would also be a modulation of both block 3 and of block 4. Similarly, inhibition of *E4* would also be

a modulation of both blocks. Consequently, in determining elasticities of either of these blocks to x the kinetics of *E3*, *E4* and *E5* must not be altered. In the example of mitochondrial oxidation, the redox state of the quinone pool is equivalent to the intermediate z, and block 3 and block 4 may represent NADH and FADH oxidation. In this case, inhibition of complex I (*E3*) would affect the kinetics of both of block 3 and block 4 and cannot be used in the determination of either of their elasticities to the redox state of the quinone pool.

If there are no allosteric effects in the system of Fig. 2a then using the classic top-down approach we would expect that there is no elasticity of block 4 to x, or of block 3 to y in Fig. 2b. As shown above this would be incorrect due to presence of cross-talk of z and *E5* between block 3 and block 4. If a change is made to the system to increase the level of intermediate x (by increasing the activity of *E1*) then there should be an increase in the rate of the reaction catalysed by *E3* and a resulting increase in the level of z. This change in the level of z will affect block 4. There will usually be a product inhibition of *E4*, and a decrease in the flux, *J2*, through block 4. This would appear as a negative elasticity of block 4 to x because the elasticity to x represents the direct effects x has on the block and the unique effects x has through y. Similarly, a negative elasticity of block 3 to y would be seen. The origin of these elasticities lies in the cross-talk that is happening through z. The size of the elasticities is given by Eq. 6.

In the case of mitochondrial respiration, grouped as in Fig. 2b, it is expected that FADH oxidation will have a negative elasticity to NADH/NAD and NADH oxidation will have a negative elasticity to FADH/FAD. This does not indicate that there are any allosteric effects occurring in either direction, but is a consequence of the way the system is divided.

Note the control exerted by *E5* will be split between the block 3 and block 4 according to the flux that goes each block. Consequently:

$$C^a_{block3} = C^a_{E3} + \frac{J1}{J1 + J2} \cdot C^a_{E5}$$

$$C^a_{block4} = C^a_{E4} + \frac{J2}{J1 + J2} \cdot C^a_{E5}$$

where a is any system variable.

3.3. Analysis of glycolytic ATP production and consumption in the presence of substrate cycling

The conceptual division of the system outlined in Fig. 2 is performed according to the flux through the system rather than to any physical division. A second example where this sort of division can be experimentally advantageous is the presence of ATP-driven substrate cycles within glycolysis or other pathways.

Consider a system of glycolytic ATP production and subsequent ATP consumption by the cell. Ideally, the division would have two reaction blocks, ATP production from glycolysis, and ATP consumption by various processes within the cell. The two blocks would be connected by the intermediate ATP.

The rate at which substrate cycles take place within glycolysis in vivo is difficult to measure. Hence, measurement of the net yield of ATP from glycolysis – and its consumption by other cellular process – is difficult to determine. One solution is to measure the net yield of glycolytic products (lactate, pyruvate) and then use known stoichiometries to determine the gross rate of ATP production from glycolysis. Any ATP-consuming back reactions in the substrate cycles of glycolysis would be included with the other ATP-consuming processes in the block of ATP-consumers. However, when we do this components of the glycolytic ATP-production block are now also components of the ATP consumption block (cf. *E5* in Fig. 2). The effects that the presence of cycling has on the division of the system can be seen by considering the reaction scheme in Fig. 3.

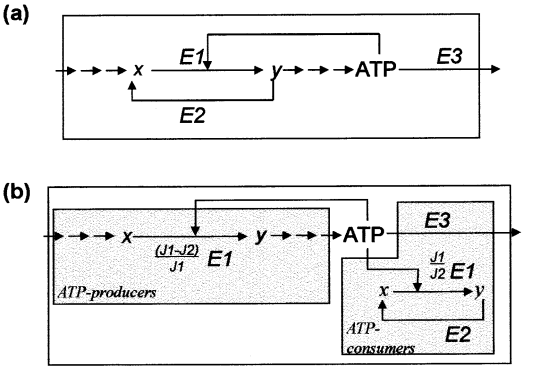

Fig. 3. A pathway with substrate cycling. (a) The pathway showing an ATP-driven substrate cycle around x and y catalysed by the enzymes *E1* and *E2*. (b) Top-down division around ATP with the substrate cycle included in the ATP consumers.

