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Control analysis of mammalian serine biosynthesis

Feedback inhibition on the final step

David A. FELL* and Keith SNELL†‡
*School of Biological and Molecular Sciences, Oxford Polytechnic, Headington, Oxford OX3 0BP, U.K.,
and †Department of Biochemistry, University of Surrey, Guildford GU2 5XH, Surrey, U.K.

The flux of serine biosynthesis in the liver of the normal rabbit, and of the rat on a low protein diet, is most sensitive to the activity of phosphoserine phosphatase (flux control coefficient up to 0.97), the last of the three enzymes in the pathway after it branches from glycolysis. The concentration of the pathway product, serine, has a strong controlling influence on the flux (response coefficient up to −0.64) through feedback inhibition at this step. The pathway is therefore controlled primarily by the demand for serine rather than the supply of the pathway precursor, 3-phosphoglycerate. Under conditions where there is a lower biosynthetic flux, the flux control coefficients of the first two enzymes of the pathway are increased, and are probably dominant in the rat on a normal diet. In rabbit liver, when ethanol is used to inhibit serine biosynthesis, control can be distributed between the three enzymes, even though the reactions catalysed by the first two remain close to equilibrium. Apart from their intrinsic value in aiding the understanding of the regulation of mammalian serine metabolism, our findings illustrate the danger of assuming that there are invariant design principles in the regulation of metabolic pathways, such as feedback control on the first step after a branch.

INTRODUCTION

Serine is an indispensable amino acid for proliferating cells, since it serves as the major intracellular source of one-carbon tetrahydrofolate adducts which donate carbon for the synthesis *de novo* of purine nucleotide and pyrimidine nucleotide bases. We have shown with mitogenically stimulated lymphocytes [1] and with hepatoma cells [2] in culture that, during cellular proliferation, the demands for increased nucleotide biosynthesis for DNA replication are matched by a corresponding increase in serine utilization for nucleotide-precursor formation. In animals, serine is a nutritionally non-essential amino acid, because it can be synthesized in substantial amounts from glucose. From studies in various neoplastic tissues of rat [2–4] and human [5] origin, we have developed the hypothesis that serine utilization for nucleotide synthesis in cancer cells is metabolically coupled to an increased capacity for the intracellular synthesis *de novo* of serine from glycolytic precursors [6], thus ensuring the autonomy of these cells in relation to proliferative capacity. Such a metabolic coupling could be accomplished either as a response to an increased availability of precursors for serine biosynthesis or as a response to an increased demand for serine for nucleotide biosynthesis. It is clearly essential to understand the nature of the control of the serine-biosynthetic pathway in order to address this problem. It is accepted that, in animals at least, there is a single biosynthetic pathway for serine, from 3-phosphoglycerate via phosphohydroxypyruvate and phosphoserine (see [7]).

It is generally expected that biosynthetic pathways will exhibit regulation by feedback inhibition of an end product on the first unique step of the pathway [8]. In the case of the three steps of serine biosynthesis from the glycolytic intermediate 3-phosphoglycerate, this expectation would appear to be fulfilled in bacteria [9,10] and peas (*Pisum sativum*) [11], where the first enzyme of the pathway, 3-phosphoglycerate dehydrogenase, is subject to inhibition by serine. However, the enzyme from animal tissues does not exhibit this inhibition at physiological concentrations of serine [12–15]; instead, the last enzyme of the pathway, phosphoserine phosphatase, is inhibited by serine [16–18]. Furthermore, Guynn and co-workers have shown that the first two reactions of the pathway are close to equilibrium in rabbit liver [19,20] and in liver from rats on a low-protein diet [21]. Indeed, this group of workers suggested that the unusual features of this pathway would make it an interesting object of study from the standpoint of more modern concepts of metabolic control (see [22]; the key concepts in that paper are presented in the current nomenclature system in [22a]). In the present paper we use such concepts to analyse the control of serine biosynthetic flux.

