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Control of frequency and amplitudes is shared by all enzymes in three models for yeast glycolytic oscillations

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Abstract

The three main existing models for glycolytic oscillations in yeast were re-examined to investigate how these oscillations are controlled. We implemented the operational definitions provided by metabolic control analysis to quantify the control properties of enzymes with regard to glycolytic oscillations. In all three models, the control of the frequency and that of the amplitudes of the metabolites were distributed among the enzymes. There was no obvious correlation between the control of the average flax and the control of the frequency. Most importantly, the so-called 'oscillophore' of the system, traditionally the enzyme primarily held responsible for the generation of the oscillation, was not the only controlling step. We conclude that just like steady-state flux control is not necessarily limited to a rate-limiting step, oscillations are not dictated by a single 'oscillophore'.

Keywords: Amplitude; Enzyme; Yeast; Glycolytic oscillation; Phosphofructokinase; Metabolic control analysis

1. Introduction

Understanding the control and regulation of cell function is a major challenge in the life sciences. Different levels of description must be combined to understand fully the behavior of metabolic systems. One can discriminate between (1) local properties, i.e., kinetics of individual enzymes; (2) systemic or control properties, which express the effect of a change in the activity of an enzyme on the overall behavior of the system, such as a steady-state flux [1–3], and (3) regulatory properties, which express the effectiveness with which enzymes sense and transduce signals from outside or inside the system, or the contribution of enzymes to homeostasis [1,4–7].

Since the development of metabolic control analysis, definitions have been formulated for these different descriptive levels that provide a quantitative foundation for statements about control and regulation of metabolism. Connectivity and summation theorems link the different levels of description, so that control properties can be understood in terms of local properties of enzymes [1,2,5], and regulatory properties can be understood in terms of both local and control properties [7,8]. The metabolic control theory has expanded its applications from simple unbranched metabolic pathways at steady state [1,2], to transients [6,9–12], branched pathways [13,14], moiety-conserved cycles [13,15], hierarchical systems (in which transcription and translation become important, so that enzyme concentrations must be treated as variables, [16]), channelling [17] and elementary reactions within an enzyme [18]. For these systems, control coefficients expressing systemic properties have been defined and connected to local properties. Moreover, time-dependent elasticity and control coefficients have been defined to treat dynamical systems (but also pseudo-steady states, see Ref. [19]).

A dynamical system that has received relatively little attention from a control analysis point of view, are limit-cycle oscillations (but see Refs. [20,21]). Limit-cycle oscillations are the stationary states among the dynamical systems [22]: they have a constant frequency and amplitude, and one may expect to be able to treat these stationary dynamical variables in a similar way to steady-state vari-

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ables. Indeed, control coefficients have been defined for the (angular) frequency (or period), for the damping and for the amplitude of oscillating metabolic systems. A frequency control coefficient is defined as the fractional change in the frequency upon a fractional change in an enzyme activity ([20,21,23]; Section 2). Moreover, summation theorems have been derived for these control coefficients [21,23,24]. However, no connectivity theorems have been derived so far that would link the control properties to the local enzyme kinetics. The lack of such a complete control theory of oscillations and hence, of clear definitions as regards control of oscillatory characteristics, may have complicated the discussion about what controls the characteristics of biochemical oscillators. Such a discussion is relevant, as for oscillating systems such as the mammalian heart, the cell cycle and certain hormonal regulatory systems, the ability to change the frequency and/or amplitude can be of vital importance [25,26]. This may be most obvious in the case of frequency-encoded information, as in neural systems [27]. Insight in the control of such variables may provide a quantitative rationale to discriminate between important and less important enzymes and ion channels.

We will here focus on one of the best studied biochemical oscillators: glycolysis in yeast [28–30]. Under certain well defined conditions, oscillations in the hexose phosphates, the adenine nucleotides and the redox couple $NADH/NAD^+$ can be observed [31,32]. The most accepted view is that phosphofructokinase (PFK) is the so-called 'oscillophore', the primary source of the oscillations; the rest of glycolysis follows the whims of this 'key enzyme' through the action of the adenine nucleotides (for review, see Ref. [29]). In the perspective of metabolic control analysis, this would seem to imply that the control of the characteristics of the oscillations should reside in this oscillophore alone. The model of Goldbeter that reduces glycolysis to the oscillophore PFK [33,34] may be seen as the culmination of this concept. Indeed, the difference in frequency when cells were fed with fructose rather than glucose has been explained by a direct effect of fructose on the kinetics of PFK [35]. Similarly, in extracts of beef heart, effectors of PFK have been used to modulate the frequency of the oscillations [36,37].

