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Control Analysis of Photosynthetic Sucrose Synthesis: Assignment of Elasticity Coefficients and Flux-Control Coefficients to the Cytosolic Fructose 1,6-Bisphosphatase and Sucrose Phosphate Synthase

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Control analysis of photosynthetic sucrose synthesis: assignment of elasticity coefficients and flux-control coefficients to the cytosolic fructose 1,6-bisphosphatase and sucrose phosphate synthase

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The use of elasticity coefficients and flux-control coefficients in a quantitative treatment of control is discussed, with photosynthetic sucrose synthesis as an example. Experimental values for elasticities for the cytosolic fructose 1,6-bisphosphatase and sucrose phosphate synthase are derived from their *in vitro* properties, and from an analysis of the *in vivo* relation between fluxes and metabolite levels. An empirical factor α, describing the response of the fructose 2,6-bisphosphate regulator cycle to fructose 6-phosphate is described, and an expression is derived relating α to the elasticities of the enzymes involved in this regulator cycle. The *in vivo* values for elasticities and α are then used in a modified form of the connectivity theorem to estimate the flux control coefficients of the cytosolic fructose 1,6-bisphosphatase and sucrose phosphate synthase during rapid photosynthetic sucrose synthesis.

In this paper I shall review how ideas about the control of metabolism have developed in other fields, and will then apply these ideas to the regulation of photosynthetic sucrose synthesis. My main aim is to explore how control in this pathway is organized and distributed between various enzymes, and to ask whether we can make quantitative statements about their response to effectors and their contribution to control *in vivo*. It is written in the belief that a proper understanding of the nature of control is an essential prerequisite for the evaluation of changes in flux and metabolite levels, and that it is essential to escape from an oversimplified use of the notion of 'limitation' in photosynthetic research. It is intended as a complement to recent papers by Dietz (1986), Heber *et al.* (1988) and Woodrow (1986).

1. Approaches to the study of control

The earliest approach to control was the concept of a 'pacemaker' reaction, in which one enzyme was seen as controlling or 'limiting' flux through the pathway. This is reminiscent of the concept of 'limiting factors' developed by Blackman at the beginning of this century. A series of criteria emerged for identifying such 'regulatory' reactions (Newsholme & Start 1973; Rolleston 1972).

(*a*) The enzyme should possess the requisite 'regulatory' properties, e.g. allosteric regulation, substrate cooperativity.

(*b*) The enzyme should be present at activities that are not greatly above ('in excess of') the required flux through the pathway.

(*c*) The enzyme should catalyse a 'non-equilibrium' or 'irreversible' reaction.

(*d*) There should be characteristic changes of the substrate concentration, which are reciprocal to the change of flux.

As these criteria were applied, a series of problems emerged. First, it soon became apparent that a pathway often contained more than one enzyme with the requisite 'regulatory' properties. For example, the above criteria were often fulfilled by hexokinase, phosphofructokinase and pyruvate kinase in glycolysis. There was also variation from tissue to tissue (see Rolleston (1972) for references). Secondly, problems arose with the simplifying notion that enzymes catalysing equilibrium reactions could be ignored. The distinction has always been somewhat arbitrary as it is not obvious when a reaction becomes 'irreversible'. It was also realized that marked changes of the individual reactants could be generated in equilibrium reactions involving pairs of substrate and product (Rolleston 1972). For example, during photosynthetic glycerate 3-phosphate (PGA) reduction, a decrease in the supply of ATP and/or NADPH will lead to an increased PGA/triose phosphate ratio because $[PGA]/[GAP] = k_{eq}\,[ADP]\,[P_i]\,[NADP]/[ATP]\,[NADPH]\,[H^+]$. (GAP, glyceraldehyde 3-phosphate.) The changed PGA and triose phosphate will in turn influence other reactions: high PGA inhibits ribulose bisphosphate carboxylase–oxygenase (Rubisco) (Foyer *et al.* 1987) and phosphoribulokinase (Gardeman *et al.* 1983), whereas low triose phosphate will restrict activity of the stromal fructose 1,6-bisphosphatase (FBPase) and other enzymes involved in the regeneration of ribulose 1,5-bisphosphate (RuBP).

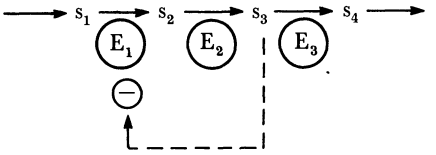
Control undoubtedly involves an interplay between several enzymes. Unfortunately, this leads to obvious complications when we try to interpret the significance of changes in metabolite levels, and assess the contributions of the various enzymes to control. Kacser & Burns (1973) and Heinrich & Rapoport (1974) made the decisive step of explicitly recognising (*a*) that control is a property of an entire pathway rather than the individual enzymes, and (*b*) that control is shared between the enzymes in a pathway. Even more important was the development of concepts that allow precise statements about control in these complex systems. Central to their approach is a clear separation between the properties of the parts of the system (e.g. the inherent properties of a particular enzyme) and the properties of the system, which emerge from the interaction of all the enzymes and metabolites.

