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Control of the rate of photosynthetic carbon dioxide fixation *

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A simplified model of the reductive pentose phosphate pathway of photosynthesis is analysed in order to quantify the degree to which each of the constituent reactions controls the rate of CO_2 fixation (given by the control coefficient). The analysis focuses on the four largely irreversible reactions of the cycle together with the first irreversible reaction in the sucrose and starch synthetic pathways. The model assumes that the other reactions are at equilibrium. The photorespiratory and electron transport systems are not included in the model. The analysis demonstrates that: (1) an analytical approach can be used to investigate the distribution of flux control in autocatalytic and moiety-conserved cycles; (2) measurements of enzyme kinetic parameters and certain fluxes and substrate concentrations can be used to solve the equations defining the enzyme control coefficients; (3) the conservation of total stromal phosphate and the intricate regulatory mechanisms of the photosynthetic system result in a relationship between the control coefficients that is complex and may defy any intuitive assessment of 'rate limitation'; (4) ribulose-1,5-bisphosphate carboxylase/oxygenase may, under certain conditions, be a major controller of the rate of CO_2 fixation and, by regulating the concentration of ribulose 1,5-bisphosphate, may be important in governing the ratio of organic to inorganic phosphate in the stroma; (5) the other enzymes may also serve an important role in determining the distribution of phosphate between organic and inorganic species because they catalyze reactions at the branch points between starch and sucrose synthesis and ribulose 1,5-bisphosphate regeneration; (6) these enzymes that catalyze 'branch-point' reactions may have negative control coefficients because of their ability to reduce the total concentration of cycle intermediates; (7) an approach combining the use of the equations presented in this paper and flux and substrate concentration measurements may be adequate for determining the control coefficients of several enzymes of the reductive pentose phosphate pathway.

Introduction

The present view of the control of photosynthetic CO_2 fixation centers very much around the reaction catalysed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase. There is substantial evidence from studies of several plant species that at relatively high light intensities the enzyme is essentially 'substrate-saturated' with regard to ribulose 1,5-bisphosphate and is therefore said to be the prime determinant of the rate of CO_2 fixation [1]. Even when the light intensity is lowered, it has been shown that there is a more or less parallel decline in the proportion of ribulose-1,5-bisphosphate carboxylase/oxygenase in the

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catalytically active state suggesting that the enzyme may also be important in flux control under these conditions [2]. This situation is complicated, however, by fact that the proportions of catalytically active fructose 1,6-bisphosphatase [3,4], sedoheptulose 1,7-bisphosphatase (Woodrow, I.E., unpublished results) and probably ribulose 5-phosphate kinase also decrease with declining light intensity. How much influence these enzymes and the electron-transport system have over the rate of CO_2 fixation, under these conditions, is not clear although relatively high quantum efficiencies at lower light intensities indicate that most of the control must reside in the electron-transport chain. It is also unclear how the rate of CO_2 fixation is controlled at elevated CO_2 concentrations [1,5]. Walker et al. [6] have shown that, as the CO_2 level is raised above the ambient concentration, one can induce oscillations in the rate of CO_2 fixation such that there are transient rates that exceed the steady-state rate. One might conclude from this observation that ribulose 1,5-bisphosphate carboxylase/oxygenase is not significantly 'substrate-saturated' and, therefore, is not the predominant factor controlling the steady state rate of CO_2 fixation. Another important situation in which the distribution of flux control is undefined is when a build-up of a product such as sucrose effects an inhibition of the rate of CO_2 fixation. It is suspected that changes in the concentration of inorganic phosphate mediate a feedback inhibition of photosynthesis, but a detailed mechanism has not yet been elaborated [7,8].

There is a need to develop a means of quantifying the degree to which the elements of the photosynthetic system contribute to flux control because it seems that there are situations in which control is shared in some manner and not simply concentrated in one element. Not only would such a quantitative hierarchy of 'importance' of the constituent enzymes be valuable in describing the regulation of steady-state photosynthesis, but it would also provide a logical framework in which to examine the implications of the modification – with regard to either catalytic capabilities or concentration – of these enzymes. The possibility of such genetic manipulation of metabolic systems makes it especially important for a mathematical model to be able to deal with the concept of 'connectivity': the complexity of changes that could be brought about within a system by an ostensibly isolated change.

The present paper outlines the way in which the analysis of flux control pioneered by Kacser and Burns [9] and Heinrich and Rapoport [10] can be applied to the photosynthetic system. This is not a full model of photosynthesis but a model that provides a base upon which a more detailed model can be built and demonstrates some fundamental properties of an autocatalytic cycle with one import and two export pathways.

Description of the model

A number of approximations are made that form the assumptions of the model of the reductive pentose phosphate pathway of photosynthesis (Fig. 1). (A) It is assumed that only enzymes catalysing reactions that are largely irreversible are important in controlling the rate of CO_2 fixation. These are: ribulose 1,5-bisphosphate carboxylase/oxygenase (1); stromal fructose 1,6-bisphosphatase (2); sedoheptulose 1,7-bisphosphatase (3); ribulose 5-phosphate kinase (4); cytosolic fructose 1,6-bisphosphatase (5); and ADP-glucose pyrophosphorylase (6). (B) The metabolite pools of the pathway that are linked by reactions close to thermodynamic equilibrium are grouped (see legend to Fig. 1) and assumed to lie at equilibrium. This means that, for the purposes of the current analysis, the linked pools behave very much as one pool [11]. It is acknowledged, however, that in reality the combined effect of the reversible reactions may be significant and that an examination of these reactions would have to be made in a more complex model. (C) Only the first largely irreversible reactions in the starch and sucrose synthetic pathways (reactions 5 and 6 of Fig. 1) are included in the model because, in a linear system, it is usually these reactions that are important in controlling the flux [9]. Nevertheless, it is acknowledged that the flux control properties attributed to these individual reactions in the ensuing analysis represent the properties of the whole sucrose or starch synthetic pathway because of the 'connections' due to feedback inhibition (e.g., sucrose phosphate synthetase is 'connected' in terms of flux control to cytosolic fructose 1,6-bis-