METHODS

The system under consideration in this analysis is that defined by the three unique enzymes of the 'phosphorylated pathway' of serine biosynthesis [7]: 3-phosphoglycerate dehydrogenase, 3-phosphoserine aminotransferase and 3-phosphoserine phosphatase. The first two enzymes in this defined pathway involve NAD^+/NADH and glutamate/2-oxoglutarate as co-factors, and the source substrate for the pathway is 3-phosphoglycerate derived from glycolysis. Since the flux through the serine-

‡ To whom correspondence and reprint requests should be addressed.

biosynthetic pathway is small compared with the major fluxes of glycolysis and carbohydrate metabolism, it is reasonable to assume that the three enzymes will have no significant determining effect on the cellular concentrations of phosphoglycerate or the levels of NAD^+/NADH and glutamate/2-oxoglutarate. Therefore it is legitimate to consider these metabolites as externally set boundary conditions (see [23]) for the serine-biosynthetic pathway and to apply the summation theorem for control coefficients only to those components (the three unique enzymes) of the system as defined.

The flux control coefficient, C_i^J, is formally defined as:

$$C_i^J = \frac{\delta J/J}{\delta e_i/e_i} \quad (1)$$

where J is the flux under consideration, and e_i is the concentration of enzyme e_i [22,24]. The elasticity coefficient (ϵ) of an enzyme e_i with respect to a metabolite X_j, is defined as:

$$\epsilon_j^i = \frac{\delta V_i/V_i}{\delta X_j/X_j} \quad (2)$$

where V_i is the velocity of the enzyme and X_j the concentration of X_j [22,24]. The response coefficient, R_k^J, for the effect of an independent metabolite, X_k (i.e. one whose concentration is not determined by the system under consideration) acting via enzyme i on the flux J is given by [22]:

$$R_k^J = C_i^J \cdot \epsilon_k^i \quad (3)$$

The elasticities of the enzymes of the serine-biosynthetic pathway to their substrates and products have been obtained in the following manner.

(*a*) 3-Phosphoglycerate dehydrogenase and phosphoserine aminotransferase were treated as a 'grouped' reaction [25] because the undetectably low tissue contents of the intermediate phosphohydroxypyruvate [20,21] prevented resolution of their separate contributions. The elasticities of this enzyme group to the pathway source, phosphoglycerate, and to the product, phosphoserine, were calculated from the expressions relating substrate and product elasticities to displacement from equilibrium for a near-equilibrium reaction [26,27]. The term contributed from the fractional saturation was neglected, since it can be shown to be much smaller than the mass-action ratio term by calculations based on the maximal activities of the enzymes in rabbit liver [19] and estimation of the pathway flux from the kinetics of the phosphatase (see below). Writing Γ for the mass-action ratio, $K_{eq.}$ for the equilibrium constant for the combined reaction [28], '1+2' to signify these first two enzymes, 3PG for phosphoglycerate and PSer for phosphoserine, we have:

$$\epsilon_{3PG}^{1+2} = 1/(1-\Gamma/K_{eq.}) \quad (4)$$

$$\epsilon_{PSer}^{1+2} = -\Gamma/K_{eq.}/(1-\Gamma/K_{eq.}) \quad (5)$$

Mass-action ratios were calculated from the average tissue metabolite contents given in [20,21] and the cytosolic NAD^+/NADH ratio as calculated from the lactate dehydrogenase reaction (see Table 1). The whole tissue contents of glutamate and 2-oxoglutarate were used in the calculations on the assumption that they closely approximate to the cytosolic contents, which have been shown to represent 80 and 76% respectively of the total cellular amounts of these metabolites [29].