The properties of the enzymes are expressed as *elasticity coefficients* ($\epsilon = (\delta v/v)/(\delta[s]/[s])$). This is a quantitative measure for the fractional change in activity ($\delta v/v$) that results from a fractional change in the concentration of a particular substrate ($\delta[s]/[s]$), when all other potential substrates and effectors are held constant. The activity of the enzyme will ultimately depend upon the sum of the interactions between the enzyme and all of its substrates and effectors, each of which has an elasticity coefficient (i.e. $\delta v/v = \epsilon_{s_1}\,\delta[s_1]/[s_1] + \epsilon_{s_2}\,\delta[s_2]/[s_2] \ldots \epsilon_{s_n}\,\delta[s_n]/[s_n]$).

The properties of the system are expressed as the *flux-control coefficient*, $C = (\delta J/J)\,(\delta E/E)$, which is defined with respect to one particular flux, and one particular enzyme. It describes the fractional change of flux through the pathway ($\delta J/J$) that results from a fractional change of enzyme activity ($\delta E/E$). This provides a quantitative expression for the contribution of the enzyme to control of flux through the pathway.

The flux-control coefficient and the elasticity coefficient are related, because any one enzyme is flanked by metabolites which interact with this enzyme in a concentration-dependent manner, and which also interact with further enzymes in the system. This means that a

perturbation at any one point in the system will be transmitted throughout the system, and can be illustrated by taking a simple pathway,



and perturbing the activity ($\delta v/v$) of the first enzyme. This will generate changes in the concentrations of the metabolites (s_1, s_2, s_3) which interact with E_1. These changed concentrations, in turn, will affect activity of E_1, and will also affect the activity of other enzymes E_2, E_3. These interactions, which depend on the elasticity coefficient for each particular enzyme and metabolite, will determine how much of the initial change of E_1 activity is transmitted as a change of flux ($\delta J/J$) through the pathway. For example, suppose that $\varepsilon_{s_2}^{E_1}$ and $\epsilon_{s_3}^{E_1}$ are large and negative (i.e. E_1 is very sensitive to product inhibition by s_2 and feedback inhibition by s_3), whereas $\epsilon_{s_2}^{E_2}$ and $\epsilon_{s_3}^{E_3}$ are small (i.e. E_2 and E_3 only respond sluggishly to rising substrate concentrations). We can envisage in this case how very little of the original increment of E_1 activity remains as a change of flux, because increasing concentrations of s_2 and s_3 just reinhibit E_1 without significantly affecting the flux through the subsequent enzymes. The flux-control coefficient of E_1, $C_{E_1} = (\delta J/J)/(\delta E_1/E_1)$, will be very low in this case.

This example illustrates how the flux-control coefficient of an enzyme emerges from the interplay of the metabolite concentrations and the elasticity coefficients of all the enzymes in the system. In particular, when a metabolite is shared by two enzymes, an enzyme with a high sensitivity to this metabolite (i.e. a high elasticity) will have a low flux-control coefficient. The relation between the flux-control coefficients and the elasticity coefficients is quantitatively expressed in the *connectivity theorem* (Kacser & Burns 1973), which states that the flux-control coefficient of two adjacent steps is inversely related to the ratio of the two elasticity coefficients for the shared substrate, i.e. $C_{E_1}/C_{E_2} = -\epsilon_{s_2}^{E_2}/\epsilon_{s_2}^{E_1}$.

It should also now be apparent that the magnitude of flux-control coefficient is not a property of the enzyme *per se*. Rather, it depends on the response of all the other enzymes in the system, i.e. it depends on the flux-control coefficients of all the other enzymes. In this case it is not surprising that, for a given pathway, the flux-control coefficient for all of its enzymes sum to a finite value, which has been shown to be unity (*flux summation property*) (Kacser & Burns 1973, 1979).

In applying this approach, it has become evident that reactions that lie close to equilibrium exert little control on flux, because an enzyme catalysing a reversible near-equilibrium reaction has a very high elasticity to its substrates and, consequently, a low flux-control coefficient. However, it has also become evident that control is shared between many enzymes of a pathway, and that the distribution of control between these enzymes can vary in a very flexible manner, depending on the conditions (Groen *et al.* (1982); see also Kacser & Porteus (1987) for further references).

