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The requirements for a steady state in the C_3 reductive pentose phosphate pathway of photosynthesis

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A simplified model of the C_3 reductive pentose phosphate pathway of photosynthesis is analysed and the steady-state equations describing the system are solved. The solutions show that certain restrictions are placed upon the magnitude of the apparent kinetic parameters of the enzymes involved in starch synthesis, export of triose phosphate from the chloroplast, and the regeneration of ribulose 1,5-bisphosphate. These restrictions probably require, over a range of cellular conditions, the 'tuning' of the apparent kinetic parameters of certain enzymes. It is shown that fructose-1,6-bisphosphatase, an enzyme catalysing one of the irreversible reactions involved in ribulose 1,5-biphosphate regeneration, undergoes such 'tuning'. A decrease in the substrate concentration concomitant with an increase in the flux indicates that the enzyme is modified by factors other than the reaction substrate and is regulatory with respect to that substrate. It is suggested that the concerted regulation of several sets of kinetic parameters, including those of fructose 1,6-bisphosphatase, is essential in determining whether and how fast a steady-state rate of photosynthesis can be attained. The importance of transient, non-steady states in determining the final rate of photosynthesis is also discussed.

Introduction

In order for the reductive pentose phosphate pathway of photosynthesis to attain a steady state, the concentration of the intermediate compounds must reach a constant level and the input of carbon via CO_2 fixation must be balanced by export via triose phosphate and 3-phosphoglycerate transport, and starch synthesis. The conditions required for a steady state in this system are not as simple as in a linear enzyme sequence, for example, where almost any combination of enzyme kinetic parameters will satisfy the steady-state equations. The partition of carbon between the regeneration of ribulose 1,5-bisphosphate, the non-gaseous substrate of the ribulose 1,5-bisphosphate carboxylase/oxygenase reaction, and the export processes are the critical phenomena and require a careful balancing of several enzyme kinetic parameters if the concentration of cycle intermediates is not to expand or contract continuously.

One enzyme that may require such careful 'tuning' is the stromal fructose-1,6-bisphosphatase, a 'light-regulated' enzyme of the C_3 reductive pentose phosphate pathway. It has been demonstrated, using leaf extracts, that the apparent maximum catalytic activity (V_{max}^{app}) of this enzyme is determined by the intensity of light to which the leaf is exposed [1,2]. Under these conditions, it has

also been predicted that the apparent $S_{0.5}$ of fructose 1,6-bisphosphatase will vary in unison with the V_{max}^{app} [3]. However, in view of the complexities of the fructose 1,6-bisphosphatase mechanism [3,4], it is by no means certain how much, over what range of conditions, and for what reasons modulation of these apparent kinetic parameters in the whole leaf occurs.

In the present study, we examine the steady-state concentration of fructose 1,6-bisphosphate over a wide range of photosynthetic fluxes as an indicator of variations in the catalytic properties of fructose 1,6-bisphosphatase [5]. The mode of control of the enzyme is examined in the broader context of the necessity of constant 'tuning' of the photosynthetic system in order to attain and maintain a steady state.

Material and Methods

Materials

Wheat (*Triticum aestivum*, cv. Timmo) was grown in a vermiculite and soil mixture in a glasshouse. Plants were used in the experiments 10–14 days after germination. Biochemicals and coupling enzymes were obtained from Boehringer, North Ryde, Australia.

