Comparison of control analysis data using different approaches: modelling and experiments with muscle extract

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Abstract Experimental and model studies have been performed to characterize the control properties of hexokinase and phosphofructokinase in muscle glycolysis and to examine the nature of error associated with experimental flux control coefficient determinations. Different approaches of metabolic control analysis, classical titration, co-response analysis and kinetic modelling indicated that flux control coefficients could be reliably estimated experimentally for the upper part of glycolysis. The kinetic parameters applied to construct the mathematical model were determined in muscle extract under similar conditions used for flux studies. If the kinetic parameters of commercial enzymes are introduced into the model the control analysis data cannot be trusted. Co-response analysis can also be successfully applied to determination of the flux control coefficients of the system. However, the involvement of a rapid-equilibrium enzyme, such as glucose 6-phosphate isomerase, could result in estimation errors for the relevant co-response coefficients that are propagated into the elasticity matrix. If the co-response coefficients related to isomerase activity are replaced by the values obtained by kinetic modelling, the values of elasticities are correct. Our data also suggest that in the upper part of glycolysis hexokinase mainly controls the pathway flux whereas phosphofructokinase exerts dominant control on the turnover of internal metabolite stocks inside the system.

Key words: Muscle; Glycolysis; Co-response analysis; Modelling; Metabolic control analysis

1. Introduction

The control structure of a metabolic pathway can be quantitatively characterized by metabolic control analysis (MCA) by means of control and elasticity coefficients (the control coefficient is the fractional change in a systemic variable that results from an infinitesimal fractional change in the rate of enzyme catalytic activity ($c_{Ei}^{Y} = \mathrm{dln}\ Y/\mathrm{dln}\ Ei$), the elasticity coefficient is the fractional change in the net rate for an individual enzymatic reaction that results from an infinitesimal fractional change in a metabolite concentration, with all other effectors of the enzyme held constant at the values they have in the metabolic pathway ($\varepsilon = \delta \ln v_i/\delta \ln S_j$)) [1–3]. Since the 1980s extensive theoretical and experimental work has been directed towards MCA using various methods (for a review see [3]). Concerning glycolysis, relatively little is known about the quantitative distribution of control of flux (J), internal metabolite concentrations (S_i) and metabolic response time (τ) under physiological conditions. One of the first attempts to apply MCA for quantification of control of J and τ in first steps of skeletal muscle glycolysis was done using a mixture of commercial enzymes [4]. In previous work [5] we analyzed the first four steps of glycolysis in muscle extract by MCA, optimized the experimental system and determined the potential errors related to control coefficients measurements. In an in vivo study the glycolytic flux performed by the heart was measured under conditions when the heart were perfused with glucose, insulin and ketone bodies and combinations of these [6]. It was concluded that the control of glucose utilization did not reside at a single step: glucose transport, hexokinase, enolase and pyruvate kinase had the major role in the control, however, but the control steps were found to vary with the conditions.

Two major types of methodology have been used to estimate control coefficients. One is experimental determination of the flux changes caused by up- or down-modulation of individual enzyme concentrations or activities (system properties) and the activity changes caused by alteration in intermediate concentrations (local properties); from these experimental data other coefficients such as co-response coefficients and the metabolite response time can be computed. The other is the evaluation of an appropriate kinetic model on the basis of information obtained from standard kinetic methods of rate measurements. Both approaches have advantages and disadvantages, but in both cases errors can be associated with experimental flux control coefficient determination. Experimental control coefficient estimates cannot be necessarily trusted for the Calvin cycle without evidence that they exhibit no significant mathematical error and without an appropriate kinetic model [7]. However, even for glycolysis there are still relatively few computer simulations that provide realistic and reliable models although the principal characteristics of most of the glycolytic enzymes are known. To make a computer model reproduce the experimental systemic behavior it is often necessary to make major changes in some of the values of the kinetic parameters of the individual enzymes obtained experimentally [3].

Cornish-Bowden and Hofmeyr [8] developed a very ingenious method, co-response analysis, which allowed one to compute MCA coefficients without the need for the component enzymes to be purified or for the changes in their activities to be known. This method is based upon measurement of the co-response coefficients ($^{Ek}O_{y_2}^{y_1}$) which relate the concomitant change in two steady-state variables (y_1, y_2) when the activity of a step (Ei) is perturbed: $^{Ek}O_{y_2}^{y_1} = \mathrm{dln}\ y_1/\mathrm{dln}\ y_2 = C_{Ei}^{y_1}/C_{Ei}^{y_2}$. However, the applicability of this method has not previously been tested experimentally.