If indeed the control of glycolytic oscillations resides exclusively in a single enzyme (PFK), this dynamic system differs completely from steady-state systems, where control tends to be distributed among the system components [6,38]. Experimental evidence, however, does not seem to be in accordance with the concept of unique control in PFK: there is control of the frequency by the substrate injection rate [39], and by the type of substrate [40]; there is control of damping by growth conditions [30,41], as well as control of amplitude and damping by the cell density [42,43] involving intercellular signalling via acetaldehyde [44]. We conjecture that this paradox of control outside the 'oscillophore' was the result of a plethora of imprecise or unusable definitions, as witnessed by terminology like 'oscillatory controlled' [45], 'efficient control site' [46], 'autodynamic regulation' [47], and 'flux-limiting in glycolytic oscillations' [48]. Metabolic control analysis offers precise and quantitative definitions of the extent to which an enzyme affects the system variables, such as the steady-state flux or the amplitude of an oscillation.

There are other quantitative approaches to study other aspects of dynamic systems. The group of Hess for instance, has used nonlinear-dynamics theory to study the response of yeast glycolysis to the frequency of an imposed oscillation in the input rate of substrate [49]. In experimental as well as computer model studies [50], a rich variety of complex dynamic behaviour could be observed. This approach focused on which of all the possible dynamic states yeast glycolysis attained. Our interest in this study is how the system variables of a *given* dynamic state, i.e., a limit cycle, are controlled by the biochemical key parameters: the enzymes. We therefore applied metabolic control analysis, which is defined around the enzymes as the relevant parameters, to analyse the control of the frequency and amplitude. This has been done for three existing mathematical models of glycolytic oscillations.

These models describe three different mechanisms for the generation of the oscillations: product activation of PFK [33,34], the autocatalytic stoichiometry of glycolysis [51] and the activation of pyruvate kinase by fructose 1,6-bisphosphate [52]. Other more complete but also more complex models [53,54] have not been used for the sake of simplicity, and because they had not been developed to demonstrate a mechanism for generating oscillations. Metabolic control analysis demonstrates that in all three models the control of the oscillations is distributed among the participating enzymes and does not reside solely in the 'oscillophore'.

2. Theory and definitions

The extent to which an enzyme i controls a steady-state variable X, is defined as the relative change in that steady-state variable, regarded as a function of all enzyme activities, upon a relative change in the activity of enzyme i [55]:

$$C_i^X \equiv \frac{v_i}{X}\left(\frac{\mathrm{d}X}{\mathrm{d}p_i}\right)_{ss} \Bigg/ \left(\frac{\partial v_i}{\partial p_i}\right)_{X_j} \qquad (1)$$

The activity of enzyme i, v_i, is specifically changed by parameter p_i (which in most cases is either a specific inhibitor or the enzyme concentration). The subscript 'ss' stands for steady-state, the subscript 'X_j' denotes the metabolites, which should be kept constant when evaluating the partial derivative. Similarly, the (angular) fre-

quency control coefficient and the amplitude control coefficient can be defined:

$$C_i^{\omega} \equiv \frac{v_i}{\omega}\frac{\mathrm{d}\omega}{\mathrm{d}p_i}\Big/\frac{\partial v_i}{\partial p_i} \tag{2a}$$

$$C_i^{A_k} \equiv \frac{v_i}{A_k}\frac{\mathrm{d}A_k}{\mathrm{d}p_i}\Big/\frac{\partial v_i}{\partial p_i} \tag{2b}$$

ω is the angular velocity (which is 2π times the frequency), A_k is the amplitude of oscillating metabolite X_k, and the differentials have the meaning defined above. It has been shown [21,23,24] that the sums of the control coefficients of all enzymes is 1 in the case of the frequency, and 0 in the case of the amplitudes:

$$\sum_i C_i^{\omega} = 1,\ \sum_i C_i^{A_k} = 0 \tag{3a,b}$$

3. Methods

All the models were programmed in SCAMP [56], as closely as possible to their literature description, i.e., with the same parameters as described. In some cases, essential parameters were missing, and missing parameters were fitted to exhibit a limit cycle that closely resembled the published one. These limit cycles were taken as the reference states of which the control distributions were studied.