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2. Quantitative analysis of flux control at the non-equilibrium reactions in photosynthetic sucrose synthesis

In previous articles I have presented empirically based models for regulation of the cytosolic FBPase (Herzog *et al.* 1984; Stitt & Heldt 1985; Stitt *et al.* 1987) and sucrose phosphate synthesis (SPS) (Stitt *et al.* 1987, 1988) which describe (*a*) how each of these enzymes is stimulated by a rising supply of photosynthate after a 'threshold' concentration is exceeded and (*b*) how this 'threshold' can be modified, e.g. to allow alterations of photosynthate partitioning (Stitt *et al.* 1987, 1988) or as an adaptation to temperature (Stitt & Groβe 1988). These earlier studies also indicated how flux at the FBPase and SPS could be integrated (see also Kerr & Huber 1987). A simplified scheme of this interaction is given in figure 1. Imagine that a perturbation (either increased flux at the FBPase, or inhibition of SPS) leads to an increase of the linking pool of hexose phosphate. This would in turn tend to stimulate SPS (Doehlert & Huber 1983; 1984) because one of its substrates (fructose 6-phosphate; F6P) and an activator (glucose 6-phosphate; G6P) rise; simultaneously the increased F6P would tend to increase fructose 2,6-bisphosphate (F2,6BP) (Stitt *et al.* 1984), and, hence, re-inhibit cytosolic FBPase. In this way, the flux at the two enzymes would be brought back into step. This account, however, is still strictly qualitative and does not reveal how control is shared between these enzymes.

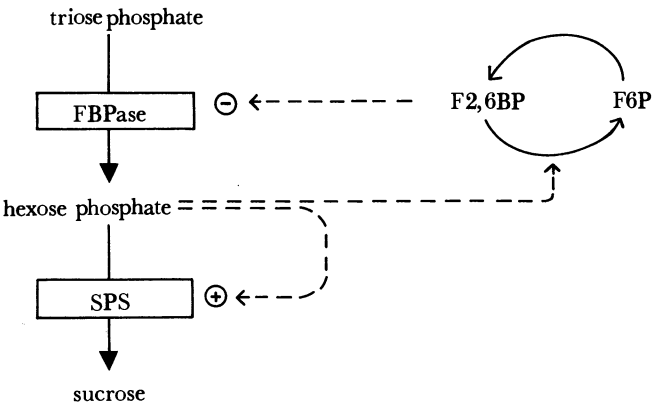

Figure 1. The integration of photosynthetic sucrose synthesis. The scheme shows the metabolic fluxes to carbon (——) and F2,6BP regulator cycle. Regulatory interactions are shown as dashed lines (----). The 'feed forward' control of F2,6BP by triose phosphate, PGA and falling P_i is omitted (see Stitt *et al.* 1987).

As already discussed, the distribution of control is inversely related to the response of two enzymes to a shared metabolite pool (the connectivity theorem). It should therefore be possible to develop a quantitative account of control in the cytosol if we know (*a*) how SPS activity responds to a change of the hexose phosphate pool, and can compare this with (*b*) the response of F2,6BP to a change of F6P and (*c*) the response of the cytosolic FBPase to a change of F2,6BP. I shall set up a simplified expression to describe each of these interactions, and shall then provide estimates for the relevant elasticity coefficients by analysis of the *in vitro* kinetic properties of the enzymes, and by analysis of the *in vivo* relation between fluxes and metabolite levels. I shall then use these values to illustrate how control is distributed between the cytosolic FBPase and SPS.

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A simplified scheme is given in figure 1, showing the metabolic fluxes to sucrose and the regulatory interactions in the cytosol. In this scheme, reactions leading from triose phosphate to hexose phosphate are treated as a group, and termed FBPase. Because triose phosphate isomerase and aldolase are near equilibrium (Gerhardt *et al.* 1987), this is probably not a major oversimplification. The reactions leading from hexose phosphate to sucrose are also treated as a group, and termed SPS. The reaction catalysed by sucrose phosphatase, and the removal of pyrophosphate (PP_i) are also significantly removed from equilibrium (Gerhardt *et al.* 1987; Weiner *et al.* 1987). This means that the estimated *in vivo* elasticities and control coefficients will refer to this group of reactions, rather than just to SPS. Nevertheless, this approach provides a useful initial approximation for investigating how control is distributed in the cytosol.

The cytosolic FBP*ase*

I shall represent the fractional response of FBPase activity ($\delta v/v$) to a fractional change of triose phosphate and F2,6BP as

$$\frac{\delta v}{v} = \frac{\delta[\text{triose phosphate}]}{[\text{triose phosphate}]}\epsilon^{\text{F}}_{\text{TP}} + \frac{\delta[\text{F2,6BP}]}{[\text{F2,6BP}]}\epsilon^{\text{F}}_{\text{26P}} + \frac{\delta[\text{F6P}]}{[\text{F6P}]}\epsilon^{\text{F}}_{\text{F6P}}$$

where $\epsilon^{\text{F}}_{\text{TP}}$, $\epsilon^{\text{F}}_{\text{26P}}$ and $\epsilon^{\text{F}}_{\text{F6P}}$ represent the elasticities for triose phosphate, F2,6BP and F6P respectively. This expression replaces the true substrate, FBP, by triose phosphate because the latter can be more readily estimated for the cytosol (see Gerhardt *et al.* 1987). As F6P has negligible effect on activity in the presence of F2,6BP (Stitt *et al.* 1985) (i.e. $\epsilon^{\text{F}}_{\text{F6P}} \rightarrow 0$), we can delete the last term. This simplified expression also assumes that other potential effectors of the FBPase (e.g. AMP, Mg^{2+}, H^+) are not changing.