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In this paper we present a systematic analysis of the upper part of glycolysis in muscle extract using experimental and theoretical methods of MCA which made it possible (1) to characterize the control role and properties of hexokinase and phosphofructokinase in the upper part of glycolysis, (2) to demonstrate the feasibility and reliability of co-response analysis, and (3) to compare the control analysis data estimated by classical titration, co-response analysis and kinetic modelling.

2. Materials and methods

2.1. Chemicals

ATP (sodium salt), NADH, glucose (Glc), glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), fructose 1,6-bisphosphate (FBP), phosphocreatine (PC), hexokinase (HK), glucose 6-phosphate isomerase (GPI), phosphofructokinase (PFK), aldolase (ALD), triose-phosphate isomerase (TPI), α-glycerol 3-phosphate dehydrogenase (GDH), glucose 6-phosphate dehydrogenase (G6PDH), and creatine kinase (CK), HEPES and MOPS were purchased from Sigma Chemical Co. All other chemical (analytical grade) were purchased from Panreac.

2.2. Preparation of muscle extracts

Leg muscle of 8–16 weeks old C57BL/6 (IFFA Credo, Spain) mice was minced with scissors and 1 g muscle was homogenized in 2.5 ml standard buffer (50 mM HEPES buffer, pH = 7.4 containing 100 mM KCl, 10 mM NH_2PO_4 and 10 mM $MgCl_2$) using a Potter-Elvehjem glass homogenizer at low speed (1000 rpm). The homogenate was centrifuged at 31 000 × *g* for 30 min. The resulting supernatant was filtered through a Sephadex G25 column (5.5 × 1.6 cm) to remove the endogenous metabolites. All procedures were carried out at 4°C.

2.3. Partial purification of HK

HK was partially purified from mouse muscle according to the method of Grossbard and Schimke [9]. The preparation was free from activities of ALD, lactate dehydrogenase, PFK and ATPase. The specific activity of the preparation was 45 U/mg.

2.4. Determination of flux for the conversion of glucose to triosephosphates

The steady-state fluxes were measured in standard buffer at 37°C by coupling the reaction with excess TPI/GDH activities. 1 ml reaction mixture contained 2 mM NADH, 2 mM MgATP, 10 mM Glc, 20 mM PC, 3 U/ml CK, 7 U/ml TPI, and 2 U/ml GDH. NADH consumption was followed at 385 nm according to a modification of the method described in [10] ($\varepsilon_{NADH}^{385nm} = 0.75\ mM^{-1}\ cm^{-1}$) using a Shimadzu UV-2101-PC spectrophotometer with 1 cm light path cells. The reaction was started by addition of 40 μl extract.

2.5. Determination of metabolite concentrations

When NADH consumption was constant the consecutive reactions were stopped at different times by addition of ice-cold $HClO_4$ at a final concentration of 10% and neutralized with an appropriated amount of KOH/MOPS (6/0.6 M). After 10 min the precipitate was removed by centrifugation for 10 min at 14 000 × *g*. The supernatants were used for the enzymatic determination of G6P, F6P and FBP according to Bergmeyer [11].

2.6. Modulation of steady-state flux and metabolite concentration by external enzymes

For classical titrations and co-response analysis the steady-state flux and metabolite concentrations were measured in the presence of different amounts of commercial enzymes (HK, GPI, PFK or ALD). Typically 10, 20 and 50% of the endogenous activities were used for titration. The reaction was started by addition of 40 μl of extract.

2.7. Measurements of enzyme activities of the extract

Activities of HK, GPI, PFK and ALD were measured in the standard buffer with 40 μl of extract at 37°C. GPI and ALD activities were determined according to Bergmeyer's methods [11]. PFK activity was measured according to the method of Brand and Sölings [12]. HK activity was measured as described by Grossbard and Schimke [9]. For determination of the inhibition constant (k_i) of HK for G6P partially purified HK was used.