In order to calculate the control coefficients of an enzyme i, a parameter p_i proportionally affecting v_i (i.e., $\partial v_i/\partial p_i = 1$) was changed by both $+\delta p_i$ and $-\delta p_i$ around the reference state, the new limit cycles were computed, and the amplitudes and angular velocity of the oscillations determined. The control coefficient is calculated as the slope of the $\ln X$-$\ln p_i$ plot in the reference state, and was approximated by:

$$C_i^x = \frac{\mathrm{d}\ln x}{\mathrm{d}lnp_i} \approx \frac{\Delta \ln x}{\Delta \ln p_i} = \frac{\ln(x_{p_i+\delta p_i}) - \ln(x_{p_i-\delta p_i})}{\ln(p_i+\delta p_i) - \ln(p_i-\delta p_i)} \tag{4}$$

where x is either the angular velocity or the amplitude of a metabolite. The angular velocity ω was calculated as the inverse of the time interval between the maxima (the period T), times 2π: $\omega = 2\pi/\mathrm{T}$.

The magnitude of the parameter change δp was balanced between linearity of the ln-ln plot (which usually requires small changes, as is seen in Fig. 1) and accuracy of determination of the change in X, and depended on the model and parameter under investigation. The magnitudes of the parameter change will be indicated for each control coefficient. It was checked that the oscillations were stationary by following the amplitudes and frequency for at least 5 cycles.

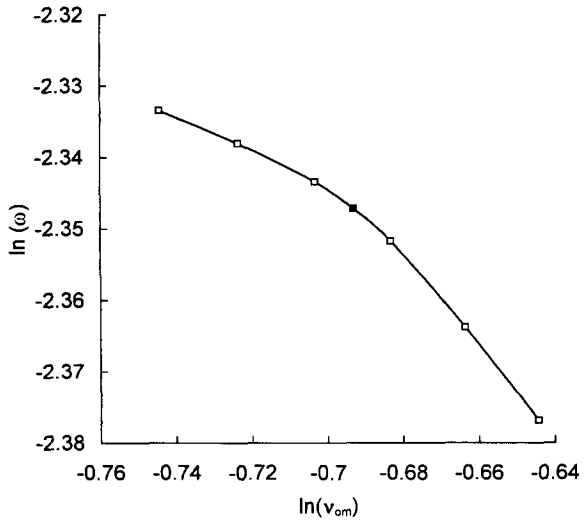

Fig. 1. A plot of the natural logarithm of the angular frequency versus the natural logarithm of v_{0m}. This example is from model 2. The black symbol corresponds to the reference state of which the control coefficient is determined. The parameter was increased and decreased by 1%, 3% and 5%.

4. Models

We here describe the equations of the models only in the form in which they have been used. Details can found in the original literature. To facilitate comparison we have kept the original symbols.

4.1. Model 1: the PFK model of Goldbeter et al. [33,34]

The PFK model of Goldbeter et al. [33,34] describes glycolysis solely in terms of the kinetics of the enzyme PFK. It consists of two variables α and γ, and three reactions (Fig. 2A):

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = \sigma_1 - \sigma_m \cdot \Phi(\alpha,\gamma) \tag{5}$$

$$\frac{\mathrm{d}\gamma}{\mathrm{d}t} = \sigma_m \cdot \Phi(\alpha,\gamma) - k_s \cdot \gamma \tag{6}$$

in which $\Phi(\alpha,\gamma)$ is a function that bears the most important kinetic details of PFK:

$$\Phi(\alpha,\gamma) = \frac{\alpha e(1+\alpha e)(1+\gamma)^2 + L\vartheta\alpha ce'(1+\alpha ce')}{L(1+\alpha ce')^2 + (1+\gamma)^2(1+\alpha e)^2} \tag{7}$$

The variables α and γ denote the concentrations of substrate (ATP or fructose 6-phosphate) and product (ADP or fructose 1,6-bisphosphate), respectively. σ_1 denotes the constant injection rate of substrate, σ_m is the rate constant (or concentration) of PFK and k_s the rate constant of removal of product. In model 1, the control of the three reactions can be determined by changing the parameters σ_1, σ_m and k_s.