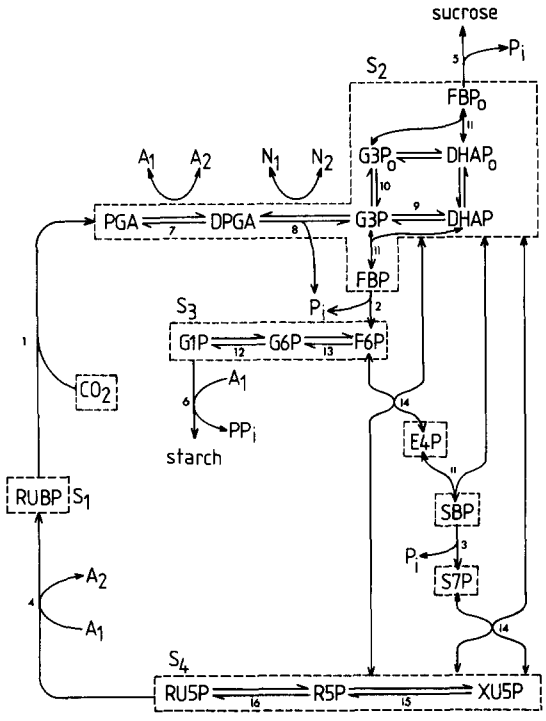

Fig. 1. Model of the C_3 reductive pentose phosphate pathway of photosynthesis excluding the electron transport and photorespiratory systems. The reactions represent: (1) ribulose 1,5-bisphosphate carboxylase/oxygenase; (2) stromal fructose 1,6-bisphosphatase; (3) sedoheptulose 1,7-bisphosphatase; (4) ribulose 5-phosphate kinase; (5) cytosolic fructose 1,6-bisphosphatase; (6) ADP-glucose pyrophosphorylase; (7) 3-phosphoglycerate kinase; (8) glyceraldehyde 3-phosphate dehydrogenase; (9) triose phosphate isomerase; (10) chloroplast envelope phosphate translocator; (11) aldolase; (12) phosphoglucomutase; (13) hexose phosphate isomerase; (14) transketolase; (15) pentose phosphate epimerase and isomerase; (16) pentose phosphate isomerase. The intermediates are abbreviated as follows: RuBP, ribulose 1,5-bisphosphate; PGA, 3-phosphoglycerate; DPGA, 1,3-diphosphoglycerate; G3P, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; FBP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate; G1P, glucose 1-phosphate; E4P, erythrose 4-phosphate; SBP, sedoheptulose 1,7-bisphosphate; S7P, sedoheptulose 7-phosphate; Xu5P, xylulose 5-phosphate; R5P, ribose 5-phosphate; Ru5P, ribulose 5-phosphate; PP_i, inorganic pyrophosphate; P_i, inorganic phosphate; A1, ATP; A2, ADP; N1, NADPH; and N2, NADP. The subscript o indicates that the compound lies in the cytosol, otherwise compounds are in the stroma. It is assumed that the export of one molecule of triose phosphate or glucose 1-phosphate is balanced by the import of one molecule of P_i into the system. It is also assumed that all of the reactions, other than those catalyzed by enzymes (1)–(6), lie at equilibrium (see Appendix). The steady-state concentrations of all the intermediates can be described using the concentrations of S_1–S_4, where S_1 is the total concentration of RuBP and S_2–S_4 are grouped pools within which the components are at equilibrium.

phosphatase by fructose 6-phosphate). (D) The stoichiometry of the model is written such that the total chloroplast phosphate pool – organic plus inorganic – remains constant [12]. The output of each fructose 1,6-bisphosphate and glucose 1-phosphate molecule is balanced by the input of two and one molecules of inorganic phosphate, respectively. The bisphosphatase reactions (2 and 3) each liberate one molecule of inorganic phosphate. (E) Two modes of interaction between inorganic phosphate and the cycle are considered: as an inhibitor of reactions 2, 3, 4, 5 and 6 and of triose phosphate export from the chloroplast; and as a requirement for the conversion of ribulose 5-phosphate to ribulose 1,5-bisphosphate. In reality, the phosphorylation of ribulose 5-phosphate involves ATP and is, therefore, coupled to the electron transport system. But, as it is not the intention of the present study to examine the role of electron transport in controlling the rate of CO_2 fixation, inorganic phosphate is simply considered as a substrate of this phosphorylation reaction in order to balance the distribution of phosphate and to make the cycle sensitive to a depletion of stromal inorganic phosphate.

It is assumed in the following analysis that the concentrations of the substrates are much greater than those of the enzymes. This is certainly not true in the case of ribulose 1,5-bisphosphate carboxylase/oxygenase [13], but the use of this assumption is justified as it greatly simplifies the mathematical analysis and does not affect the conclusions drawn from this analysis in a qualitative manner. A relatively high K_m for ribulose 1,5-bisphosphate was chosen for the carboxylase so as to produce steady state concentrations of this substrate that are comparable to those measured in vivo.

The fluxes through the various reactions in the steady state are given by the following equations:

$$v_1 = 6v_5 + 6v_6 \quad (1)$$

$$v_2 = v_6 + v_3 \quad (2)$$

$$v_4 = 3\,v_3 \quad (3)$$

$$v_1 = v_4. \quad (4)$$

The subscripts refer to the reaction numbers shown in Fig. 1. These represent the fluxes expected in

the reductive pentose phosphate pathway in the absence of photorespiration (Fig. 1). The photorespiratory system did not have a significant effect on the results when included in a simplified form (data not shown) and was therefore excluded from the present model. The rate equations used in the computer simulations are given in the Appendix.