2.8. Protocol for co-response analysis

1. Determine the steady-state concentrations of G6P, F6P and steady-state rates of the consecutive reactions catalyzed by HK, GPI, PFK and aldolase in the extract
2. Perturb the basal flux and intermediate concentrations by adding commercial muscle enzymes, HK, GPI, PFK, ALD and determine the new steady-state rate and metabolite concentrations
3. Prepare log S_i against log J (or against log S_j) plots for each perturbation to obtain co-response coefficients ($^{Ek}O_J^{S_i} = d\ln S_i / d\ln J$ or $^{Ek}O_{S_j}^{S_i} = d\ln S_i / d\ln S_J$) for each enzyme
4. Construct co-responses matrices from the whole set of co-response coefficients
5. Compute elasticities, control coefficients and time response coefficients [8].

2.9. Computer modelling of the pathway

The rate equations of HK, GPI, PFK and ALD used for the kinetic model are listed in Table 2. The model was constructed using a IBM-PC version of the simulation and control package MIST [13]. Control and elasticity coefficients were also computed by numerical differentiation using the same program. Co-response coefficients were obtained from elasticity and control coefficients using the appropriate matrix equations [8].

3. Results and discussion

3.1. Classical titration

Fig. 1 shows typical time courses of triosephosphate production by muscle extract from glucose used for determination of control coefficients by classical titration [5]. The basal rate (47.8 nmol/mg protein × min), which proceeds at constant rate for several minutes, was significantly increased by addition of HK. The concentrations of G6P and F6P determined at 200 and 400 s without external HK were constant (30.8 ± 0.2 μM for G6P and 11.2 ± 0.2 μM for F6P) suggesting that the glucose conversion is proceeding at the steady-state rate. The concentration of FBP was not measurable under similar conditions [11], in agreement with literature data (e.g. [6]). Similar sets of experiments were carried out with muscle extract at different dilutions showed that the steady-state rates (J) were proportional to the amount of extract (cf. Fig. 1, inset). In addition, no alterations in the steady-state concentrations of G6P and F6P could be detected at different dilutions (data not shown). These findings provide evidence that the summation theorem for flux control coefficients is fulfilled ($\Sigma[C_{Ei}^{J} = 1$), therefore, control coefficients, co-response coefficients and elasticity coefficients are related by simple matrix equations [3,8,14].

The values of the control coefficients determined by classical titration [4] were obtained from the straight lines of log J vs. log (E) plots are 0.82, 0.01, 0.17 and 0.01, for HK, GPI, PFK and ALD respectively. These data suggest that HK has the major control of the upper part of glycolysis in muscle extract, in agreement with the data reported in the literature for other cell types (see for a review [15]).

3.2. Co-response analysis

To make an experimental test of co-response analysis [8] and to determine its advantages and disadvantages with respect to other control analysis approaches, the elasticity coefficients as well as control coefficients were computed from

the co-response coefficients. Co-response coefficients were obtained from the experimental curves that compare the variations in the steady-state values of each pair of systemic variables (J, G6P, F6P) in response to perturbations of the basal steady-state rates by titration with exogenous enzymes. In each case the co-response coefficient was computed from the slope at the operating point. Because of technical difficulties in the determination of the FBP concentration, co-response coefficients involving this metabolite were not measured, and consequently they were omitted from the co-response matrices. We observed that changes in aldolase concentration by addition of exogenous enzyme to the extract altered neither the basal flux nor the concentrations of G6P and F6P. Therefore, we concluded that this enzyme did not control the systemic variables and any co-response coefficients related to perturbation of ALD activity. It should be noted that for any pair of variables (J, G6P, F6P) the log-log plots obtained by adding HK or PFK can be fitted very nicely with straight lines ($r = 0.99$). However, this was not the case for the plots obtained as a response to a perturbation in GPI ($r = 0.92$–0.95). The best-fit data for the log-log plots were used to compute the co-response coefficients (Table 1A).

To compute elasticity and control coefficients from the experimental co-response coefficients we need to choose an appropriate set of co-response coefficients to construct adequate co-response matrices. In principle, from the set of co-response coefficients obtained in our experimental system 27 different co-response matrices can be constructed. Selection of the most reliable ones for computing control coefficients is not easy, because co-response coefficients can have any values associated with very different errors. If the co-response coefficient value is very low then the experimental error associated with this co-response coefficient could be very high [8]. This is the case in our experimental system for GPI where $^{GPI}O^{J}_{G6P} = 0.058$.