Gas-exchange measurements

Measurements of the net rate of CO_2 assimilation were made in an open gas-exchange system. For each measurement, 8–10 leaves were arranged in a parallel fashion and clamped into an aluminium gas-exchange chamber. The total leaf area in the chamber was 8 cm^2, irradiance at the leaf surface was 760 μmol photon per m^2/s photosynthetically active radiation (in experiments where the CO_2 concentration was varied), the leaf temperature was 21–23°C, and air flow through the chamber was 1.14 $mmol \cdot s^{-1}$. Differences in the partial pressures of CO_2 and H_2O entering and leaving the chamber were measured with a Beckman 865 CO_2 and an ADC Series 225 H_2O infrared gas analyser. The light source consisted of two slide projectors with 150 W quartz-iodine bulbs. Two fibre-optic bundles were used to direct the light onto the upper surface of the leaves. Light intensity was varied by inserting neutral density filters into the light path. Different CO_2 concentrations were obtained by mixing CO_2-free air with either 10% or 1% CO_2 in known proportions using Tylan flow controllers (Models FC-260 and FC-261). The gas phase was 21% O_2 and 0.032% CO_2 in experiments where the light intensity was varied. Gas-exchange parameters were calculated according to Von Caemmerer and Farquhar [6].

Metabolite measurements

Wheat leaves were illuminated for 1 h in the chamber to allow photosynthesis to reach a steady state and were then 'freeze-clamped' in situ. The leaf chamber [7] had windows of Nescofilm (Bando Chemical Ind. Ltd.) and was mounted halfway between two spring-loaded copper rods. The latter were cooled with liquid nitrogen. When a steady state rate of CO_2 fixation was obtained, the rods were allowed to smash through the chamber windows and freeze-clamp the leaves. The frozen leaf tissue was quickly transferred from between the copper rods to a ceramic mortar, containing liquid nitrogen, and was then ground to a fine powder in the presence of 5% $HClO_4$. After thawing, the leaf extract was centrifuged for 15 min at $12\,000 \times g$ and the supernatant neutralised was with KOH. The pellet was retained for chlorophyll determination [8]. Metabolites were assayed enzymically with a Shimadzu UV-240 spectrophotometer as described by Lowry and Passoneau [9]. Recovery, after adding a known amount of metabolite to plant tissue during grinding, was never less than 92%.

Estimation of enzyme activity

Wheat leaves were illuminated, frozen and ground as described above except that the $HCLO_4$ was replaced by a solution (1 ml) containing 1 mM fructose 1,6-bisphosphate/2 mM 2-mercaptoethanol/10 mM $MgCl_2$/1 mM ethylenediaminetetraacetic acid/0.05% Triton X-100/100 mM 2-amino-2-hydroxymethylpropane-1,3-diol-HCl (Tris-HCl) (pH 8.2) [1]. Fructose 1,6-bisphosphatase activity was estimated using a continuous spectrophotometric assay at 20°C. The reaction mixture contained, in a final volume of 1 ml, two units of glucose 6-phosphate dehydrogenase/two units of phosphoglucose isomerase/100 mM Tris-HCl (pH 8.2)/1 mM fructose 1,6-bisphosphate/1 mM ethylenediaminetetraacetic acid/ 10 mM

$MgCl_2$/0.05% Triton X-100/enzyme solution (equivalent to about 1 μg of chlorophyll) [10]. As in previous studies of this kind [1], it is assumed that the low cytosolic fructose 1,6-bisphosphatase activity present under these conditions [11] is constant.

Results

Requirements for steady-state photosynthesis

In order to attain a steady state in an autocatalytic cycle, the amount of metabolite entering and leaving the cycle must be balanced. The nature of this balance is determined by the kinetic properties of enzymes catalysing reactions in both the export and recycling sequences. In general, the reactions up to and including the first essentially irreversible reaction in each sequence must be considered when determining whether and in what way a steady state can be approached. The critical reactions in the C_3 reductive pentose phosphate pathway * include, in the recycling sequence, all the reactions connecting the triose phosphate and ribulose 5-phosphate pools in addition to the reaction catalysed by ribulose 5-phosphate kinase, and in the export sequences, all the reactions up to and including those catalysed by cytosolic fructose 1,6-bisphosphatase and stromal ADP-glucose pyrophosphorylase.