(*b*) The elasticities of phosphoserine phosphatase (denoted reaction '3') to phosphoserine and serine were calculated by numerical differentiation of the enzyme rate law fitted to the kinetics results reported in [18] for the rat liver enzyme. The non-linear least-squares fitting was accomplished using a BASIC program for the BBC microcomputer (D. A. Fell & H. M. Sauro, unpublished work). Fits were compared for the serine inhibition of phosphoserine phosphatase treated as mixed, non-

Table 1. Concentrations of intermediary metabolites in the serine-biosynthetic pathway in rabbit liver *in vivo*

The mean values (from six to eight animals per group) for the metabolite contents *in vivo* in freeze-clamped rabbit liver were taken from LaBaume *et al.* [20] and converted into concentrations by assuming an intracellular water content of 0.8 ml/g of liver. The cytoplasmic free [NAD^+]/[NADH] ratio was calculated from mean values of pyruvate and lactate concentrations [20] and from the equilibrium constant for L-lactate dehydrogenase (1.11×10^{-11} M). Γ^{1+2} is the mass-action ratio calculated for the combined 3-phosphoglycerate dehydrogenase and phosphoserine aminotransferase reactions ([NADH]·[phosphoserine][2-oxoglutarate]/[NAD]·[3-phosphoglycerate]·[L-glutamate]). $\Gamma^{1+2}/K_{eq.}^{1+2}$ was then calculated from the combined $K_{eq.}$ of 2.44×10^{-4} M for the two enzymes [20]. All rabbits were starved for 24 h before being injected intraperitoneally with 0.9% (w/v) NaCl (saline control), ethanol (1 g/kg) in saline, glucose (7.2 g/kg) in saline, or ethanol and glucose together. Livers were freeze-clamped, within 10 s of cervical dislocation, 1 h after the injections [20]. The statistical significance of differences from values in the control group are taken from LaBaume *et al.* [20] and indicated as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

		[Metabolite] (mM)			
Metabolite	Treatment...	Control	Glucose	Ethanol	Glucose + ethanol
Serine		2.71	4.08**	2.19	2.69
Phosphoserine		0.446	0.815**	0.025**	0.014***
2-Oxoglutarate		0.158	0.120	0.094	0.055***
Glutamate		3.84	4.39	6.48**	5.86*
3-Phosphoglycerate		0.255	0.291	0.016***	0.024***
[NAD^+]/[NADH]...		500	863	146	55
$\Gamma^{1+2}10^4$...		1.44	0.886	1.53	0.988
$\Gamma^{1+2}/K_{eq.}^{1+2}$...		0.588	0.364	0.625	0.405

competitive, simple uncompetitive and uncompetitive behaviour [30], with the latter characterized by the equation:

$$v = \frac{V_{max} \cdot [PSer] \cdot a}{[PSer] + K_m \cdot a} \quad (6)$$

where $a = (1 + [Ser]/K_i')/(1 + [Ser]/K_i)$, and resulting in the best fit, when $K_m = 0.089$ mM, $K_i = 0.60$ mM and $K_i' = 16.5\ \mu$M. The kinetics results for the chicken liver enzyme [17] were also fitted best by this equation with similar parameters, except that K_i' was smaller, but the experiments had used concentrations of serine below those normally found in liver. Using the elasticities calculated from this alternative set of parameters (for the chicken liver enzyme) made little difference to the control coefficients reported in the present paper; the parameters from the results with rat liver in [18] were used because they are for a mammalian liver enzyme and they cover the range of serine concentrations observed *in vivo*.

The flux control coefficients were obtained by the matrix method [23,25] as the numerical solutions of:

$$\begin{bmatrix} C^J_{1+2} \\ C^J_3 \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ \epsilon^{1+2}_{PSer} & \epsilon^{3}_{PSer} \end{bmatrix}^{-1} \begin{bmatrix} 1 \\ 0 \end{bmatrix} \quad (7)$$

where J = flux through the pathway. Note that the control coefficients for the first two reactions in the pathway cannot be separately resolved and the reported coefficients, C^J_{1+2}, are the sum of their individual contributions.