Tables 1 and 2 provide estimates of the elasticities for F2,6BP and triose phosphate from *in vitro* data. They are estimated from the slope of the relation between enzyme activity and the concentration of a particular effector, and were estimated for a range of conditions. The elasticity for F2,6BP ($\epsilon^{\text{F}}_{\text{26P}}$) is negative, because increasing F2,6BP inhibits activity. The values are large (about -2) at limiting substrate concentration and decrease as the enzyme becomes more substrate-saturated. This, of course, just reflects the fact that F2,6BP inhibits in a competitive manner to the substrate. The elasticity coefficient for triose phosphate is positive, because activity is stimulated by rising substrate. As triose phosphate is increased, $\epsilon^{\text{F}}_{\text{TP}}$ rises to a maximum of about four, and then declines to zero, reflecting the sigmoidal substrate saturation curve for FBP (Herzog *et al.* 1984). The elasticities for triose phosphate are further increased because FBP varies as the square of the triose phosphate concentration. These results predict that if FBPase operates *in vivo* in the region where it is partly saturated by substrate,

TABLE 1. ELASTICITY COEFFICIENTS OF FBPASE FOR F2,6BP, ESTIMATED FROM *IN VITRO* DATA

(Values were estimated from the slope of the inhibition curves in data replotted from Herzog *et al.* (1984).)

		saturation with FBP (%)			
[F2,6BP]/μM	[AMP]/mM	20	40	60	80
1	0	−2.3	−1.8	−1.3	−0.6
1	0.2	−1.5	−1.4	−1.0	−0.3
5	0	−2.0	−1.6	−1.3	—
5	0.2	−1.9	−1.5	−1.2	—

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TABLE 2. ELASTICITY COEFFICIENTS OF THE CYTOSOLIC FBPase FOR TRIOSE PHOSPHATE, ESTIMATED FROM *IN VITRO* DATA

(Values of triose phosphate were estimated from the slope of the substrate saturation curves in figure 2 of Herzog *et al.* (1984). The FBP concentrations were converted to concentrations assuming equilibrium reactions at aldolase and triose phosphate isomerase (k_{eq} 10100 and 22 respectively, [FBP] = 460 [DHAP]^2). DHAP, dihydroxycacetone phosphate. Values for ϵ^F_{FBP} were converted to ϵ^F_{TP} with the relation $\epsilon^F_{TP} = 2\epsilon^F_{FBP}$. This follows because [FBP] $\propto$ [triose phosphate]^2, which can be differentiated to give δ[FBP]/[FBP] = 2 δ[triose phosphate]/[triose phosphate], and substituted into the expression $\epsilon^F_{FBP} = (\delta v/v)/\delta$[FBP]/[FBP].)

[F2,6BP]/μM	[AMP]/mM	0.15	0.21	0.29	0.40	0.51	0.59	0.72	0.83	0.94	1.08	1.18
1	0	0.6	1.3	3.4	4.1	1.9	1.0	0.4	—	—	—	—
1	0.2	—	—	—	0.9	2.3	2.9	4.3	1.2	—	—	—
5	0	—	—	—	0.3	1.2	3.4	4.0	3.5	2.9	0.3	—
5	0.2	—	—	—	—	—	—	—	1.0	2.2	3.4	4.4

it would respond much more sensitively to changes of triose phosphate ($\epsilon^F_{TP} = 2$–4) than to changes of F2,6BP ($\epsilon^F_{26P} = -0.5$ to -1.8).

Experimental values for the *in vivo* elasticities can be obtained if metabolism is peturbed and the changes of flux ($\delta J/J$) are compared with the changes in the levels of the effectors ($\delta[s_1]/[s_1]$, $\delta[s_2]/[s_2] \ldots \delta[s_n]/[s_n]$). For a given experimental treatment, $\delta J/J = \epsilon_{s_1}\, \delta[s_1]/[s_1] + \epsilon_{s_2}\, \delta[s_2]/[s_2] \ldots \epsilon_{s_n}\, \delta[s_n]/[s_n]$.

In the simplest case, with two effectors this expression contains two unknowns, ϵ_{s_1} and ϵ_{s_2}. If two independent perturbations are carried out, it is possible to write two simultaneous equations that can then be solved. Table 3 summarizes the fractional changes in the rate of sucrose synthesis and the levels of various metabolites when sucrose synthesis is restricted by a falling rate of CO_2 fixation, or by accumulation of sucrose in the leaf. The values are derived from the empirical model in Stitt & Heldt (1985) and allow elasticities of +1.0 for triose

TABLE 3. FRACTIONAL CHANGES OF METABOLITES, SPS ACTIVATION AND FLUXES TO SUCROSE AND EXPERIMENTAL ESTIMATES OF ELASTICITY COEFFICIENTS