The enzyme kinetic parameters used in the computer simulations were estimated from data derived from studies of isolated enzymes (see, e.g., Refs. 14–18). In the cases where few data are available, the parameters were selected such that they resulted in substrate pool sizes similar to those measured in intact leaves and protoplasts (e.g., Ref. 19). The standard parameters are given in the legend to Fig. 2.

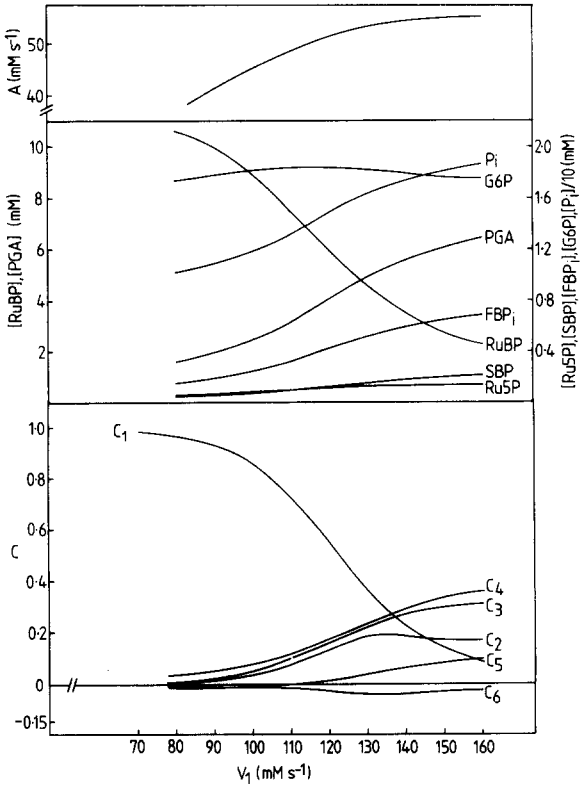

Fig. 2. Changes in the control coefficients of the largely irreversible enzymes, the concentrations of several intermediates and the rate of CO_2 fixation (A) in response to changes in the activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (V_1). The abbreviations are the same as those defined in the legend to Fig. 1. The model was analyzed by computer simulation using a fourth order Runge-Kutta method of numerical integration. The enzyme rate equations and equilibrium criteria are given in the Appendix. The initial conditions were: $V1 = 80$; $V2 = 30$; $V3 = 23$; $V4 = 90$; $V5 = 25$; $V6 = 8$ (all $mM \cdot s^{-1}$); $K_1^C = 1$; $K_1^R = 1$; $K_2 = 0.07$; $K_i^2 = 12$; $K_3 = 0.013$; $K_i^3 = 6.6$; $K_4^P = 5$; $K_4^R = 0.01$; $K_i^4 = 2$; $K_i^5 = 4$; $K_i^6 = 5$; $K_5 = 0.04$; $K_a^6 = 3$; $K_i^7 = 5$; $K_i^0 = 1$; $S_1 = 9.93$; $S_2 = 3.04$; $S_3 = 2.63$; $S_4 = 0.31$ and $P_i = 10.81$ (all mM).

Derivation of control equations

The two principal types of coefficients used in the ensuing analysis are the control (C_P^V) and the elasticity (ϵ_S^v) coefficients. The nomenclature follows that outlined by Burns et al. [20]. The coefficients are defined as follows:

$$C_P^V = \frac{\partial \ln V}{\partial \ln P} = \frac{\partial V}{\partial P}\frac{P}{V} \tag{5}$$

where V, in the present case, stands for the rate of CO_2 fixation (the superscript is omitted in the ensuing analysis as all the control coefficients refer to CO_2 fixation) and P for any parameter (independent variable) whose change causes the change in the rate of CO_2 fixation;

$$\epsilon_S^v = \frac{\partial \ln v}{\partial \ln S} = \frac{\partial v}{\partial S}\frac{S}{v} \tag{6}$$

where v, in the present case, stands for the catalytic rates of the enzymes of the model, and S for any molecular species (effector) that affects the catalytic rate of the enzyme. The elasticities can be calculated by partial differentiation of the enzyme rate equations with respect to the appropriate metabolite [9]. There is a fundamental difference between the control and elasticity coefficients that is central to the theory of metabolic control. The elasticity coefficient defines the response of the rate of catalysis of the isolated enzyme to changes in the concentration of molecular species involved in or affecting the reaction. In contrast, the control coefficient defines the response of the whole system to changes in the kinetic properties of any one constituent enzyme.

The summation theorem [9] underlies the current analysis and states that the sum of flux control coefficients for the enzyme in the system is unity:

$$\sum_{P=1}^{6} C_P^v = 1 \tag{7}$$

A modified version of the summation theorem may be needed in a model of photosynthesis where the concentrations of enzymes are comparable to those of their respective substrates because sequestration of some compounds would alter the level of total, free phosphate (organic plus inorganic) in the stroma (Woodrow, I.E. and Farquhar, G.D., unpublished data). Eqn. 7 is valid in the present analysis, however, since it was assumed that enzyme concentrations are such that no significant substrate sequestration can occur. The use of this assumption does not affect the results of this study in a qualitative sense.

Complications in the derivation of the control coefficients arise in the present model of photosynthesis for two reasons: (1) there are two branch points in the pathway; and (2) the conservation of phosphate makes it impossible to adjust a particular metabolite concentration independently [11,21]. The derivation of the equations describing the relationship between the control coefficients of the two export reactions and that of reaction 2 is given in the Appendix. The second problem was overcome by applying a modified version of the connectivity theorem [11], the results of which are also presented in the Appendix. This analysis provides equations from which general expressions for the control coefficients for the six key enzymes of the cycle can be derived. These equations involve measurable quantities: fluxes, substrate concentrations and elasticities.