The flux response coefficients [22] to phosphoglycerate and serine are given by:

$$R^J_{3PG} = C^J_{1+2} \cdot {}^{\kappa}\epsilon^{1+2}_{3PG};$$
$$R^J_{Ser} = C^J_3 \cdot {}^{\kappa}\epsilon^{3}_{Ser} \quad (8)$$

RESULTS AND DISCUSSION

LaBaume *et al.* [20] reported the tissue contents *in vivo* of the metabolites involved in the serine-biosynthetic pathway for rabbit liver under control conditions (24 h-fasted animals) and 1 h after the intraperitoneal injection of glucose, ethanol, or glucose plus ethanol together (see Table 1). This gave a set of results for the operation of the pathway over a range of 3-phosphoglycerate concentrations and various redox ratios. We have determined the elasticities of the pathway enzymes towards the metabolites for the average values of the metabolite concentrations in each treatment group (Table 1); the calculated elasticities and the flux control and response coefficients are shown in Table 2.

In the control and glucose treatments, the flux control coefficient of phosphoserine phosphatase is close to 1 (Table 2), so it behaves like a 'rate-limiting enzyme' for pathway flux, albeit one that is unusual in being the last step of a biosynthetic pathway. That it has the larger flux control coefficient can be understood from the connectivity theorem [22]; its elasticity with respect to its substrate phosphoserine is small, as this metabolite concentration is well above the K_m value (an effect which is in fact accentuated by the uncompetitive inhibition), whereas the elasticity of the first two pathway enzymes to this metabolite is large because they are close to equilibrium. Serine inhibits the enzyme with the largest flux control coefficient (phosphoserine phosphatase), giving a response coefficient for pathway flux close to $-\frac{2}{3}$; i.e. a 3% rise in serine concentration will cause a 2% decrease in biosynthetic flux. On the other hand, although the elasticity of the first two enzymes with respect to 3-phosphoglycerate is relatively large, the very low value of their flux control coefficient results in a very small flux response coefficient, so that the biosynthetic flux will be relatively unaffected by changes in glycolysis/gluconeogenesis which might alter the concentration of 3-phosphoglycerate.

Under the unusual (for a rabbit) conditions of the ethanol treatments (with and without glucose), the flux control coefficient of phosphoserine phosphatase falls because of the increase in its elasticity with respect to

Table 2. Elasticities, flux control coefficients and flux response coefficients for the serine-biosynthetic pathway in rabbit liver *in vivo*

The control functions were calculated and defined as described in the Methods section. J refers to overall pathway flux; $^{1+2}$ refers to the combined pathway enzymes, 3-phosphoglycerate dehydrogenase and phosphoserine aminotransferase; ^{3} refers to phosphoserine phosphatase. Other abbreviations: 3PG, 3-phosphoglycerate; PSer, L-3-phosphoserine; Ser, L-serine. Details of the experimental treatment of rabbits *in vivo* are given in the legend to Table 1.

Control function	Experimental treatment...	Parameter value: Control	Glucose	Ethanol	Glucose + ethanol
Elasticities					
${}^{\kappa}\epsilon^{1+2}_{3PG}$		2.43	1.57	2.67	1.68
ϵ^{1+2}_{PSer}		−1.43	−0.57	−1.67	−0.68
ϵ^{3}_{PSer}		0.041	0.017	0.466	0.576
${}^{\kappa}\epsilon^{3}_{Ser}$		−0.649	−0.661	−0.356	−0.287
Control coefficients					
C^J_{1+2}		0.03	0.03	0.22	0.46
C^J_3		0.97	0.97	0.78	0.54
Response coefficients					
R^J_{3PG}		0.07	0.05	0.58	0.77
R^J_{Ser}		−0.63	−0.64	−0.28	−0.16

phosphoserine, which now has a concentration near to the apparent K_m. At the same time the decrease in the elasticity for serine inhibition accentuates the loss of response of the pathway flux to control by the serine level (although the biosynthetic flux can be calculated to be about half of its control value). The summation theorem [22] requires that the decrease in the flux control coefficient of phosphoserine phosphatase is accompanied by a rise in the flux control coefficients of the other two enzymes, so that the control of pathway flux is now distributed between all of the enzymes, none of which can be described as 'rate-limiting'. Note that the first two enzymes can contribute to the control of flux even though they remain close to equilibrium, providing a concrete example of a possibility predicted on theoretical grounds [22,31]. The consequence of this shift of control is that the response coefficient for 3-phosphoglycerate on the pathway flux increases, showing that the low 3-phosphoglycerate concentration under these conditions (presumably reflecting the adverse change in the redox state) is limiting the flux.