The balance between reactions required for a steady state can be described by considering a simplified scheme of the C_3 reductive pentose phosphate pathway (Fig. 1) which contains the following essential features. (i) The cycle can be autocatalytic, since there is an input of substrate via S_5. The two molecules of S_2 produced from an S_1 and an S_5 molecule are partitioned between regeneration of an S_1 molecule and export from the cycle. (ii) The first export pathway, consisting of a reversible and an irreversible reaction, represents the phosphate translocator/cytosolic fructose 1,6-bisphosphatase sequence in the photosynthetic system. It is assumed that only one intermediate is exported by this means. (iii) The second export pathway represents the irreversible reaction catalysed by ADP-glucose pyrophosphorylase. It is assumed, in this case, that the rate of starch degradation to glucose 6-phosphate is negligible. (iv) The reactions producing S_3 and S_1 are irreversible in the simplified scheme. The analogy with the reductive pentose phosphate pathway is less clear in this case because of the complexity of the sequence converting triose phosphate (analogous to S_2) to ribulose 1,5-bisphosphate (analogous to S_1). Nevertheless, this sequence is ultimately irreversible because it involves fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase and ribulose 5-phosphate kinase, all of which catalyse essentially irreversible reactions. The reversible reactions of the photosynthetic system, the apparent kinetic parameters of which are not variable, have been omitted because they would only appear as constants in the rate equations. Moreover, steps analogous to the 3-phosphoglycerate kinase and NADP-glyceraldehyde 3-phosphate dehydrogenase catalysed reactions were excluded from the scheme (Fig. 1) for reasons that will become evident in the ensuing discussion. The scheme presented in Fig. 1, although relatively simple, encapsulates all of the features germane to this discussion of the requirements for a steady

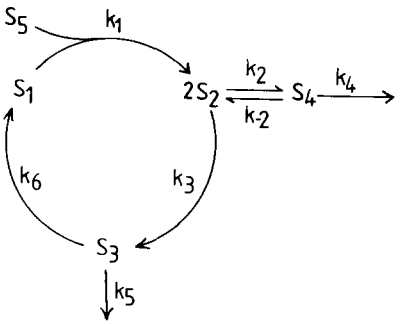

Fig. 1. Simplified scheme of the C_3 reductive pentose phosphate pathway of photosynthesis incorporating the first irreversible reactions of carbon export. The scheme is analogous to the photosynthetic system in the following ways: (i) there is a carbon input via the irreversible reaction (analogous to ribulose 1,5-bisphosphate carboxylase) that produces two S_2 molecules from an S_1 and an S_5; (ii) one output involves a reversible (analogous to the phosphate translocator) and an irreversible (analogous to cytosolic fructose 1,6-bisphosphatase) reaction; (iii) the other output consists of an irreversible (analogous to ADP-glucose pyrophosphorylase) reaction; and (iv) the two molecules of S_2 must be divided between export and S_1 regeneration in order for the system to attain a steady state. *k* represents the rate constant describing a given reaction.

* Carbon loss due to photorespiration will not be considered in the ensuing analysis.

state in an autocatalytic cycle with two basic export pathways.

In the scheme shown in Fig. 1, rate constants have been used to describe the reactions. The use of such linear rate equations is valid, in the case of Michaelis-Menten mechanisms, when substrate concentrations are less than the respective Michaelis constants. This situation can arise in a cyclic system since, unlike linear sequences, a steady state can occur when all the enzyme substrate concentrations are well below saturation [12].

A steady state can occur in the scheme shown in Fig. 1 if the import and export processes are balanced:

$$k_1[S_1][S_5] = k_5[S_3] + k_4[S_4] \tag{1}$$

The two molecules of S_2 that are produced by the reaction using S_1 and S_5 are divided ultimately such that one is exported and the other is involved in the regeneration of one molecule of S_1. Certain constraints must be placed upon the rate constants that characterise regeneration and export before S_2 can be partitioned in this way. These constraints are defined by solving the steady state equations for the scheme shown in Fig. 1:

$$k_3 = \frac{k_2 k_4 (k_5 + k_6)}{(k_{-2} + k_4)(k_6 - k_5)} \tag{2}$$

This equation shows that the kinetic parameters of all the enzymes, other than that catalysing S_2 production, must be coordinated in a specific fashion. Choices of rate constants not defined by Eqn. 2 result in non-steady states, i.e., continuous expansion or contraction of the concentration of intermediates in the cycle.