Control by ribulose-1,5-bisphosphate carboxylase/oxygenase

Ribulose-1,5-bisphosphate carboxylase/oxygenase has both a unique and an important role in controlling the rate of photosynthetic CO_2 fixation. Computer simulation of the model shows that as the ribulose 1,5-bisphosphate concentration rises – effected by inactivation of the carboxylase – the control coefficient of the carboxylase (C_1) approaches unity and those of the other enzymes approach zero (Fig. 2). Similar decreases in the activity of the five other enzymes, however, do not result in such a clear-cut change in the distribution of flux control. For example, when the concentration of active stromal fructose 1,6-bisphosphatase is lowered, the control coefficient of the carboxylase and kinase decline and the magnitude of all the other coefficients increases (Fig. 3). This pattern reflects two fundamental properties at the reductive pentose phosphate pathway: (1) the substrate pools of all of the enzymes, other than the carboxylase, are linked by the reversible reactions such that changes in the steady state concentration of one pool must be accompanied by changes in others; and (2) several substrate pools are directly – or indirectly as components of the grouped pools – involved in both metabolite export and ribulose 1,5-bisphosphate regeneration. These points will be discussed in more detail in the next section.

In the present model, it is clear that when the carboxylase is completely saturated with ribulose 1,5-bisphosphate, the enzyme must have total control over the rate of CO_2 fixation. But since the amount of stromal phosphate is limited, it is necessary to pose the question: how closely must saturation with ribulose 1,5-bisphosphate be approached before the carboxylase assumes a control coefficient close to unity? Solution of the connectivity equations derived from the general connectivity expression (see Appendix) shows that the control coefficient of the carboxylase is a function of not only the ribulose 1,5-bisphosphate con-

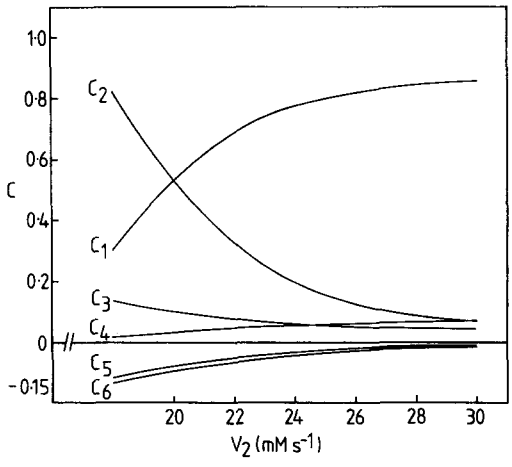

Fig. 3. The change in the control coefficients of the enzymes catalysing the six largely irreversible reactions of the model system effected by changing the activity of the stromal fructose 1,6-bisphosphatase (V_2). The initial conditions of the computer simulation were as described in the legend to Fig. 2.

centration and the kinetic properties of the enzyme (ϵ^1_{RuBP}) but also pool sizes and elasticity terms related to the other reactions of the cycle. Thus, the degree of saturation of the carboxylase required for a control coefficient of a given magnitude is dependent upon the state of the whole system and it may be quite misleading to assess the importance of the enzyme in controlling the rate of CO_2 fixation using estimates of ribulose 1,5-bisphosphate levels and carboxylase activities alone. The data in Fig. 4 substantiates this point showing how a change in the relationship between C_1 and the ribulose 1,5-bisphosphate concentration is affected by changing the constant describing feedback inhibition of ribulose 5-phosphate kinase by ribulose 1,5-bisphosphate.

The connectivity equations show that, over a wide range of conditions, the state of the whole photosynthetic system must be considered when assessing the control coefficient of the carboxylase. In a more realistic model of photosynthesis,

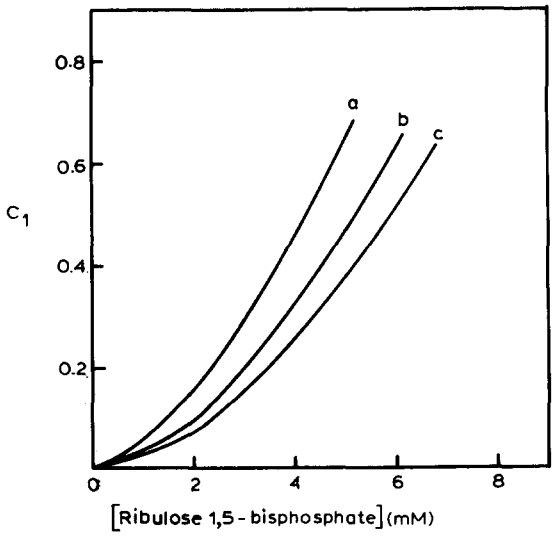

Fig. 4. The effect of the magnitude of the constant describing inhibition of ribulose 5-phosphate kinase by ribulose 1,5-bisphosphate on the relationship between the control coefficient of the carboxylase (C_1) and the concentration of ribulose 1,5-bisphosphate. The inhibition constants (K_i^5) used were: (a) 0.5 mM; (b) 2 mM; and (c) 20 mM. Changes in the concentration of ribulose 1,5-bisphosphate were effected by changing the activity of ribulose bisphosphate carboxylase/oxygenase (V_1). The initial conditions were as described in the legend to Fig. 2, except that: $V_1 = 160$ mM s^{-1}; $S_1 = 2.24$; $S_2 = 10.33$; $S_3 = 2.59$; $S_4 = 0.70$; and $P_i = 18.61$ (all mM).