We now computed each of the 27 possible co-response matrices from the whole set of experimentally determined co-response coefficients. Analysis of the data resulted in different elasticity and control coefficient matrices. We found that in most cases the elasticity coefficients calculated from co-response matrix are very similar and qualitatively give quite correct values, positive for substrates and negative for products. However, in some cases some unexpected elasticity coefficient values different from zero give spurious suggestions of weak activation of PFK by G6P, weak inhibition of HK by F6P and activation of HK by G6P, which are not supported by experimental observations. Also it should be noted that the value obtained for the elasticity of HK with respect to G6P is always close to zero, being in some cases slightly positive, a spurious suggestion of activation of HK by G6P. Taking into account these results, we selected nine of the 27 elasticity matrices computed from the co-response matrix, namely those that give a negative value for the elasticity of HK with regard to glucose and that give the lowest values of the spurious elasticities of PFK with respect to G6P and of HK with respect to F6P. The averaged elasticity coefficients computed from these nine selected elasticity matrices are showed in Table 1B. From these results we concluded that the choice of the co-response matrix is not very critical in our case. However, we can draw the important conclusion that all 27 matrices must have an appreciable mathematical error associated with the perturbation of GPI activity, because the reaction catalyzed by GPI is in rapid equilibrium and causes spurious elasticity coefficients to be observed. So GPI, as expected, has relatively high absolute values for ε^{GPI}_{G6P} and ε^{GPI}_{F6P} which are comparable to each other. Indeed, this assumption is justified experimentally in our system: PFK activity of the extract measured with a mixture of G6P and F6P (30 μM/10 μM) or G6P alone (40 μM) was practically identical (data not shown). This is the first experimental demonstration that a rapid equilibrium enzyme with high elasticity coefficients can cause large experimental errors in the determination of the corresponding co-response coefficients that are propagated into the computed elasticity coefficients. Therefore, if the GPI-related co-response coefficients are included into the matrices then all 27 possible matrices have a high mathematical error on account of the kinetic behavior of GPI.

Inversion of the elasticity matrices constructed using the averaged elasticity coefficients listed in Table 1B allowed evaluation of the flux and concentration control coefficients shown in Table 1C. Our data suggest that the values of flux control coefficients obtained by co-response analysis are very similar to those estimated directly by the classical titration. This result also justifies the theoretical prediction of Cornish-Bowden and Hofmeyr [8] that even if errors in the co-response matrix result in anomalies in the elasticity matrix the control coefficients matrix obtained by inversion usually gives qualitatively correct information and the values of control coefficients are correct in order of magnitude.

The control coefficients with respect to σ (C^{σ}_{Ei} being

Table 1
Characteristic coefficients of the MCA for the upper part of glycolysis in muscle extract obtained by co-response analysis. A: Co-response coefficients. B: Elasticity coefficients. C: Control coefficients with regard flux and internal metabolites. D: Control coefficients with regard to the whole pool (σ = G6P+F6P) and the metabolic response time (τ = σ/J).

A		B		C		D	
$^{HK}O^{J}_{G6P}$	0.949	ε^{HK}_{G6P}	−0.025	c^{J}_{HK}	0.832	c^{σ}_{HK}	0.920
$^{HK}O^{J}_{F6P}$	0.803	ε^{HK}_{F6P}	−0.140	c^{J}_{PGI}	−0.009	c^{σ}_{PGI}	−0.089
$^{HK}O^{F6P}_{G6P}$	1.167	ε^{PGI}_{G6P}	4.602	c^{J}_{PFK}	0.177	c^{σ}_{PFK}	−0.830
$^{PGI}O^{J}_{G6P}$	0.058	ε^{PGI}_{F6P}	−3.119	c^{G6P}_{HK}	0.879	c^{τ}_{HK}	0.087
$^{PGI}O^{J}_{F6P}$	−0.249	ε^{PFK}_{G6P}	0.359	c^{G6P}_{PGI}	−0.155	c^{τ}_{PGI}	−0.080
$^{PGI}O^{F6P}_{G6P}$	−0.599	ε^{PFK}_{F6P}	0.500	c^{G6P}_{PFK}	−0.723	c^{τ}_{PFK}	−1.007
$^{PFK}O^{J}_{G6P}$	−0.244			c^{F6P}_{HK}	1.031		
$^{PFK}O^{J}_{F6P}$	−0.157			c^{F6P}_{PGI}	0.093		
$^{PFK}O^{F6P}_{G6P}$	1.552			c^{F6P}_{PFK}	−1.124		