(The results in spinach leaf discs are derived from the slope of the model shown in Heldt & Stitt (1985) in the region where CO_2 fixation decreases because of lower light or CO_2, and the region where sucrose synthesis decreases in response to accumulation of sucrose. The changes of SPS activity ($\delta SPS/SPS$) are estimated from Stitt *et al.* (1988) and unpublished results that compare the change of SPS activity (*SPS*) in selective assay conditions (2 mM F6P, 10 mM G6P, 5 mM P_i) as sucrose synthesis decreases in response to falling light, or accumulation of sucrose. The data for *Clarkia xantiana* are from E. Neuhaus, A. L. Kruckeberg, R. Feil and M. Stitt (unpublished data) and show the response of metabolite levels and fluxes to a decrease of cytosolic phosphoglucose isomerase (PGI) activity to 18% of the wild type level (Jones *et al.* 1986) in low light (125 μmol quanta m^{-2} s^{-1}) or high light (1000 μmol quanta m^{-2} s^{-1}). The pools of F6P (*) and G6P (**) are shown separately.)

		fractional metabolite change					
material	treatment	$\frac{\delta J}{J}$	$\frac{\delta[\text{triose phosphate}]}{[\text{triose phosphate}]}$	$\frac{\delta[\text{F2,6BP}]}{[\text{F2,6BP}]}$	$\frac{\delta[\text{hexose phosphate}]}{[\text{hexose phosphate}]}$	$\frac{\delta[\text{sucrose}]}{[\text{sucrose}]}$	$\frac{\delta SPS}{SPS}$
spinach	decreased CO_2 fixation	−0.33	−0.2	+0.3	−0.2	0	−0.15
	accumulation of sucrose	−0.33	+0.5	+1.7	+0.5	+8	−0.55
Clarkia	decreased PGI (high light)	−0.08	+0.22	+1.1	+0.5** −0.1*	—	—
	decreased PGI (low light)	−0.23	−0.01	+0.6	+0.3** −0.13*	—	—

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phosphate and −0.5 for F2,6BP to be estimated (table 4). Table 3 also shows the fractional changes (compared with the wild type) of sucrose synthesis and metabolites in mutants of *Clarkia xantiana* with low cytosolic phosphoglucose isomerase activity (E. Neuhaus, A. Kruckeberg, R. Feil and M. Stitt, unpublished data). The estimated elasticities for triose phosphate and FBP are +1.6 and −0.4, respectively (table 4). These experimental estimates are provisional, as they are based on overall metabolite levels and we have excluded other possible effectors of the cytosolic FBPase. Nevertheless, they are in agreement with the analysis of the *in vitro* data in predicting that the cytosolic FBPase may respond three to fourfold more sensitively to changes of triose phosphate than to changes of F2,6BP. They also predict that ϵ^{F}_{26P} will be less than unity.

TABLE 4. EXPERIMENTAL ESTIMATION OF ELASTICITIES

(Values are estimated from the fractional changes of fluxes and metabolites shown in table 3, by using two simultaneous equations of the form $\delta J/J = \epsilon_{s_1}\,\delta[s_1]/[s_1] + \epsilon_{s_2}\,\delta[s_2]/[s_2]$, as described in the text. For *Clarkia*, separate elasticities of SPS to G6P (**) and F6P (*) are shown.)

	ϵ^{F}_{TP}	ϵ^{F}_{26P}	ϵ^{SPS}_{HP}	$\epsilon^{SPS}_{sucrose}$
spinach	+1.0	−0.5	+1.0	0.03
Clarkia	+1.5	−0.4	+2.00** / +0.4*	—

Sucrose phosphate synthase

The activity of SPS can be described as

$$\frac{dv}{v} = \frac{\delta[SPS]}{[SPS]} + \frac{\delta[F6P]}{[F6P]}\epsilon^{SPS}_{F6P} + \frac{\delta[UDPG]}{[UDPG]}\epsilon^{SPS}_{UDPG} + \frac{\delta[G6P]}{[G6P]}\epsilon^{SPS}_{G6P} + \frac{\delta[P_i]}{[P_i]}\epsilon^{SPS}_{P_i}.$$

The term δ[SPS]/[SPS] is included because SPS is subject to 'coarse' control. This can be represented as equivalent to changes in the amount of the enzyme, with an enzyme concentration elasticity coefficient of unity (Kacser & Burns 1987). This approach is valid provided the 'coarse' control involves (*a*) changes in the amount of the enzyme, or (*b*) interconversion between an active form and an inactive form that has negligible activity *in vivo*. Available evidence suggests the former is true in soybean (Kerr & Huber 1987) and the latter in spinach (Stitt *et al.* 1988), although this interpretation will remain tentative until the molecular mechanisms have been characterized. The remaining terms reflect the dependence on the substrates (F6P, UDPG), and the allosteric activator (G6P) and inhibitor (P_i) (Doehlert & Huber 1987).