it would be necessary to incorporate the following features: (1) a higher concentration of carboxylase active sites and a higher affinity for ribulose 1,5-bisphosphate than used here (see Appendix); (2) the influence of the electron-transport system over the catalytic properties of the carboxylase (this would include the influence of the H^+, Mg^{2+} and 'inhibitor' [22] concentrations in addition to that of other mechanisms effecting a change in the activation state of the carboxylase in response to light-intensity changes [2]); (3) the effect of compounds such as inorganic phosphate and 3-phosphoglycerate on the carboxylase; and (4) changes in the concentration of stromal CO_2 that may occur in response to alterations in stomatal conductance. The inclusion of the first three features would further complicate the expression for the control coefficient for the carboxylase, but would be essential when considering the control of photosynthetic CO_2 fixation at 'limiting' light intensities. It has been shown that, as the light intensity is lowered, the amount of active carboxylase declines and the ribulose 1,5-bisphosphate pool remains relatively high [2]. It would be illogical to expect a high control coefficient for the carboxylase under these conditions, and this may be a case where there is not an obvious correlation between saturation of the carboxylase with ribulose 1,5-bisphosphate and the enzyme control coefficient.

The inclusion of stomata in the present model would change the absolute values of the control coefficients significantly because the stomata can constitute a considerable 'limitation' to the rate of CO_2 fixation. Control coefficients for stomata in the 0.1–0.6 range have been measured for C_3 species (Woodrow, I.E., Ball, T.J.. and Berry, J.A., unpublished results). Therefore, when one speaks of 'total control' in the present context, one refers to the control that resides in the 'biochemistry' of the photosynthetic system. If the stomata and boundary layer have a combined control coefficient of 0.5, for example, then the control coefficient of the carboxylase, in the context of the present model, would approach 0.5 as ribulose

* It is assumed for convenience that the sequestration of ribulose 1,5-bisphosphate by the carboxylase does not affect the summation property (Eqn. 7) significantly.

1,5-bisphosphate approached a saturating concentration *.

The photosynthetic system within the chloroplast may be described as a moiety conserved cycle with respect to phosphate ¶ because the total chloroplast pool remains essentially constant and additions to and subtractions from that pool are relatively slow compared to the rates of reductive pentose phosphate pathway reactions. Woodrow et al. [4] showed that it may be feasible to redistribute the total stromal phosphate pool such that none of the enzymes are saturated with their cycle substrates and the inorganic phosphate pool is relatively large. Under these conditions, the cycle could be said to be 'metabolite limited' and an increase in the total stromal phosphate concentration would result in an increase in the rate of CO_2 fixation [25,26]. Since the summation theorem still holds under conditions of metabolite limitation, the carboxylase could exert considerable control over the rate of CO_2 fixation without approaching saturation with its cycle substrate.

Control by the other enzymes

There is no clear, general relationship between the concentration of the substrates of the enzymes catalyzing reactions 2 to 6 (Fig. 1) and their respective control coefficients. Several studies have examined the changes in the concentrations of many of the intermediates in this part of the reductive pentose phosphate pathway (e.g., Refs. 4 and 19), but in the absence of an adequate quantitative analysis one can only identify the regulatory enzymes [27]. And these regulatory enzymes are not necessarily important in flux control.

Kacser [28] demonstrated that at a branch-point in a linear metabolic system the two alternate pathways compete for the common intermediate and that the flux in one pathway will inversely affect the flux in the other. The reductive pentose phosphate pathway has two branch-points but is more complex than a linear pathway because: (1) the output flux is strictly related to the rate of CO_2 fixation *; and (2) the two export pathways (starch and sucrose synthesis) do not carry true export fluxes because they are coupled to the release of inorganic phosphate which, in turn, can affect several reactions in the cycle. Nevertheless, under most conditions an increase in the activity of cytosolic fructose 1,6-bisphosphatase and ADP-glucose pyrophosphorylase inhibits the rate of CO_2 fixation. This is reflected by the negative control coefficients for both enzymes (Figs. 2, 3, 6 and 7). The coefficients may become positive when the cycle becomes 'phosphate limited', since an increase in the activity of the export reactions would increase in the concentration of stromal inorganic phosphate (Fig. 6). This phenomenon was also discussed by Laisk and Walker [8] who, using a model of photosynthesis, showed that the rate of CO_2 fixation becomes highly sensitive to triose phosphate export and inorganic phosphate import at high light intensities and CO_2 concentrations.

Fig. 6 shows an example where the control coefficient of sedoheptulose 1,7-bisphosphatase becomes negative at the same time as those of ADP-glucose pyrophosphorylase and cytosolic fructose 1,6-bisphosphatase become positive. This is consistent with the relationship between the two fluxes at a branch-point discussed by Kacser [28]. The sedoheptulose 1,7-bisphosphatase-catalysed reaction competes with the starch synthetic pathway for substrate and has, therefore, an opposite effect on the rate of CO_2 fixation. The stromal fructose 1,6-bisphosphatase can also adopt a negative control coefficient (Fig. 7) by competing with the sucrose synthetic pathway for substrate in a similar manner. This property can be seen by examining one of the connectivity equations (see Appendix):

$$\frac{C_2}{v_2} + \frac{C_6}{v_6} = \frac{C_5}{v_5}.$$

It is evident that C_2 is not restricted to positive

¶ The essence of moiety conservation is that synthesis and degradation of the moiety is very slow in comparison to the interconversion of the different forms of the moiety. The total concentration of the moiety, therefore, remains essentially constant [23–25].

* The relationship between the fluxes through the largely irreversible reactions is given by Eqns. 1–4. In the steady state, the net output flux is one-sixth of that through the reaction catalysed by the carboxylase.