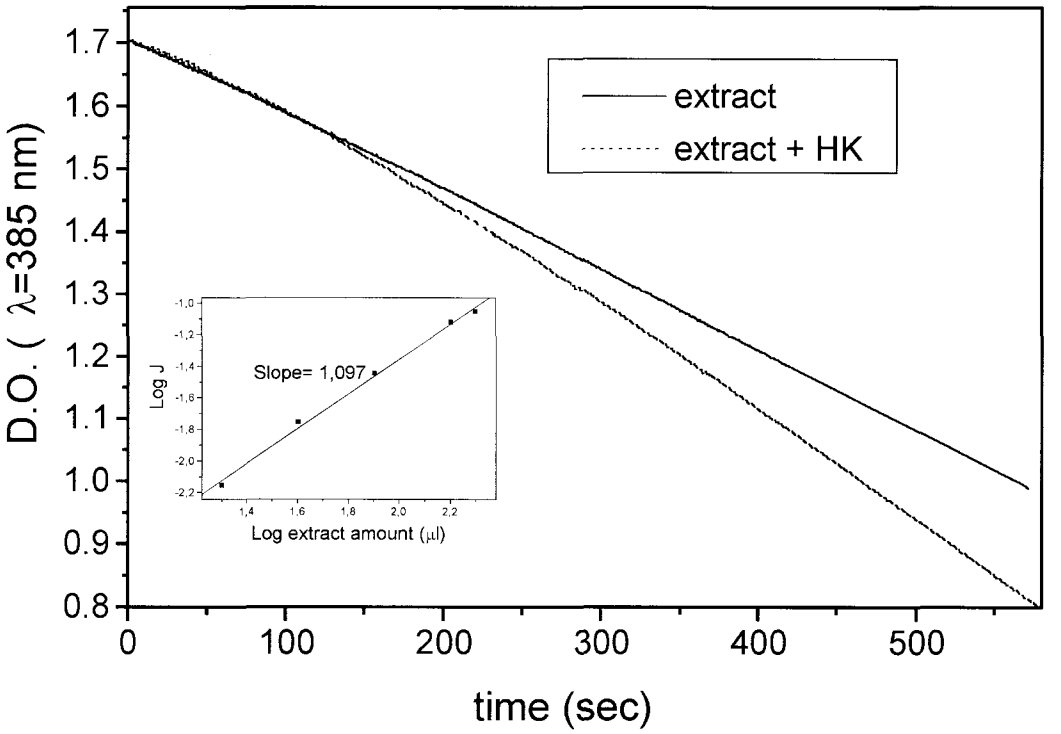

Fig. 1. Time courses of NADH consumption in muscle extract in the absence (dotted line) and presence (solid line) of exogenous HK. The reaction of trisephosphate formation from glucose was coupled with excess TPI/GDH. For other details see Section 2. The inset shows the change of flux as a function of concentration of muscle extract without external enzymes.

σ = G6P+F6P) and with regard to τ (C^{τ}_{Ei}, being $\tau = \sigma/J$), according to Easterby's definition [16], were computed from the flux and concentration coefficients [17] (cf. Table 1D). The time response coefficient is an important property of the system because it can be related to the time necessary to attain a given steady-state rate and its inverse gives an estimate of the average turnover of stocks of internal metabolites [18]. Therefore, it can be concluded that in the upper part of glycolysis, PFK controls mainly the metabolic response time.