The absolute and relative activities of the enzymes of serine biosynthesis vary in different mammalian livers according to dietary, hormonal and developmental factors [6,7]. The study analysed above [20] had used rabbit liver because the pattern of serine metabolism in this species may more closely resemble that of the human than does that of the rat. However, there are comparable data for rat liver [21] that allow some assessment of the effects of altered enzyme levels on the distribution of control in this species. In the rat on a normal laboratory diet, the activities of the first two enzymes of the serine-biosynthetic pathway are low, and the concentration of phosphoserine is undetectable in liver. It is not possible to repeat the calculations shown above [see the Methods section, paragraph (*a*)] because the first two enzymes are no longer close to equilibrium. It is likely, however, that ϵ^{1+2}_{PSer} has a small negative value; this follows from eqn. (5), which shows that the elasticity must approach closer to zero as the displacement from equilibrium increases, although an exact value cannot be given because eqn. (5) will be a poor approximation in these circumstances. ϵ^{3}_{PSer} can be calculated [see the Methods section, paragraph (*b*)] to be in the range from 0.76 to (a maximum of) 1.0. With these considerations in mind, the connectivity theorem requires that the flux control coefficient of phosphoserine phosphatase will now be smaller than that of the first two pathway enzymes. The distribution of control is thus different from that in normal rabbit liver, and the two enzymes that operate near to equilibrium in the rabbit are non-equilibrium in the rat. There is therefore no single principle of control that can, *a priori*, be applied to this pathway. The first two enzymes of the pathway are induced over 10-fold in rats fed on a low-protein diet; phosphoserine phosphatase activity increases only about 2-fold [21]. The metabolite concentrations indicate that the first two enzymes will bring the mass-action ratio nearer to equilibrium, close enough for the simple elasticity calculated used above to be accurate to within about 10%. Under these circumstances the elasticity of phosphoserine phosphatase with respect to phosphoserine is decreased because of the increased phosphoserine concentration, so that the flux control coefficient of phosphoserine phosphatase will be about 0.8; that of the other two enzymes must therefore be about 0.2. Thus under conditions where the flux of serine biosynthesis is increased in the rat, the pattern of control approaches more closely to that in the control experiments with the rabbit.

General conclusions

The present control analysis has revealed that, at high flux, the pathway of serine biosynthesis is regulated by demand rather than supply. This conclusion follows from the flux response coefficients with respect to the pathway precursor, 3-phosphoglycerate, and the pathway product, serine. Thus the demand for serine utilization will control serine biosynthetic flux, and the extent to which this utilization involves serine in the provision of precursors for nucleotide synthesis will determine the metabolic coupling between these processes. In cancer cells, with greatly increased serine biosynthesis and with an enzymic pattern that favours utilization of serine for nucleotide formation [2–6], there will be a high degree of metabolic coupling.

Finally, the results show that it is practicable to apply modern concepts of control analysis, e.g. [22], to pathways such as serine biosynthesis on the basis of entirely conventional experiments in enzymology and metabolism. This pathway provides a further example that control need not be focused entirely on a single step, but can be distributed between steps, and that the distribution can change with alterations in flux, and in metabolite and enzyme levels. Under various circumstances it provides counter-examples to concepts such as key regulatory enzymes being at the beginning of a pathway rather than at the end, and enzymes operating close to equilibrium having little regulatory significance [32].

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