Under most conditions it is more likely that the rate of photosynthesis will be optimised with respect to the amount of nitrogen invested in stromal enzymes. This means that the first-order assumption used in the foregoing analysis is probably not valid and a significant degree of substrate saturation will occur in some reactions [12]. In this way, the flux will be limited ultimately by the concentration of enzymes rather than that of intermediates. In order to analyse this scenario, the rate equations for the two irreversible export reactions (reactions 4 and 5 in Fig. 1) and the recycling reactions using S_2 and S_3 (reactions 3 and 6 in Fig. 1) were rewritten:

$$V_j = \frac{V_j[S_j]}{K_j + [S_j]} \tag{3}$$

where S_j is the reaction substrate, and V_j and K_j are the maximum velocity and Michaelis constant, respectively. It is assumed that the concentration of S_5 is constant. The equations defining the steady state are too complex to be expressed here. Their essential quality, however, is that, unlike Eqn. 2, they are not independent of substrate terms. Furthermore, certain restrictions must be placed upon the magnitude of the kinetic constants before a steady state can be attained. These constants must be arranged so that:

(i) less than 50% of S_2 leaves the system via S_4; and:

(ii) the balance of S_3 export and recycling results in the production of one S_1 for each S_5 fixed.

Fig. 2 shows that the change in the total concentration of the cycle (Fig. 1) intermediates with

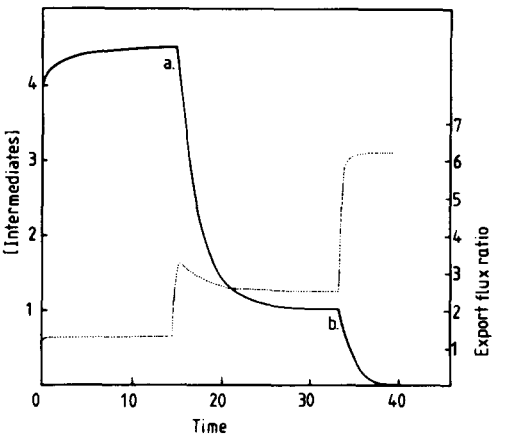

Fig. 2. The change in the total concentration of intermediates and the ratio of fluxes through the two export pathways with time in the simplified scheme (Fig. 1). Michaelis-Menten rate equations were used to describe reactions 3, 4, 5 and 6 (see Fig. 1). The initial conditions were: $k_1 = 1$, $k_2 = k_{-2} = 5$, $V_3 = K_3 = 0.5$, $V_4 = K_4 = 1$, $V_5 = K_5 = 3$, $V_6 = 2$ and $K_6 = 0.5$ (arbitrary units). At time (a), V_3 was increased to 1. At time (b), K_3 was increased to 1.5. The graph shows the total concentration at intermediates (———) and the ratio of fluxes through reactions 4 and 5 (·····). The rate equations were solved numerically using a fifth-order Runge-Kutta method.

time under the following conditions:

(i) the K_m of reaction 3 is less than that of reaction 4, whereas the V_{max} of the former reaction is greater than that of the latter;

(ii) the equilibrium constant for reaction 2 is 1, and the rate constants are such that the reaction remains close to equilibrium;

(iii) both the K_m and V_{max} of reaction 5 are greater than those of reaction 6.

The system approaches a steady state and the ratio of fluxes through reactions 4 and 5 is about 1.25. When the V_{max} of reaction 3 is lowered to that of reaction 4, a steady state is again approached, but the total concentration of intermediates drops by a factor of about 3 and the ratio of fluxes through reactions 4 and 5 changes to about 2. However, when the relative affinity of reaction 4 becomes greater than that of reaction 3 a non-steady state results and the total concentration of intermediates drops to zero (Fig. 2).