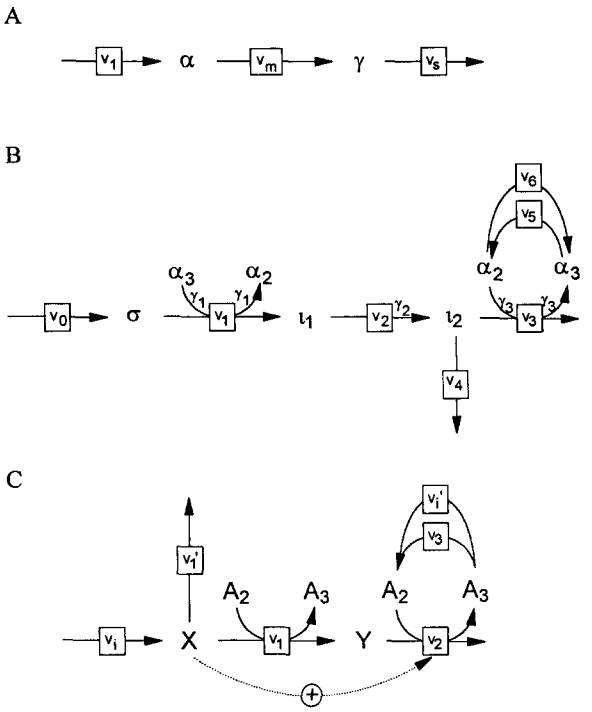

Fig. 2. Reaction schemes of models 1(A), 2(B), and 3(C). See the text for explanation of the symbols and the rate equations.

4.2. Model 2: a stoichiometric model by Sel'kov [51]

Sel'kov [51] designed a model with simple Michaelis-Menten kinetics, where the oscillations were generated by the autocatalytic stoichiometry of glycolysis (Fig. 2B). Here the scaled version of the model will be used. All scaling factors can be found in the original paper. This model consists of two scaled free metabolite concentrations, σ and α_3, and seven reactions. Two intermediate concentrations, ι_1 and ι_2 are assumed to be in quasi-steady-state at each moment, so that they merely follow the oscillations of σ and α_3. σ, ι_1 and ι_2 may represent the concentrations of glucose, fructose 1,6-bisphosphate and the triosephosphates divided by their K_m values, respectively. α_3 and α_2 represent the scaled concentrations of ATP and ADP, respectively:

$$\alpha_3 = \frac{[\mathrm{ATP}]}{[\mathrm{ATP}]+[\mathrm{ADP}]} \tag{8}$$

$$\alpha_2 = \frac{[\mathrm{ADP}]}{[\mathrm{ATP}]+[\mathrm{ADP}]} = 1-\alpha_3 \tag{9}$$

The reduced set of differential equations reads:

$$\frac{d\sigma}{d\tau} = v_0 - v_1 \tag{10}$$

$$\epsilon_3 \frac{d\alpha_3}{d\tau} = \left(\frac{\gamma_2\gamma_3}{\gamma_1} - 1\right)v_1 - \frac{\gamma_3}{\gamma_1}v_4 - v_5 + v_6 \tag{11}$$

Here τ is the scaled time, ϵ_3 is a scaling constant, and γ_1, γ_2 and γ_3 are stoichiometric coefficients. γ_1 is the number of molecules of ATP converted to ADP per number of molecules of σ (glucose) consumed in reaction 1, γ_2 is the number of molecules of ι_2 produced per number of molecules of ι_1 consumed in reaction 2 and γ_3 is the number of molecules of ADP converted to ATP per number of molecules of ι_2 consumed in reaction 3. The rate equations are:

$$v_0 = v_{0m} \tag{12}$$

$$v_1 = \frac{\beta_1\sigma\alpha_3}{(1+\sigma)(\kappa_1+\alpha_3)} \tag{13}$$

$$v_2 = \frac{\beta_2\iota_1}{1+\iota_1} \tag{14}$$

$$v_3 = \frac{\beta_3\iota_2\alpha_2}{(1+\iota_2)(\kappa_3+\alpha_2)} \tag{15}$$

$$v_4 = \frac{\beta_4\iota_2}{\kappa_4+\iota_2} \tag{16}$$

$$v_5 = \frac{\beta_5\alpha_3}{\kappa_5+\alpha_3} \tag{17}$$

$$v_6 = \beta_6\alpha_2 \tag{18}$$

The intermediates ι_1 and ι_2 are taken to be in quasi-stationary state, such that $v_1 = v_2 = (v_3+v_4)/\gamma_2$. Their quasi-stationary concentrations are then:

$$\iota_1 = \frac{v_1}{\beta_2 - v_1} \tag{19}$$

$$\iota_2 = \frac{0.5\left(b+\sqrt{b^2+4\gamma_2 v_1\kappa_4 c}\right)}{c} \tag{20}$$

with:

$$b = \gamma_2 v_1(1+\kappa_4) - d\kappa_4 - \beta_4 \tag{21}$$

$$c = d + \beta_4 - \gamma_2 v_1 \tag{22}$$

$$d = \frac{\beta_3\alpha_2}{k_3+\alpha_2} \tag{23}$$

The control coefficients of all reactions except v_2 and v_3 were calculated by modulating v_{0m}, β_1, β_4, β_5 and β_6. Reactions v_2 and v_3 drop from the reduced set of differential equations and, therefore, the summation theorems do not comprise them.