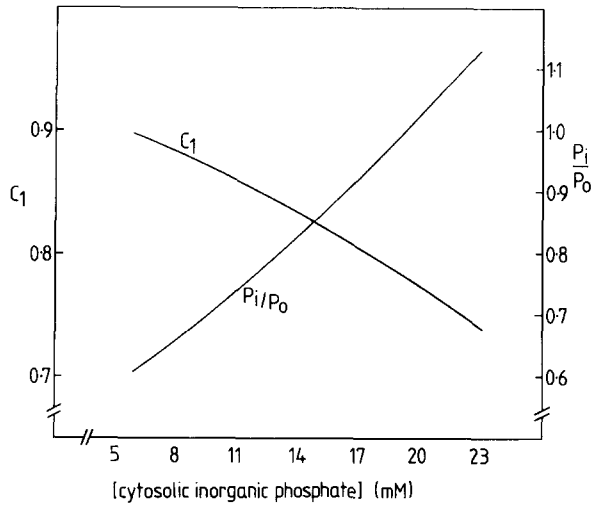

Fig. 5. The effect of the cytosolic inorganic phosphate concentration on the control coefficient of ribulose 1,5-bisphosphate carboxylase/oxygenase (C_1) and the distribution of stromal phosphate between inorganic (P_i) and organic (P_o) compounds. The initial conditions of the computer simulation were as described in the legend to Fig. 2.

values if one accepts that C_5 and C_6 are not tied together in a specific manner. The equation also demonstrates another important property of the reductive pentose phosphate pathway. Should v_5

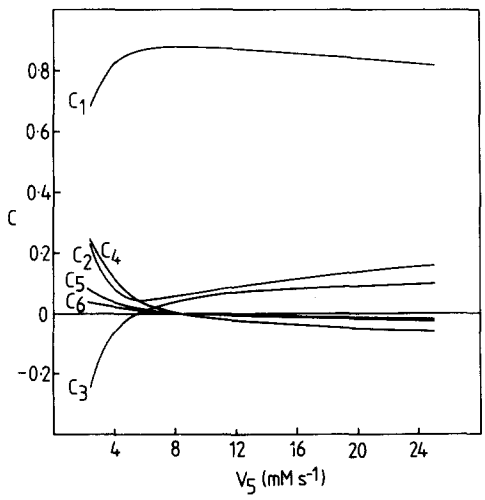

Fig. 6. The change in the control coefficients of the enzymes catalysing the six largely irreversible reactions of the model system effected by changing the activity of cytosolic fructose 1,6-bisphosphatase (V_5). The initial conditions of the computer simulation were as described in the legend to Fig. 2.

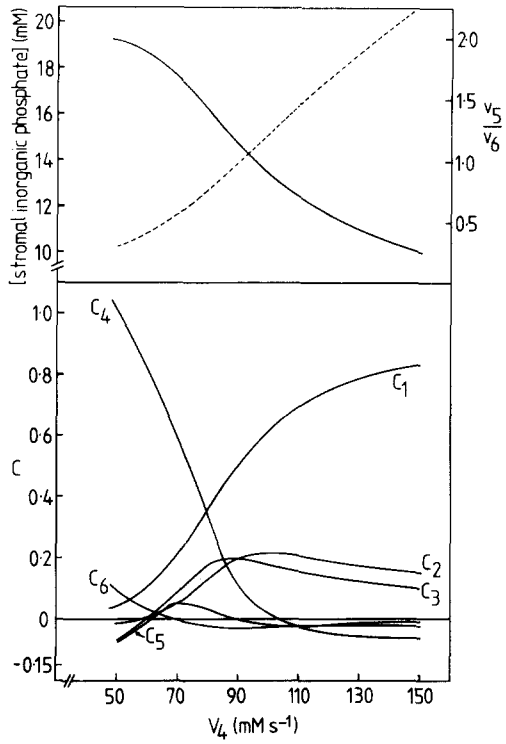

Fig. 7. The change in the control coefficients of the enzymes catalysing the six largely irreversible reactions of the model system, the stromal inorganic phosphate concentration, and the ratio (— — —) of the rate of sucrose synthesis (v_5) to starch synthesis (v_6) effected by changing the activity of ribulose 5-phosphate kinase (V_4). The initial conditions were as described in the legend to Fig. 2, except that: $V_4 = 150$ mM·s^{-1}; $S_1 = 10.10$; $S_2 = 4.60$; $S_3 = 1.56$; $S_4 = 0.17$; and $P_i = 9.98$ (all mM).

or v_6 become very small relative to their respective control coefficients, then some coefficients could become very large and greatly exceed one. Such 'hyper-rate-limitation' [28] can be demonstrated under conditions where almost all of the output flux is through either the starch or the sucrose synthetic pathway (Woodrow, I.E., unpublished data).

The control coefficient for ribulose 5-phosphate kinase may also adopt both positive and negative values (Figs. 6 and 7). The negative coefficients essentially reflect the capacity of this enzyme to influence the balance between starch and sucrose synthesis (Fig. 7). Therefore, under certain conditions, the kinase can promote the export of intermediates and therefore inhibit the rate of CO_2

fixation. But as the activity of the kinase is lowered, the role of this enzyme in regenerating the carboxylase substrate outweights any negative impact due to metabolite export and the enzyme assumes a positive control coefficients (Fig. 7).