This example illustrates several basic points concerning the regulation of autocatalytic cycles:

(i) Coordinated regulation of several enzymes is required in order to attain a steady state.

(ii) Transient non-steady states are important in changing the total concentration of intermediates and thus, under some conditions, the rate of product formation [12]. This phenomenon is probably very important during the induction phase of photosynthesis [13].

(iii) The relative fluxes through the two export pathways can – and under certain circumstances must – be controlled by variations in the kinetic properties of several enzymes [14].

In the present model, a very simple stoichiometry of one molecule of S_2 exported to one molecule recycled was used. Farquhar and Von Caemmerer [15], however, demonstrated that of the total dihydroxyacetone phosphate pool in the chloroplast (defined as $1 + 0.5\phi$, where ϕ is the number of ribulose 1,5-bisphosphate oxygenations per carboxylation), $(2 + 2\phi)/3$ must be recycled and $(1 - 0.5\phi)/3$ must be exported in the steady state. Despite the more complex ratio of export to recycling, the principle that applies to the simplified model also applies to the C_3 reductive pentose phosphate pathway of photosynthesis: the attainment of a steady state requires the balancing of several reactions in both the exporting and recycling directions.

Modulation of the kinetic parameters of fructose 1,6-bisphosphatase

The foregoing analysis of the criteria for a steady state in the C_3 reductive pentose phosphate pathway shows that, in order to maintain the required balance of carbon fluxes, changes in the magnitude of a kinetic constant may need to be counteracted by changes in at least one other constant. If one considers, for example, the first irreversible step in starch synthesis – the reaction catalysed by ADP-glucose pyrophosphorylase – then a decrease in the stromal inorganic phosphate concentration effects an increase in the V_{max}/K_m ratio of this enzyme. Woodrow et al. [14] have already discussed how this effect, in addition to the inhibition of sedoheptulose 1,7- and fructose 1,6-bisphosphatases by inorganic phosphate, could produce an increase in the steady state carbon flux into starch compared to that into the cytosol. In view of the analysis in the last section, the concerted action of inorganic phosphate on these enzymes may also be necessary for maintaining the balance of kinetic parameters required for a steady state. In the following experiments, we examine whether, as a possible mechanism to maintain steady state photosynthesis, changes in the apparent kinetic properties of stromal fructose 1,6-bisphosphatase occur in response to alterations in cellular conditions.

Fig. 3 shows the relationship between the light intensity and the rate of CO_2 assimilation for wheat leves under conditions of essentially steady state photosynthesis. Half the maximum rate was attained at a light intensity of about 150 μmol photon per m^2/s, which is about 8% of full sunlight. The V_{max}^{app} of fructose 1,6-bisphosphatase, measured in leaf extracts, is also related to light intensity (Fig. 4), and like CO_2 assimilation, the half-maximum V_{max}^{app} was attained at a little less than 10% of full sunlight. A similar relationship was observed for spinach protoplasts [2]. The current measurements of V_{max}^{app} were made under conditions that are thought to approximate those obtained in the illuminated stroma. It is also assumed that the dilution of proteins in the reaction mixture does not permit a net oxidation of reduction of fructose 1,6-bisphosphatase to occur during the assay period

The relationship between the steady state concentration of fructose 1,6-bisphosphate and the

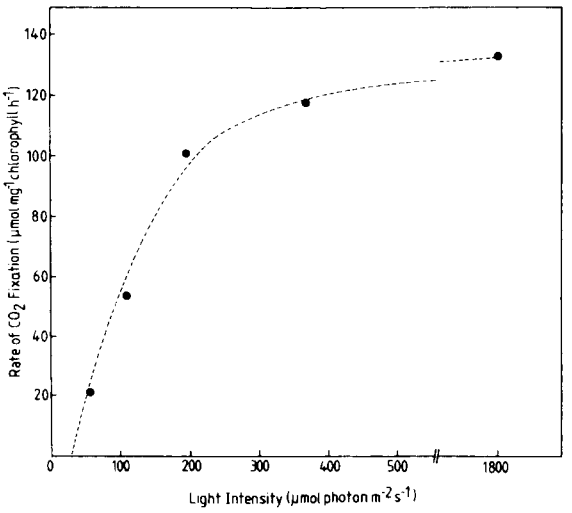

Fig. 3. The relationship between light intensity and the rate of CO_2 assimilation by wheat leaves. Assimilation was measured using a gas-exchange apparatus. The leaves were illuminated in the chamber until a steady-state rate of photosynthesis was attained.