4.3. Model 3: the lower part of glycolysis according to Dynnik and Sel'kov [52]

Dynnik and Sel'kov proposed a model in which the lower part of glycolysis acted as an 'oscillophore' via the activation of pyruvate kinase by fructose 1,6-bisphosphate [52]. The model consists of 3 independent metabolites and

6 reactions. The unscaled version of the model has been used:

$$d[X]/dt = v_i - v_1 - v_1' \quad (24)$$

$$d[Y]/dt = v_1 - v_2 \quad (25)$$

$$d[A_2]/dt = v_3 + v_i' - v_1 - v_2 \quad (26)$$

in which $[X]$ denotes the pool of fructose 1,6-bisphosphate, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, $[Y]$ stands for the pool of 3-phosphoglycerate to phospho*enol*pyruvate and $[A_2]$ stands for ADP. The rate equations are:

$$v_1 = k_1[X][A_2] \quad (27)$$

$$v_1' = k_1'[X] \quad (28)$$

$$v_2 = k_2[Y][A_2]\left(v_0 + ([X]/K_a)^\gamma\right) \quad (29)$$

$$v_3 = k_3(\Sigma_A - [A_2]) \quad (30)$$

The reaction rates v_i and v_i' are constant. The parameter values of k_1 and Σ_A (the sum of ATP and ADP) were not given by Dynnik and Sel'kov, and have been taken to yield a limit-cycle. The parameters to be modulated for a metabolic control analysis are v_i, v_i' and the rate constants k_1, k_1', k_2 and k_3.

5. Results

For each model we first confirmed that limit-cycle oscillations were obtained and then calculated the extent to which the various steps controlled the frequency and amplitudes of these limit cycles.

5.1. Model 1: the PFK model of Goldbeter et al.

For the parameter set given in Ref. [57] (the legend to Fig. 3. in this paper), model 1 exhibited sustained oscillations in α and γ (Fig. 3). Table 1 gives the control exerted by the three reactions on the amplitude of the oscillations

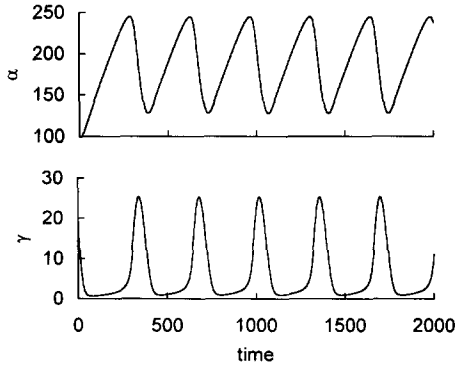

Fig. 3. Results of a simulation of model 1. System parameter values from Goldbeter and Caplan [57]: $L = 10^6$; $c = 10^{-5}$; $e = e' = 0.9090909$; $\theta = 1$; $\sigma_i = 0.7$; $\sigma_m = 4$; $k_s = 0.1$.

Table 1
Control distribution of frequency and amplitude of the oscillations of model 1

Parameter	C_i^ω	$C_i^{A_\alpha}$	$C_i^{A_\gamma}$	$C_i^{\bar{J}}$
σ_i	0.65	0.23	0.37	1.00
σ_m	0.30	−0.33	−1.19	0.00
k_s	0.06	0.11	0.82	0.00
Sum	1.01	0.01	0.00	1.00

The parameters of the reference state are given in the legend to Fig. 3. The relative modulation of the parameters used to calculate the frequency control coefficients was 5%. For the amplitude this was 0.5%. $C_i^{\bar{J}}$ is the control on the average flux.