Table 5 summarizes effector elasticity coefficients estimated from the *in vitro* data of Doehlert & Huber (1987). The elasticity coefficients for F6P (ϵ^{SPS}_{F6P}) and UDPG (ϵ^{SPS}_{UDPG}) are always below unity and fall towards zero with rising substrate concentration. This reflects the hyperbolic substrate saturation response to both substrates. When G6P is included in equilibrium with F6P, a combined elasticity coefficient for hexose phosphates (ϵ^{SPS}_{HP}) is obtained that reaches peak values of about +1.7. This reflects the apparent sigmoidal response to F6P in the presence of phosphoglucose isomerase (Doehlert & Huber 1984; Stitt *et al.* 1988) and will represent the case *in vivo*. I also estimated the elasticity coefficient for a marginal shift of G6P (ϵ^{SPS}_{G6P}) at a 3:1 ratio, representing what would happen as the reaction catalysed by phosphoglucose isomerase moved away from equilibrium, and obtained a value of about +0.8. The elasticity coefficient for P_i ($\epsilon^{SPS}_{P_i}$) was about −0.4.

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Table 5. Elasticity coefficients of SPS for UDPG, combined hexose monophosphates, for F6P and G6P separately, and for P_i

(ϵ^{SPS}_{UDPG} was estimated from assays with 8 mM F6P, 10 mM P_i (figure 4 of Doehlert & Huber (1984)); ϵ^{SPS}_{F6P} was estimated at 8 mM UDPG, zero P_i, from figure 5 of Doehlert & Huber (1984). ϵ^{SPS}_{HP} represents the response to an equilibrium mixture of F6P and G6P, with F6P at the concentrations shown in the table and G6P in fourfold excess, 8 mM UDPG and zero P_i (figure 5 of Doehlert & Huber 1984). $\epsilon^{SPS}_{P_i}$ was estimated from an assay containing 1 mM F6P, 3 mM G6P, 8 mM UDPG, varying P_i from 5 to 10 mM (Doehlert & Huber 1983). ϵ^{SPS}_{G6P} is estimated for conditions of 1 mM F6P, 3 mM G6P, 8 mM UDPG, with P_i as shown in the table (figure 3 of Doehlert & Huber (1983)) and represents the response to a marginal displacement of G6P away from the equilibrium with F6P.)

[UDPG]/mM	ϵ^{SPS}_{UDPG}	[F6P]/mM	ϵ^{SPS}_{F6P}	ϵ^{SPS}_{HMP}	P_i/mM	ϵ^{SPS}_{G6P}	$\epsilon^{SPS}_{P_i}$
1	0.7	0.1	—	0.3	0	0.1	—
2	0.5	0.3	—	1.3	1	0.24	—
4	0.5	0.5	0.7	1.6	5	0.65	−0.43
8	0.4	0.7	—	1.6	10	0.75	—
16	0.3	1.0	—	1.0	20	0.60	—
—	—	1.5	0.6	0.3	—	—	—
—	—	2.5	0.5	—	—	—	—
—	—	3.5	0.5	—	—	—	—
—	—	4.5	0.4	—	—	—	—

The *in vivo* elasticities of SPS were estimated from the data in table 3. For spinach, we eliminated the term for uridine diphosphoglucose (UDPG), and included G6P and F6P as a composite term to yield the elasticity for hexose phosphate. This is justified provided UDPG does not change significantly (i.e. $\delta[UDPG]/[UDPG] \rightarrow 0$), and G6P and F6P remain close to their expected equilibrium (Stitt & Heldt 1985; Stitt *et al.* 1987). We included a notional term, $\epsilon^{SPS}_{sucrose}$ for restriction of the enzyme by sucrose or sucrose phosphate as sucrose accumulates in the leaf. It was also necessary to include values for the changes of SPS activity that result from 'coarse' control. These were obtained by measuring the SPS activity in rapidly prepared extracts by using assay conditions that are selective for the active form of SPS (Stitt *et al.* 1988). From the resulting equation

$$\frac{\delta J}{J} = \frac{\delta[SPS]}{[SPS]} + \frac{\delta[\text{hexose phosphate}]}{[\text{hexose phosphate}]}\epsilon^{SPS}_{HP} + \frac{\delta[sucrose]}{[sucrose]}\epsilon^{SPS}_{sucrose},$$

we estimated an elasticity for hexose phosphate (ϵ^{SPS}_{HP}) of +1.0, whereas the notional elasticity for inhibition by sucrose was negligible. The *Clarkia xantiana* phosphoglucose isomerase dosage mutants allowed estimation of the separate elasticities for F6P and G6P, yielding values of +0.4 and +2.0 respectively (table 4).