The analysis of the model in this section has elucidated several important points concerning the regulation of photosynthesis. (1) The regulation of the enzymes involved in the export and recycling of cycle intermediates is critical in determining the distribution of stromal phosphate between organic and inorganic compounds [4,29]. The light-activated enzymes of the stroma, which constitute a link between the electron-transport system and the reductive pentose phosphate pathway, may be very important in this context. (2) Under certain conditions, both the cytosolic fructose 1,6-bisphosphatase and the ADP-glucose pyrophosphorylase may have a significant effect on the rate of the CO_2 fixation. A build-up of products such as starch and sucrose, therefore, could affect the rate of CO_2 fixation by affecting the first, and probably other, irreversible reactions in their synthetic pathways. This effect is intimately tied to the phenomenon of 'metabolite limitation', where stromal organic phosphates are lowered such that no enzyme approaches saturation with its cycle substrate (see last section and Ref. 7). (3) There is a complex association between the control of the rate of CO_2 fixation and the distribution of carbon between starch and sucrose synthesis. Because the net output flux is tied to the rate of CO_2 fixation, enzymes with high control coefficients with respect to fixation must also have a great influence over the output flux. A high control coefficient with respect to CO_2 fixation, however, is not necessarily related to a large influence over the ratio of the rates of starch to sucrose synthesis.

Application of the control equations and conclusions

One of the intentions of presenting this analysis is to point out, in terms of control parameters, the many unique properties of the photosynthetic system and the dangers associated with an over-simplistic approach to analysing the control of the rate of CO_2 fixation. The analysis shows how, once detailed rate laws for the component reactions have been elucidated, the relevant elasticity, pool size and flux data can be used to quantify the various control coefficients. This is a formidable task and, in the interim, it would be desirable to use relatively indirect methods to calculate the parameters using in vivo systems. The way this problem can be approached has been dealt with, in general terms, by Kacser and Burners [30] and applied, for example, by Groen et al. [31]. The photosynthetic system has the advantage that it can be affected relatively easily by changing the light intensity and the CO_2 and O_2 concentrations. It should be possible to quantify the control coefficients of certain components of this system using variations of these environmental parameters to induce changes in flux and metabolite concentration. When analysing the data, it will be necessary to pay particular attention to the complex connectivity equations that result from both the intricate regulatory mechanisms of the photosynthetic system and the conservation of total stromal phosphate.

The control analysis could also be extended to an examination of the optimisation of the system with respect to the rate of CO_2 fixation. The constraints within which optimisation should be considered will vary under different environmental conditions. If, for example, one assumes that the total quantity of protein nitrogen is fixed, then the optimisation analysis would indicate the most effective way to distribute the nitrogen between the protein components of the system. Consider a simple linear system of two reactions. If the proteins have the same specific activities and nitrogen contents, then the optimal situation would be an equal sharing of flux control, i.e., both enzymes would have a control coefficient of 0.5. The present model is somewhat more complex, however, and an optimisation analysis must take into account the optimal distribution of both nitrogen and the conserved moiety, phosphate. In a system optimised with respect to the rate of CO_2 fixation, one might expect that the proteins with the highest molecular weights and lowest specific activities will have the highest control coefficients. Ribulose 1,5-bisphosphate carboxylase/oxygenase may be the most obvious example of this rule.

Appendix

Expressions for the control coefficients of the six largely irreversible reactions in the pathway (Fig. 1) can be derived by simultaneous solution of the summation and connectivity equations. The latter were derived by considering small changes in a given pool (δ[S]/[S]), the compensating changes needed to maintain the total stromal phosphate level, and the adjustments in the enzyme concentrations required to balance the metabolite concentration changes such that the rate of CO_2 fixation is unchanged [9,11]. In the limit as δ[RuBP] $\rightarrow$ 0:

$$\frac{\delta v_1}{v_1} = \frac{\delta[E_1]}{[E_1]} + \epsilon^1_{RuBP}\frac{\delta[RuBP]}{[RuBP]} = 0$$

$$\frac{\delta v_2}{v_2} = \frac{\delta[E_2]}{[E_2]} + \epsilon^2_{FBP_i}\frac{\delta[FBP_i]}{[FBP_i]} + \epsilon^2_{P_i}\frac{\delta[P_i]}{[P_i]} = 0$$

$$\frac{\delta v_3}{v_3} = \frac{\delta[E_3]}{[E_3]} + \epsilon^3_{SBP}\frac{\delta[SBP]}{[SBP]} + \epsilon^3_{P_i}\frac{\delta[P_i]}{[P_i]} = 0$$

$$\frac{\delta v_4}{v_4} = \frac{\delta[E_4]}{[E_4]} + \epsilon^4_{Ru5P}\frac{\delta[Ru5P]}{[Ru5P]} + \epsilon^4_{RuBP}\frac{\delta[RuBP]}{[RuBP]} + \epsilon^4_{P_i}\frac{\delta[P_i]}{[P_i]} + \epsilon^4_{PGA}\frac{\delta[PGA]}{[PGA]} = 0$$

$$\frac{\delta v_5}{v_5} = \frac{\delta[E_5]}{[E_5]} + \epsilon^5_{FBP_0}\frac{\delta[FBP_0]}{[FBP_0]} = 0$$

$$\frac{\delta v_6}{v_6} = \frac{\delta[E_6]}{[E_6]} + \epsilon^6_{G1P}\frac{\delta[G1P]}{[G1P]} + \epsilon^6_{PGA}\frac{\delta[PGA]}{[PGA]} + \epsilon^6_{P_i}\frac{\delta[P_i]}{[P_i]} = 0$$

The fractional changes in enzyme amounts can then be multiplied by the respective flux control coefficients to obtain the effects on the fractional changes in flux. The connectivity equations can be derived from the following general equation:

$$C_1\epsilon^1_{RuBP}\frac{\delta[RuBP]}{[RuBP]} + C_2\left(\epsilon^2_{FBP_i}\frac{\delta[FBP_i]}{[FBP_i]} + \epsilon^2_{P_i}\frac{\delta[P_i]}{[P_i]}\right) + C_3\left(\epsilon^1_{SBP}\frac{\delta[SBP]}{[SBP]} + \epsilon^3_{P_i}\frac{\delta[P_i]}{[P_i]}\right)$$

$$+ C_4\left(\epsilon^4_{Ru5P}\frac{\delta[Ru5P]}{[Ru5P]} + \epsilon^4_{RuBP}\frac{\delta[RuBP]}{[RuBP]} + \epsilon^4_{P_i}\frac{\delta[P_i]}{[P_i]} + \epsilon^4_{PGA}\frac{\delta[PGA]}{[PGA]}\right)$$

$$+ C_5\epsilon^5_{FBP_0}\frac{\delta[FBP]_0}{[FBP]} + C_6\left(\epsilon^6_{G1P}\frac{\delta[G1P]}{[G1P]} + \epsilon^6_{PGA}\frac{\delta[PGA]}{[PGA]} + \epsilon^6_{P_i}\frac{\delta[P_i]}{[P_i]}\right) = 0$$