3.3. Modelling

To compare the experimental data obtained by two different methods of analysis with that of mathematical modelling, we set up a realistic model using kinetic parameters and rate equations for enzymatic reactions. The rate equations used and the kinetic parameters determined experimentally using the same amount of muscle extract as for the flux measurements appear in Table 2. Using these data the steady-state flux rate (45.4 nmol/mg protein×min) as well as the concentrations of G6P and F6P (38 μM and 14 μM) were computed, with results in good agreement with the experimental data. The correctness of the estimates is supported by experimental observations that the initial rates of reactions catalyzed by HK, GPI and PFK at the endogenous substrate concentrations were similar to the steady-state flux. This was not the case when the kinetic parameters of the commercial muscle enzymes or 10-fold diluted extract (compare to the flux measurements) were used for the model. Using the correct kinetic parameters for modelling all characteristic coefficients of the MCA were computed (cf. Table 3A,B,C). Comparison with the data obtained by co-response analysis (cf. Table 1) shows that the two approaches result in quite similar data sets in most cases. This is especially true for the flux control coefficients, which are perfectly in concordance. The concentration control coefficients are also qualitatively concordant, however, in some cases the experimental and the modelling values (e.g. C^{F6P}_{HK}) have significant differences (such as 60%). This is not surprising, as the error associated with measurements of metabolite levels is larger than that associated with the flux measurements. In the case of elasticity coefficients, important discrepancies were found between the experimental and the modelling data probably because GPI is a rapid-equilibrium enzyme that is virtually without any control role. To check this hypothesis, co-response coefficients obtained as a re-

Table 2
Rate equations used in the kinetic model and kinetic parameters measured for HK, GPI, PFK and ALD in the extract

$v = \dfrac{V^{HK}_{max}[Glu]}{K^{HK}_m + [Glu]\left(1 + \dfrac{[G6P]}{K^{G6P}_I}\right)}$	$K^{HK}_m = 0.4019$ $K^{G6P}_I = 0.111$ $V^{HK}_{max} = 63.0$
$v = \dfrac{\left(\dfrac{V^{GPI_f}_{max}[G6P]}{K^{GPI_f}_m}\right) - \left(\dfrac{V^{GPI_b}_{max}[F6P]}{K^{GPI_b}_m}\right)}{1 + \dfrac{[G6P]}{K^{GPI_f}_m} + \dfrac{[F6P]}{K^{GPI_b}_m}}$	$K^{GPI_f}_m = 0.48$ $K^{GPI_b}_m = 0.272$ $V^{GPI_f}_{max} = 12474$ $V^{GPI_b}_{max} = 18125$
$v = \dfrac{V^{PFK}_{max}[F6P]^n}{(S^{PFK}_{0.5})^n + [F6P]^n}$	$S^{PFK}_{0.5} = 0.061$ $V^{PFK}_{max} = 434$ $n = 1.4744$
$v = \dfrac{V^{ALD}_{max}[FBP]}{K^{ALD}_m + [FBP]}$	$K^{ALD}_m = 0.1297$ $V^{ALD}_{max} = 6000$

V_{max} and K_M are expressed in nmol/min×mg protein and mM respectively

Table 3
Characteristic coefficients of the MCA for the upper part of glycolysis in muscle extract obtained from the kinetic model

A		B		C		D	
$^{HK}O^{J}_{G6P}$	1.290	ε^{HK}_{G6P}	−0.250	c^{J}_{HK}	0.837	c^{σ}_{HK}	0.644
$^{HK}O^{J}_{F6P}$	1.320	ε^{HK}_{F6P}	0	c^{J}_{PFK}	0.010	c^{σ}_{PGI}	−0.029
$^{HK}O^{F6P}_{G6P}$	0.977	ε^{PGI}_{G6P}	19.3	c^{J}_{PFK}	0.151	c^{σ}_{PFK}	−0.615
$^{PGI}O^{J}_{G6P}$	−0.249	ε^{PGI}_{F6P}	−18.4	c^{G6P}_{HK}	0.649	c^{τ}_{HK}	−0.192
$^{PGI}O^{J}_{F6P}$	1.317	ε^{PFK}_{G6P}	0	c^{G6P}_{PGI}	−0.043	c^{τ}_{PGI}	−0.040
$^{PGI}O^{F6P}_{G6P}$	−0.189	ε^{PFK}_{F6P}	1.32	c^{G6P}_{PFK}	−0.605	c^{τ}_{PFK}	−0.766
$^{PFK}O^{J}_{G6P}$	−0.250			c^{F6P}_{HK}	0.634		
$^{PFK}O^{J}_{F6P}$	−0.235			c^{F6P}_{PGI}	0.0082		
$^{PFK}O^{F6P}_{G6P}$	1.061			c^{F6P}_{PFK}	−0.642		