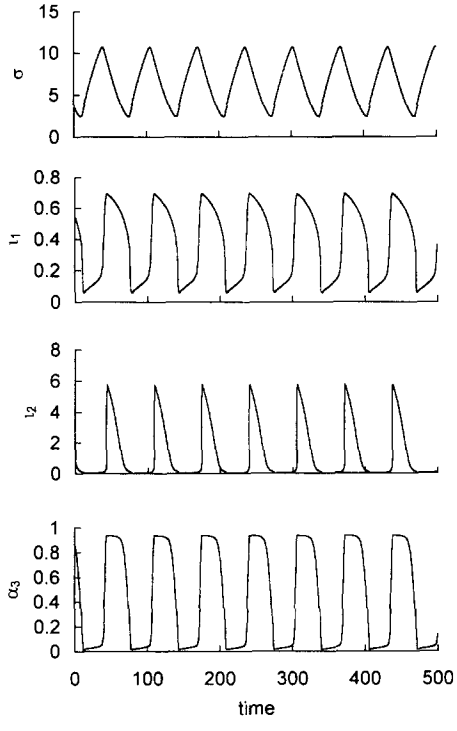

Fig. 4. Sustained oscillations in $[X]$, $[Y]$ and $[A_2]$ of model 3. System parameters were: $\Sigma_A = 2$; $K_a = 2$; $v_0 = 0.01$; $\gamma = 4$; $v_i = 0.2$; $k_1 = 0.3$; $k_1' = 0.07$; $k_2 = 0.3$; $k_3 = 0.07$; $v_i' = 0.1$.

Table 2
Control distribution of frequency, amplitude and average flux of the oscillations of model 2

Parameter	C_i^ω	$C_i^{A_\sigma}$	$C_i^{A_{\alpha_3}}$	$C_i^{\bar{J}}$
v_{0m}	−0.42	0.03	0.01	1.00
β_1	10.65	−11.73	0.00	0.00
β_4	−0.03	0.04	0.01	0.00
β_5	−12.54	16.11	−0.01	0.00
β_6	3.33	−4.41	−0.05	0.00
Sum	0.99	0.04	−0.04	1.00

The parameters of the reference state are given in the legend to Fig. 4. The relative modulation of the parameters were 1% for v_{0m}, β_4 and β_6, and 0.25% for β_1 and β_5.

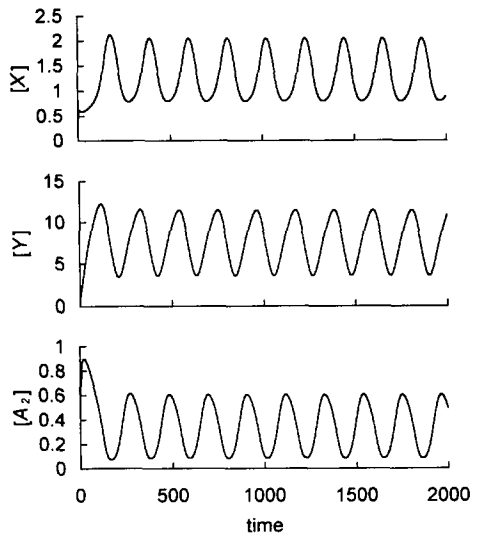

Fig. 5. Results of a simulation of model 2. System parameters were: $\epsilon_3 = 0.5$; $\gamma_1 = 1$; $\gamma_2 = 2$; $\gamma_3 = 1$; $\kappa_1 = 0.1$; $\kappa_3 = 0.5$; $\kappa_4 = 100$; $\kappa_5 = 0.045$; $v_{0m} = 0.5$; $\beta_1 = 1$; $\beta_2 = 2$; $\beta_3 = 16$; $\beta_4 = 2$; $\beta_5 = 0.75$; $\beta_6 = 0.1$.

in α and γ, on the angular frequency and on the average flux through the system. Whereas all the control on the average flux through the system resided completely in the input step σ_1, the control of the frequency and amplitudes of α and γ was distributed over the three reactions, and was certainly not limited to the phosphofructokinase reaction (σ_m). The sums of the control coefficients were equal to their expected values of 1 and 0 for the control of frequency and amplitude, respectively.