Interaction between F6P *and* F2,6BP

The response of F2,6BP to rising F6P will depend on (*a*) the stimulation of F2,6BP kinase and (*b*) the inhibition of F2,6BPase. The change of F2,6BP will be even greater than the changes in the individual enzyme activities, because they operate as a cycle. Taking the simplest case, where further effectors do not change, the rate of F2,6BP synthesis (v^{+1}) and degradation (v^{-1}) can be expressed as

$$\frac{\delta v^{+1}}{v^{+1}} = \frac{\delta[F6P]}{[F6P]}\epsilon^{2\text{-k}}_{F6P} + \frac{\delta[F2,6BP]}{[F2,6BP]}\epsilon^{2\text{-k}}_{26P},$$

$$\frac{\delta v^{-1}}{v^{-1}} = \frac{\delta[F6P]}{[F6P]}\epsilon^{2\text{-P}}_{F6P} + \frac{\delta[F2,6BP]}{[F2,6BP]}\epsilon^{2\text{-P}}_{26P},$$

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where $\epsilon^{2\text{-k}}_{\text{F6P}}$ and $\epsilon^{2\text{-k}}_{\text{26P}}$ are the elasticites of F6P 2-kinase for F6P and F2,6BP, and $\epsilon^{2\text{-P}}_{\text{F6P}}$ and $\epsilon^{2\text{-P}}_{\text{26P}}$ are the elasticities of F2,6BPase for F6P and F2,6BP. As we are comparing steady states, $\delta v^{+1}/v^{+1} = \delta v^{-1}/v^{-1}$ and the above expression can be arranged as

$$\frac{\delta[\text{F2,6BP}]}{[\text{F2,6BP}]} \Big/ \frac{\delta[\text{F6P}]}{[\text{F6P}]} = \frac{\epsilon^{2\text{-k}}_{\text{F6P}} - \epsilon^{2\text{-P}}_{\text{F6P}}}{\epsilon^{2\text{-P}}_{\text{26P}} - \epsilon^{2\text{-k}}_{\text{26P}}} = \alpha.$$

This relation allows us to relate the amplification factor, α, to the elasticites of the synthesizing and degrading enzymes for F6P and F2,6BP. It might be noted that this expression can be readily adapted to provide an estimate of the maximum potential activation for each other effector (e.g. PGA, triose phosphate, P_i) of this regulator cycle.

Analysis of the *in vitro* properties of F6P 2-kinase (Stitt *et al.* 1984) shows there is a sigmoidal response to F6P in the presence of PGA or triose phosphate, the elasticity for F6P ($\epsilon^{2\text{-k}}_{\text{F6P}}$) reaching maximum values of about 1.6. This value will be decreased by rising P_i, because the substrate saturation becomes hyperbolic. I shall assume there is negligible product inhibition of F6P 2-kinase (i.e. $\epsilon^{2\text{-k}}_{\text{26P}} \rightarrow 0$). F2,6BPase is non-competitively inhibited by F6P with an elasticity ($\epsilon^{2\text{-P}}_{\text{F6P}}$) of about -0.4, which decreases to -0.1 or even lower if P_i is included. Substrate saturation of F2,6BPase is hyperbolic at zero or low P_i (i.e. $\epsilon^{2\text{-P}}_{\text{26P}}$ decreases from 1 to 0, with a value of about 0.5 in the region where it is half saturated), but becomes sigmoidal at higher P_i ($\epsilon^{2\text{-P}}_{\text{26P}}$ has values above unity).

Obviously, α can adopt a wide range of values, depending on the conditions. If we substitute the maximum values for $\epsilon^{2\text{-k}}_{\text{F6P}}$ and $e^{2\text{-P}}_{\text{F6P}}$ into the above expression, and assume F2,6BPase is operating at about half substrate saturation, the amplification factor, α, would be about four. This would apply to conditions of low P_i. It may be noted that α decreases markedly if P_i is present at 5–10 mM (see below for discussion of the possible significance). It could obviously be much higher if F2,6BPase were to become substrate saturated.

This wide range of potential values underlines the importance of reducing the relation between F6P and F2,6BP to a simple term that can easily be given an empirical value. Table 6 lists several empirical values for α, measured in conditions where rising F6P is leading to an increase of F2,6BP. The measured values lie between 2 and 3. This suggests that our theoretical estimate is not unrealistic; indeed somewhat lower values would be expected *in vivo* because (*a*) P_i will be present (see above) and (*b*) rising F2,6BP will inhibit the cytosolic FBPase and this, in turn, would lead to higher triose phosphate and PGA, which themselves act to inhibit F6P 2-kinase and dampen the response to rising F6P.

Table 6. Experimental values for α, the amplification factor for the relation between changes of F2,6BP and of F6P.

(The results are taken from (*a*) the rise of F2,6BP and F6P as sucrose accumulates in leaf discs during photosynthesis in saturating light and CO_2 (Stitt & Heldt 1985), (*b*) the relation between F2,6BP (Stitt *et al.* 1983) and cytosolic F6P (Gerhardt *et al.* 1987) levels in spinach leaves in ambient conditions, and (*c*) the relation between F6P and F2,6BP in *Clarkia* mutants with decreased PGI activity (E. Neuhaus, A. Kruckeberg, R. Feil and M. Stitt, unpublished data), in high (1000 μmol quanta m^{-2} s^{-1}) or low (125 μmol quanta m^{-2} s^{-1}) light.)