In Fig. 3a *E1* converts *x* to *y* and in the process consumes ATP. *y* can subsequently yield ATP, or can be converted back into *x* by *E2* with a net dephosphorylation of ATP in a substrate cycle. The remaining ATP is consumed by *E3*, which may represent all other ATP-consuming processes in a cell.

The net flux through the system is equal to the flux through *E1* (i.e. *J1*) minus the flux through *E2* (*J2*). Consequently, the fraction of *E1* that contributes to the net flux is given by $(J1-J2)/J1$. The remainder $(J2/J1)$ of the flux through *E1* is used to drive the cycling process together with *E2*.

The presence of cycling results in a division of the system as shown in Fig. 3b. The two blocks in Fig. 3b share *x*, *y*, *E1* and *E2*, so there will be extensive cross-talk between them. This can be dealt with as discussed above. Direct modulations of either block would be an invalid means to analyse the control pattern, but modulating a branch feeding in to (or out from) ATP would be valid. One such branch could be ATP provided by oxidative phosphorylation.

ATP consumption has two components to it: the reactions that drive the cycling process and the cellular ATP-consuming processes. They are grouped together in one block (the ATP consumers), represented by the shaded area. The control exerted by this block will be equal to the sum of the control by the reactions that compose it. Without knowledge of the cycling flux the contribution of the control over the system variables by the two parts of the ATP consumers block cannot be separated.

The control pattern of the system in Fig. 3b will relate only to the measured flux, here the net flux through the ATP-producers. For example, consider the control exerted by the steps leading to the production of *x* over the net flux. The *x* producers will control the flux through both *E1* and *E2*. However, in the system division of Fig. 3b the control by the *x* producers would be determined as control over the net flux ($J_{net} = J1-J2$). The size of this control is related to the control over *E1* and *E2* and the cycling rate by the following equation:

$$C^{J_{net}}_{x\,\text{producers}} = \frac{C^{J1}_{x\,\text{producers}} \cdot J1 - C^{J2}_{x\,\text{producers}} \cdot J2}{J_{net}}$$

Thus the control the *x* producers have over J_{net} and *J1* may be very different.

4. Discussion

Using branches as a means to modulate systems is a general way to get around the problem of 'cross-talk' between metabolic blocks. It opens up the possibility of analysing systems that are inaccessible using inhibitors alone. These systems are inaccessible because it may be difficult to measure some important intermediates and so hard to make all the relevant intermediates explicit.

In real complex systems there is likely to be a limit on the number of metabolic branches and inhibitors available that are selective enough to be of use. If in a system there is a mixture of blocks that contain intermediates that cross-talk and 'simple' ones that do not, then it would be valid to inhibit the 'simple' blocks. In such a system a combination of branches and inhibitions may provide sufficient information to solve the requisite elasticities.

Where system division means that different blocks share some common intermediates or enzymes, the control coefficients must be understood to relate only to the fraction of the enzymes which are deemed to be inside that block. For example, in Section 3, the fraction of a glycolytic enzyme that opposes substrate cycling was considered to be part of the ATP consumption block rather than the ATP production block, and thus contributes to the control by the ATP consumption block.

Where 'cross-talk' occurs, changes within blocks that have cross-talking elements are not considered equivalent [15]. For example, in Fig. 1c, an inhibition of *E2* is not equivalent to an inhibition of *E3*, since they have different effects on the cross-talking intermediate. The control coefficients represent system changes if a *uniform* change in activity were made to *all* the enzymes/processes that contribute to a block. The modulation is then the same fractional change in activity of all of the processes. Where an enzyme is in two different blocks, the fractional change relates to the part of the activity found within the block in question.