5.2. Model 2: a stoichiometric model by Sel'kov

In Fig. 4 the limit cycles generated by model 2 are shown. Table 2 gives the control coefficients of the enzymes. Again the control of the average flux fully resided in the input step v_{0m}, while the control of the frequency and the amplitude of σ was distributed. The differences between the individual control coefficients were very large:

Table 3
Control distribution of frequency, amplitude and average flux of the oscillations of model 3

Parameter	C_i^{ω}	$C_i^{A_X}$	$C_i^{A_Y}$	$C_i^{A_{A_2}}$	$C_i^{\bar{J}}$
v_i	3.19	−3.19	−6.62	−6.18	1.00
v_1	−0.76	1.66	2.33	1.00	0.00
v_1'	−0.76	−1.36	0.25	0.38	0.00
v_2	0.57	−0.59	−1.12	−0.61	0.00
v_3	−0.61	1.61	2.50	2.61	0.00
v_i'	−0.62	1.88	2.66	2.80	0.00
Sum	1.01	0.01	0.00	0.00	1.00

The parameters of the reference state are given in the legend to Fig. 5. The relative changes in the parameters used to calculate the amplitude control coefficients were 0.5%. For the frequency control coefficients the relative changes in the parameters were 0.1% for v_i, 0.25% for v_2 and v_3, and 0.5% for v_1, v_1' and v_i'.

the control coefficients of β_1 and β_5 on the frequency and the amplitude of σ exceeded 10, while the amplitude of α_3 was virtually insensitive to changes of any of the parameters. Nevertheless the sums of the control coefficients were 1 for the frequency and 0 for the amplitudes.

5.3. Model 3: the lower part of glycolysis by Dynnik and Sel'kov

Fig. 5 shows the oscillations for a set of parameters that showed limit-cycle behavior, and that was used as the reference state. The control coefficients can be found in Table 3. The frequency control coefficients sum up to 1, and the amplitude control coefficients sum up to 0. Again, both types of control were distributed over the various steps.

6. Discussion

Metabolic control analysis has shown that the control of steady-state fluxes and concentrations tends to be distributed over the enzymes that constitute a system, and does not often reside in a single step. In this paper we have used numerical methods to demonstrate that the control of metabolic oscillations also tends to be distributed, even in model 1, which represents the proposed 'oscillophore' PFK. It may be useful to note that a distribution of control of frequency and amplitudes is in line with, but not demanded by metabolic control analysis. The summation theorem for the frequency control dictates that the sum of the frequency control coefficients of all enzymes is 1 (see subsequent paragraphs) which allows distribution of control but also allows control to be confined to a single step. The latter possibility exists even though, for instance, in the case of limit cycles close to the Hopf bifurcation, the frequency, i.e., the imaginary part of the positive eigenvalues in the fixed point, may depend simultaneously on many parameters [23]. Metabolic control analysis focuses on the *sensitivity* of this frequency (or other system variable) to small changes in these parameters. The conclusion that control of metabolic oscillations may be distributed should have implications for cell biology research. Studies on the control of the cell cycle should expect to find many proteins in the living cell that affect the process. Similarly, other metabolic or biological oscillations, such as the heart beat, calcium oscillations in neural systems or even circadian rhythms are expected to be controlled by many parameters, and differently depending on whether frequency or amplitude is concerned.

In steady-state analysis of ideal systems [58], the sum of all control coefficients of a certain variable has a distinct value (1 for the flux, 0 for the steady-state metabolite concentrations). In the case of oscillations, similar summation theorems have been derived [21,23,24]. In the three mathematical models of glycolytic oscillations in yeast, the

sums of the control coefficients were in accordance with the summation theorems, as they were in earlier studies on the control of the frequency and amplitudes. Also the total control of the average flux was 1 for all three models, as expected [23]. For these models the average flux was fully controlled by the first step, simply because its rate was taken constant.

Das and Busse [59] have shown experimentally that, in series of dilutions of yeast extracts, the period of the oscillation was inversely proportional to the protein concentration of the extract. The dilutions were done with a buffer containing fixed concentrations of AMP, ADP, ATP, NAD^+ and NADH. When their data are transformed to a $\ln(\omega)$-$\ln(c_{prot})$ plot, the slope is 1, in accordance with a theorem (Eq. (3a)) that did not yet exist, provided that the dilution procedure did not significantly affect other conserved sums of metabolite concentrations.

Although the sums of the control coefficients for the different variables were always the same, the individual control coefficients could be as high as 16, as low as -12.5, or as negligible as 0.00. These extreme values do not often occur in steady-state analysis. These extreme coefficients were most notable in model 2: σ is extremely sensitive to changes in the enzyme activities (especially to β_1 and β_5, Table 2), whereas α_3 is almost inert to changes in any parameter. These findings do not seem to be specific for autocatalytic stoichiometry, since an earlier control analysis of a computer model based on the autocatalytic stoichiometry of glycolysis yielded control coefficients between -1 and 1 [23].