The rate of CO_2 fixation and the concentrations of the metabolites do not change if the amounts of enzymes 2, 5 and 6 are adjusted such that $\delta v_5 = -\delta v_2$ and $\delta v_2 = \delta v_6$. Since $\delta v_2/v_2 = \delta[E_2]/[E_2]$, $\delta v_5/v_5 = \delta[E_5]/[E_5]$ and $\delta v_6/v_6 = \delta[E_6]/[E_6]$, it follows that

$$v_2\frac{\delta[E_2]}{[E_2]} = v_6\frac{\delta[E_6]}{[E_6]} = -v_5\frac{\delta[E_5]}{[E_5]}.$$

And since there is no fractional change in either the metabolite concentrations or the rate of CO_2 fixation, in the limit as δ[E] $\rightarrow$ 0:

$$\frac{\delta J_c}{J_c} = 0 = C_5\frac{\delta[E_5]}{[E_5]} + C_2\frac{\delta[E_2]}{[E_2]} + C_6\frac{\delta[E_6]}{[E_6]}$$

where J_c is the rate of CO_2 fixation. Hence

$$\frac{C_2}{v_2} + \frac{C_6}{v_6} - \frac{C_5}{v_5} = 0$$

is an additional connectivity equation which can be used in the derivation of the control coefficients.

The rate equations used in the computer simulations of the system are:

$$v_1 = \frac{V_1[\text{RuBP}][\text{CO}_2]}{[\text{RuBP}][\text{CO}_2] + K_1^{\text{C}}[\text{RuBP}] + K_1^{\text{R}}[\text{CO}_2] + K_1^{\text{R}}K_1^{\text{C}}}$$

$$v_2 = \frac{V_2[\text{FBP}_i]}{K_2\left(1 + \frac{[\text{P}_i]}{K_i^2}\right) + [\text{FBP}_i]}$$

$$v_3 = \frac{V_3[\text{SBP}]}{K_3\left(1 + \frac{[\text{P}_i]}{K_i^3}\right) + [\text{SBP}]}$$

$$v_4 = \frac{V_4[\text{Ru5P}][\text{P}_i]}{[\text{Ru5P}][\text{P}_i] + K_4^{\text{P}}[\text{Ru5P}] + K_4^{\text{P}}K_4^{\text{R}}\left(1 + \frac{[\text{PGA}]}{K_i^4} + \frac{[\text{P}_i]}{K_i^5} + \frac{[\text{RuBP}]}{K_i^6}\right)}$$

$$v_5 = \frac{V_5[\text{FBP}_0]}{K_5\left(1 + \frac{[\text{P}_i^0]}{K_i^0}\right) + [\text{FBP}_0]}$$

$$v_6 = \frac{V_6[\text{G1P}]K_i^7\left(K_a^6 + 3[\text{PGA}]\right)}{K_i^7[\text{G1P}][\text{PGA}] + K_i^7K_a^6[\text{G1P}] + K_i^7K_a^6K_6 + [\text{P}_i]K_6K_a^6}$$

where V_j is the maximum velocity of reaction j; K_1^{C}, K_1^{R}, K_2, K_3, K_4^{P}, K_4^{R}, K_5 and K_6 are the Michaelis constants for CO_2, RuBP (reaction 1), FBP_i (reaction 2), SBP (reaction 3), P_i, Ru5P (reaction 4), FBP_0 (reaction 5) and G1P (reaction 6), respectively; K_i^0, K_i^2, K_i^3, K_i^4, K_i^5, K_i^6 and K_i^7 are the inhibition constants for P_i^0 (reaction 5), P_i (reaction 2), P_i (reaction 3), PGA, P_i, RuBP (reaction 4), and P_i (reaction 6), respectively; and K_a^6 is a constant describing the activation of ADP-glucose pyrophosphorylase by PGA.

The following equilibrium conditions were also assumed:

$[\text{X5P}]/[\text{Ru5P}] = 1.5$

$[\text{Ru5P}]/[\text{R5P}] = 0.4$

$[\text{S7P}][\text{G3P}]/[\text{X5P}][\text{R5P}] = 1.18$

$[\text{F6P}][\text{G3P}]/[\text{X5P}][\text{E4P}] = 11.9$

$[\text{DHAP}][\text{E4P}]/[\text{SBP}] = 1.4 \cdot 10^{-4}$ M

$[\text{G6P}]/[\text{G1P}] = 19$

$[\text{F6P}]/[\text{G6P}] = 0.43$

$[\text{G3P}]/[\text{DHAP}] = 4.5 \cdot 10^{-2}$

$$[G3P][DHAP]/[FBP] = 1.4 \cdot 10^{-4}\ M$$

$$[DHAP_i]/[DHAP_0] = 1.05\,[P_i^i]/[P_i^0]$$

$$[DHAP][P_i]/[PGA] = 4.22 \cdot 10^{-3}\ M.$$

In the derivation of the latter expression, it is assumed that $[H^+] = 10^{-8}$ M, [NADPH]/[NADP] = 1.8 and [ATP]/[ADP] = 2.3 [19].

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