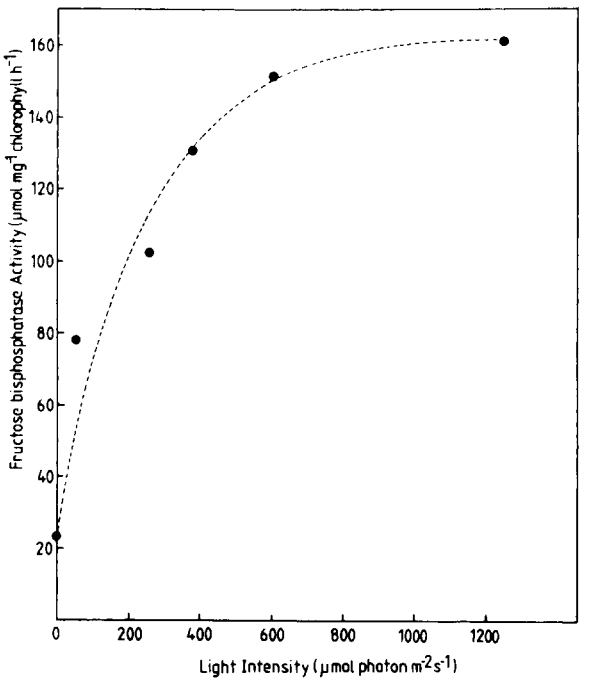

Fig. 4. The relationship between light intensity and the apparent maximum catalytic velocity (V^{app}) of stromal fructose 1,6-bisphosphatase. Measurements were made using extracts from wheat leaves that were frozen immediately after a steady-state rate of photosynthesis was attained. The V^{app} is thought to reflect the amount of enzyme in the active (reduced) form.

rate of CO_2 assimilation is shown in Fig. 5. Both parameters were modulated by varying the light intensity. The total leaf fructose 1,6-bisphosphate concentration measured in these experiments includes, in addition to the pool available for binding to stromal fructose 1,6-bisphosphatase, the pool in the cytosol and that bound to ribulose 1,5-bisphosphate carboxylase [16]. It has been shown that the cytosolic pool is quite small relative to that in the chloroplast [17] and that at light intensities greater than about 80 μmol photon per m^2/s very little fructose 1,6-bisphosphate could bind to the carboxylase enzyme [16]. It is, therefore, assumed that the abscissa coordinates of Fig. 5 approximate the stromal fructose 1,6-bisphosphate concentration. It is also assumed in the ensuing discussion that there is an approximate proportionality between the rate of CO_2 assimilation and the flux through the fructose 1,6-bisphosphatase reaction [6]. Fig. 5 shows that an increase in the