sponse of a perturbation in GPI activity (cf. Table 1A, lines 4, 5 and 6) were replaced by the values evaluated from the kinetic modelling (cf. Table 3A, lines 4, 5 and 6) and then the elasticities, and flux and concentration control coefficients were computed from this corrected co-response matrix (see Table 4A,B,C). As shown in Table 4A, the new set of elasticity coefficients agrees well with those obtained from the model and the spurious elasticities disappeared, indicating that the intrinsic error was associated with the experimental measurement of co-response coefficients with respect to GPI. Therefore, to avoid this inconvenience in the co-response analysis we suggest that experimenters should first test whether there is any rapid-equilibrium enzyme in the pathway and to exclude co-response coefficients with respect to such enzymes from the co-response matrix for computation elasticity and control coefficients.

Comparison of the data obtained in this study allows us to evaluate the advantages and disadvantages of the different approaches. The easiest way to determine the flux control coefficients of a metabolic pathway is to use the 'classical titration' approach, although there are potential errors related to control coefficient determinations [5]. In fact, the coefficients obtained for the skeletal muscle system by this approach under optimized experimental conditions appeared to be correct and similar to those evaluated from the kinetic model. This justifies the conclusion that the flux control coefficient estimates can be directly extracted from experimental observations and they can be trusted. However, it has to be emphasized that if the aim is to determine not only flux control coefficients but also other coefficients for characterization of the control structure of a pathway, then the co-response analysis has the advantage as the enzyme activities used for perturbation of the system do not need to be measured.

Table 4
Characteristic coefficients of the MCA for the upper part of glycolysis in muscle extract obtained from a set of co-response coefficients which includes co-response coefficients for HK and PFK obtained experimentally and co-response coefficients for GPI obtained from the model

A		B		C	
ε^{HK}_{G6P}	−0.248	c^{J}_{HK}	0.793	c^{σ}_{HK}	0.877
ε^{HK}_{F6P}	0.002	c^{J}_{GPI}	0.045	c^{σ}_{GPI}	−0.125
ε^{GPI}_{G6P}	4.60	c^{J}_{PFK}	0.160	c^{σ}_{PFK}	−0.751
ε^{GPI}_{6FP}	−3.11	c^{G6P}_{HK}	0.839	c^{τ}_{HK}	0.084
ε^{PFK}_{G6P}	−0.083	c^{G6P}_{GPI}	−0.183	c^{τ}_{GPI}	−0.170
ε^{PFK}_{F6P}	0.877	c^{G6P}_{PFK}	−0.655	c^{τ}_{PFK}	−0.912
		c^{F6P}_{HK}	0.983		
		c^{F6P}_{GPI}	0.034		
		c^{F6P}_{PFK}	−1.01		

On the other hand, the kinetic parameters applied for modelling were determined in muscle extract at similar conditions as used for control analysis. This is a crucial point in our studies. We also proved that if the kinetic parameters were taken from data obtained with commercial enzyme preparations or even with diluted extract the steady-state values of systemic variables and some coefficients were significantly changed. This could be due to either 'abnormal' kinetics of some of the steps or effects of allosteric ligands resulting in these incorrect values. In addition, if the isolation of enzymes or dilution of the extract destroys important structural aspects such as compartmentations of glycolytic enzymes [19] the behavior of the system does not give necessarily similar estimates of the control properties.

Finally, we can draw some conclusions on the role of HK and PFK in the control of a central pathway. We suggest that HK mainly controls pathway flux, and that consequently changes in the activity of this enzyme can result in relatively large changes in the pathway flux. PFK exerts only small control on the flux and it mainly controls τ. So, an increase in PFK activity affects the flux very little but results in a decrease of the internal metabolite stocks inside the system. Therefore, it is likely that the turnover of the stocks of internal metabolites is higher and the metabolic response time of the overall pathway is decreased by elevation of PFK activity. The fact that the control of J and the control of τ lie in different steps of the pathway explains the early observation of Bücher and Rüsman [20] that large changes in flux, which are characteristic between resting and working states, are possible with only small changes in the relative concentrations of the intermediate metabolites.

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