Not only were the control coefficients very different for different parameters, but also the control exerted by one parameter on the different variables could differ extensively. Thus, in model 1, the control of PFK (σ_m) on the amplitude of α is much smaller than that on the amplitude of γ (-0.33 and -1.19, respectively (Table 1)). Even more drastically, in model 3, the control of v_1' on the amplitude of $[X]$ could be negative and large (-1.36), but the control on the amplitude of $[Y]$ could be positive and relatively small ($+0.25$). A statement that a certain enzyme has a high control on the oscillations, therefore, has no meaning, unless the variable of which the control is examined, is specified.

Fig. 1 demonstrates that the choice of reference state will also affect the values of the control coefficients. Had we been interested in the control of the frequency in model 2 with v_{0m} being only 2.5% higher than it was in the actual study, the control coefficient of v_{0m} had been much more negative (cf. the slope of Fig. 1 around the reference state (black symbol) with that two symbols to the right). Because the sum of the frequency control coefficients should remain 1, the control of the other enzymes must also change. These shifts in control upon non-infinitesimal parameter changes are well-known for steady-state analyses [38]. They illustrate that the state of the system of which the control distribution is studied should be specified and that special care should be taken when control in different organisms is compared (see, e.g., Ref. [48]).

When comparing the control of the average flux with the control of the frequency, it is clear that they are not the same: even though in all three models control of the average flux resides in the first step, this step does not have all control on the frequency. Moreover, no obvious correlation between the control of the frequency and that of the average flux was found: in model 1 and 3, the control on ω by the flux controlling step was positive, in model 2 this control was negative.

In the literature, the term 'oscillophore' has been used for an enzyme that is supposed to be primarily responsible for the generation of oscillations. It is clear from this model study, that any such an 'oscillophore', if it were to exist, does not necessarily have a high control on the oscillatory variables. This is most obvious in model 1, where only PFK was modelled, together with some input reaction and a sink reaction to prevent the system from reaching equilibrium. Even in that model, the proposed 'oscillophore' controlled the frequency for not more than 30%. In model 3, where reaction v_2 might be proposed to be the 'oscillophore', that 'oscillophore' alone controlled neither the frequency nor the amplitudes (Table 3).

Clearly, an 'oscillophore' does not completely control the characteristics of the oscillations it is supposed to generate. Moreover, the 'oscillophore' is not the only enzyme responsible for the generation of oscillations. For oscillations to occur, all the parameters of the system should be within certain boundaries. The input rate, for example, should be within the 'oscillatory window' [39]. Thus, all enzymes are important in generating oscillations. Is there any use then for the term 'oscillophore', or should it be regarded as an archaic term, as is the term 'rate-limiting step' in the steady-state situation? With respect to steady states, an enzyme may still be called 'rate-limiting step' if its control on that steady-state rate (flux) is close to 1. For oscillations, one might wish to propose that an enzyme can be called an 'oscillophore' if its control on the frequency is close to 1, whereas that of all other enzymes is close to 0. Such a definition, however, does not guarantee a control close to 1 on the amplitudes of the metabolites. Moreover, neither the enzyme PFK, nor any other enzyme would then be an 'oscillophore', as shown in all three models and suggested by experimental results [60].

The term 'oscillophore' ('carrier of oscillations') suggests that *one enzyme* can be held solely responsible for the oscillatory behavior, forcing the other enzymes to follow. A minimum testable prediction of an enzyme being an 'oscillophore' would then be that small changes in the activity of any other enzyme should not affect the oscillatory behaviour. In this paper we have demonstrated numerically that the other enzymes do have an effect and that therefore, the oscillophore picture is too simple. Both the occurrence of oscillations and their characteristics are properties of the whole system, rather than properties of

only one enzyme. A similar conclusion could have been drawn from earlier work, most notably from the fact that the glucose injection rate in studies of yeast extracts determined the form and frequency of the NADH oscillation [39,49]. Yet, these findings were considered compatible with the idea of an 'oscillophore', or with the 'PFK oscillator theory'. If so, then the 'oscillophore' would have to be of a diffuse nature comprising many if not all enzymes in the system. Such a concept does not seem very useful. Hence, we propose not to discuss (control of) oscillations in terms of 'oscillophores', but in the quantitative terms provided by systems theory [61], (Mosaic) nonequilibrium thermodynamics [6] and metabolic control analysis.

7. Note added in proof

Recently, a paper [62] was brought to our attention, in which the control coefficient was generalized to oscillations and in which, for a different model, distributed control on frequency and amplitude was observed.

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