Thus, it is possible to analyse systems that have blocks that contain intermediates that are not made explicit, yet pass information to other blocks. It can be done by introducing branches that alter the levels of the explicit intermediates, and by treating the effects of the non-explicit intermediates as functions of

the explicit intermediates. This alters the meaning of the elasticities to the explicit intermediates but allows valid control and regulation analysis to be performed. The method turns out to be particularly helpful in the analysis of system where it is useful to divide enzymes between different blocks so that they appear in more than once in the system description.

We have used the concepts presented in this paper to analyse the control of ATP production and consumption in isolated hepatocytes (Ainscow and Brand, in preparation). The division of the system into metabolic blocks was such that many blocks contained 'cross-talking' intermediates. NADH and pyruvate oxidation were treated as two separate blocks despite the processes sharing many common elements, such as the respiratory chain (cf. Section 2). ATP consumption and glycolysis were also treated as separate reaction blocks. The presence of cycling within glycolysis was treated essentially as described in Section 3. The theories discussed in this paper justify the validity of analysing hepatocyte metabolism in this way.

Acknowledgements

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References

[1] H. Kacser, J.A. Burns, The control of flux, Symp. Soc. Exp. Biol. 27 (1973) 65–104.

[2] H. Kacser, J.A. Burns, Molecular democracy; who shares the controls?, Biochem. Soc. Trans. 7 (1979) 1149–1161.

[3] D.A. Fell, Metabolic control analysis, a survey of its theoretical and experimental development, Biochem. J. 286 (1992) 313–330.

[4] C. Reder, Metabolic control theory: a structural approach, J. Theor. Biol. 135 (1988) 175–201.

[5] C. Giersch, Determining elasticities from multiple measurements of steady state flux rates and metabolite concentrations – theory, J. Theor. Biol. 169 (1994) 88–99.

[6] L. Acerenza, A. Cornish-Bowden, Generalization of the double-modulation methods for in situ determination of elasticities, Biochem. J. 327 (1997) 217–223.

[7] G.C. Brown, R.P. Hafner, M.D. Brand, A 'top-down' approach to the determination of control coefficients in metabolic control theory, Eur. J. Biochem. 188 (1990) 321–325.

[8] R.P. Hafner, G.C. Brown, M.D. Brand, Analysis of the control of respiration rate, phosphorylation rate, proton leak rate and protonmotive force in isolated mitochondria using the 'top-down' approach of metabolic control theory, Eur. J. Biochem. 188 (1990) 313–319.

[9] M.D. Brand, Top down metabolic control analysis, J. Theor. Biol. 182 (1996) 351–360.

[10] S. Schuster, D. Kahn, H.V. Westerhoff, Modular analysis of the control of complex metabolic pathways, Biophys. Chem. 48 (1993) 1–17.

[11] E.K. Ainscow, M.D. Brand, Top-down control analysis of systems with more than one common intermediate, Eur. J. Biochem. 231 (1995) 579–586.

[12] R. Bohnensack, Theory of steady-state control in complex metabolic networks, Biomed. Biochim. Acta 11/12 (1985) 1567–1578.

[13] G.C. Brown, P.L. Lakin-Thomas, M.D. Brand, Control of respiration and oxidative phosphorylation in isolated rat liver cells, Eur. J. Biochem. 192 (1990) 355–362.

[14] J.H.S. Hofmeyr, H. Kacser, K.J. van der Merwe, Metabolic control analysis of moiety-conserved cycles, Eur. J. Biochem. 155 (1986) 631–641.

[15] J.M. Rohwer, S. Schuster, H.V. Westerhoff, How to recognise monofunctional units in a metabolic system, J. Theor. Biol. 179 (1996) 213–228.