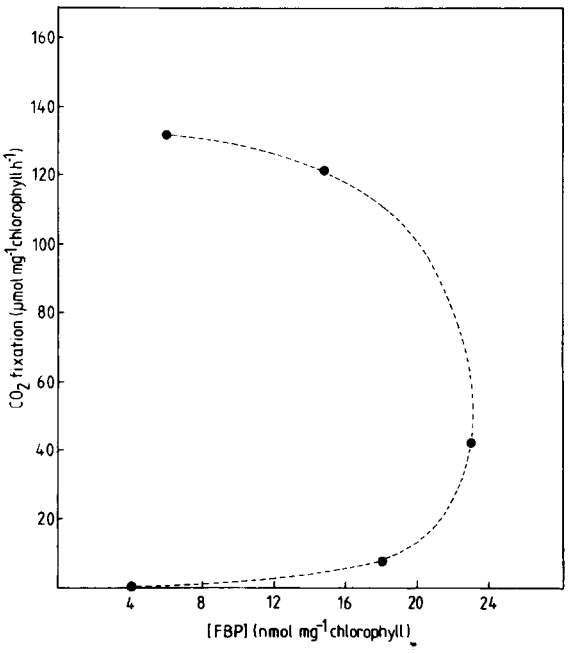

Fig. 5. The relationship between the rate of CO_2 assimilation and the steady-state concentration of total leaf fructose 1,6-bisphosphate measured in wheat leaf extracts. Different irradiances were used to modulate the rate of CO_2 assimilation at 21% O_2, 0.32% CO_2, and 21–23°C. It is assumed that the total fructose 1,6-bisphosphate concentrations approximate those occurring in the chloroplast stroma. The rate of CO_2 fixation is also assumed to reflect the flux through the fructose 1,6-bisphosphatase reaction.

flux through the fructose 1,6-bisphosphatase-catalysed reaction is correlated with first a rise and then a fall in the substrate concentration as the maximum flux is approached. This pattern is contrary to that which occurs with enzyme mechanisms, where an increase in the reaction velocity is accompanied by an increase in substrate concentration. Badger et al. [7] also measured the total leaf fructose 1,6-bisphosphate under conditions of varying light intensity and CO_2 concentration. However, the scatter in their data does not permit a comparison to be made with the results of this study.

The relationship between the steady-state fructose 1,6-bisphosphate concentration and the calculated carbon flux through the fructose 1,6-bisphosphatase-catalysed reaction under conditions of constant light intensity is shown in Fig. 6. In these experiments, the CO_2 concentration was used to vary the flux. The patterns shown in Figs. 5 and 6 are similar in both shape and maximum fructose 1,6-bisphosphate concentration.

Fluxes through the fructose 1,6-bisphosphatase reaction (Fig. 6) were calculated using the measured rates of assimilation and intercellular CO_2 concentrations [16]. The values of R_d (day respiration) and Γ_* (the CO_2 compensation point in the absence of day respiration) required for these calculations were estimated from other gas-exchange measurements on wheat (data not shown) and from the effect of temperature on the CO_2/O_2 specificity factor of spinach ribulose 1,5-bisphosphate carboxylase/oxygenase [18].

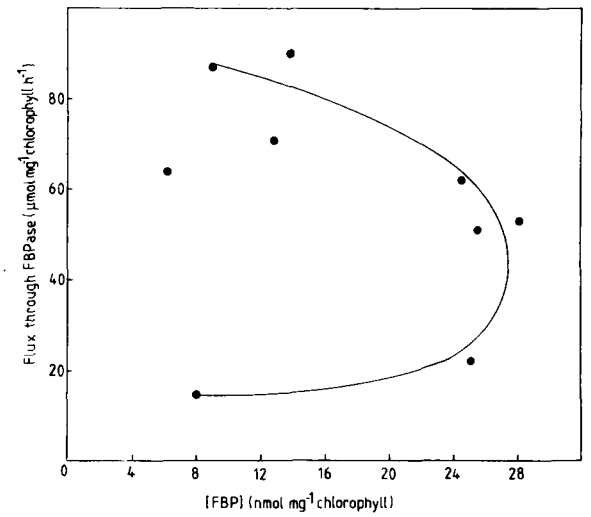

Fig. 6. Relationship between the flux through the fructose 1,6-bisphosphatase reaction and the steady-state concentration of total leaf fructose 1,6-bisphosphate in wheat leaf extracts. Different CO_2 concentrations were used to modulate the rate of CO_2 assimilation. It is assumed that the total fructose 1,6-bisphosphate concentrations approximate those occurring in the stroma. The flux through the fructose 1,6-bisphosphatase reaction was calculated according to Farquhar and von Caemmerer [6].