treatment	$\frac{\delta[\text{F2,6BP}]/[\text{F2,6BP}]}{\delta[\text{F6P}]/[\text{F6P}]}$
sucrose accumulation in saturating light and CO_2	3.1
sucrose accumulation in ambient conditions	1.8
decreased PGI activity in high light	2.2
decreased PGI activity in low light	2.0

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Estimation of flux-control coefficients

The connectivity theorem can now be applied to estimate how control is shared between FBPase and SPS. In doing this, a modification is included to take account of the indirect action of the shared pool (hexose phosphate) on the cytosolic FBPase. The distribution of control between SPS ($C_{SPS}^{sucrose}$) and the FBPase ($C_{F}^{sucrose}$) during sucrose synthesis will be given as

$$C_{F}^{sucrose}/C_{SPS}^{sucrose} = -\epsilon_{HP}^{SPS}/(\epsilon_{26P}^{F}\alpha),$$

reflecting the fact that a fractional change, δ[hexose phosphate]/[hexose phosphate] of hexose phosphate will be accompanied by a fractional change, αδ[hexose phosphate]/[hexose phosphate] of F2,6BP. Figure 2 shows how the relative control strengths of SPS and FBPase will vary as α increases at three different values (−1, −2.5, −5) of the $\epsilon_{HP}^{SPS}/\epsilon_{26P}^{F}$ ratio. At the *in vivo* values during rapid photosynthesis ($\epsilon_{HP}^{SPS} = 1.0$; $\epsilon_{26P}^{F} = -0.4$; $\alpha = 2$–3), we find that control is shared almost equally between SPS and the cytosolic FBPase.

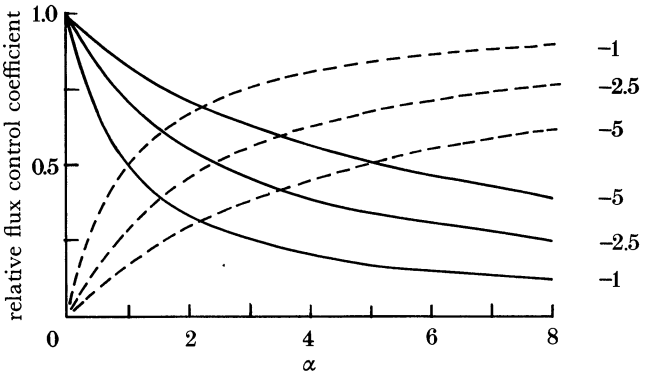

FIGURE 2. Model for the relative control strengths of SPS and the cytosolic FBPase during photosynthetic sucrose synthesis. C_F (——) and C_{SPS} (----) are shown for three different values of the $\epsilon_{HP}^{SPS}/\epsilon_{26P}^{F}$ ratio as the value of α is increased. The values are computed as $C_F = x/(1+x)$ where $x = \epsilon_{HP}^{SPS}/\epsilon_{26P\,\alpha}^{F}$, the expression being derived from the modified connectivity theorem $C_F/C_{SPS} = \epsilon_{HP}^{SPS}/C_{26P\,\alpha}^{F}$. The term α = (δ[F2,6BP]/[F2,6BP)]/(δ[F6P]/[F6P]).

The previous discussion has emphasized that the elasticity coefficients of complex enzymes can adopt a wide range of values. We must therefore expect that control will shift between the FBPase and SPS depending on the conditions. Control will shift towards the cytosolic FBPase if (*a*) SPS becomes more sensitive to activation by hexose phosphate, i.e. ϵ_{HP}^{SPS} increases; (*b*) FBPase becomes less sensitive to inhibition by F2,6BP, i.e. ϵ_{26P}^{F} decreases; or (*c*) F2,6BP responds less sensitively to a change of F6P, i.e. α decreases. Detailed studies, including the use of mutants, will be needed to analyse these interactions.

However, predictions can already be made about how control may shift between these enzymes. For example, high P_i will decrease the response of F2,6BP to a change of F6P (see above). This means that the flux-control coefficient of the FBPase may increase in conditions when metabolites are low and P_i is high (e.g. low light or CO_2). This is understandable because control at the FBPase would ensure a first priority for maintaining high metabolite pools in the stroma. Conversely, during very rapid photosynthesis and starch accumulation, metabolite levels are high and P_i is probably low (Gerhardt *et al.* 1987). In these conditions,

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F2,6BP may respond more sensitively to changes of F6P, and control would shift towards SPS.

This idea might be expressed non-rigorously by saying that SPS may 'limit' the maximal rate of sucrose synthesis, whereas control of FBPase becomes increasingly important at lower flux rates and allows 'regulation' of sucrose synthesis to maintain adequate levels of metabolites for Calvin cycle turnover. However, I hope that it is apparent why a more rigorous treatment is worthwhile. It allows us to see the distribution of control in any given condition as the product of interactions within the entire pathway. It also encourages us to carry out experiments and process the results in a way that allows simple conclusions to be drawn from a bewildering welter of data. Further, it forces us to integrate our knowledge about the properties of individual enzymes, and their operation within a pathway. Last, but not least, it allows us to replace emotive words with numerical concepts.

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