Discussion

For non-equilibrium enzymes, Krebs [19] suggested that if changes in the flux occur in the opposite direction to changes in the concentration of the substrate of the enzyme, then that enzyme must be regulatory with respect to that substrate. In this context, therefore, a regulatory enzyme is essentially an enzyme that possesses kinetic parameters determined by factors other than the reaction substrate, and that is capable of exerting an influence over a metabolic pathway [20,21]. This influence covers both rate limitation and determination of substrate concentrations.

The analysis of the total fructose 1,6-bisphosphate pool shows that, at least over the upper range of photosynthetic fluxes, the fructose 1,6-bisphosphatase enzyme is regulatory. The continuous decline in the substrate concentration under conditions where the flux was modulated by either light intensity (Fig. 5) or CO_2 concentration (Fig. 6) demonstrates that the enzyme undergoes a continuous modification by substances other than the reaction substrate. It should be emphasized that Krebs' [19] criterion is only positive; that is, if changes in flux and substrate concentration are not opposite, then the enzyme is not excluded from being regulatory. Therefore, it is possible that modification of fructose 1,6-bisphosphatase also occurs at the lower photosynthetic fluxes.

The kinetic properties of fructose 1,6-bisphosphatase are regulated primarily by the *R/O* ratio *, and the H^+, Mg^{2+} and inorganic phos-

* The *R/O* ratio was first used to describe the overall ratio of the concentrations of reductants and oxidants which activate and inactivate, respectively, the light-regulated enzymes of the reductive pentose phosphate pathway [22]. Thioredoxin-*f* has been identified as one such redox effector [23]. The proportion of reduced thioredoxin-*f* appears to be determined by the redox state of ferredoxin, and is therefore linked to the rate of photosynthetic electron transport.

phate concentrations [3]. It is likely that inorganic phosphate and the R/O ratio are the important regulators under a variable light regime because changes in the H^+ and Mg^{2+} concentrations appear to occur only at relatively low light intensities (up to about 80 μmol photon per m^2/s) [2]. This view is supported by the results of the experiment showing the dependence of the V_{max}^{app} of fructose 1,6-bisphosphatase upon the light intensity (Fig. 3). When the flux is determined by the CO_2 concentration, however, inorganic phosphate alone is probably the main effector. Under these conditions it was shown that the V_{max}^{app} of fructose 1,6-bisphosphatase does not decline with decreasing flux [24] and, therefore, that large variations in the R/O ratio are unlikely. The exact relationship between the flux, the stromal inorganic phosphate concentration, and the R/O ratio remains to be determined. However, it is known that the inorganic phosphate concentration [25] and the proportion of oxidised thioredoxin-*m* [26] – which presumably also reflects the level of oxidised thioredoxin-*f* – in the stroma both decline significantly during a dark-to-saturating-light transition. It would, therefore, be reasonable to expect intermediate levels of these effectors at intermediate fluxes and continuous modification of enzyme activity over the whole spectrum of available fluxes.

We have demonstrated that the kinetic properties of stromal fructose 1,6-bisphosphatase are modulated over a wide range of conditions by factors other than the reaction substrate. The regulation of this enzyme and, in view of the similar regulatory mechanisms, the regulation of sedoheptulose 1,7-bisphosphatase and ribulose 5-phosphate kinase [3], appears to be of importance in controlling both the rate of CO_2 fixation and the destiny of the fixed carbon. The influence of these enzymes over the flux is at two basic levels: firstly, their ability to generate non-steady states allows them to control the flux by changing the total concentration of cycle intermediates [12], and secondly, since the reactions catalysed by these enzymes are significantly displaced from equilibrium [27], it is probable that they contribute significantly to the control of the steady-state flux under certain conditions. Woodrow et al. [14] discussed the role of sedoheptulose 1,7- and fructose 1,6-bisphosphatase, as well as ribulose 5-phosphate kinase, in directing the flow of photosynthetically fixed carbon between starch synthesis and export from the chloroplast. The current analysis demonstrates that a carefully coordinated regulation of these enzymes, and of cytosolic fructose 1,6-bisphosphatase and ADP-glucose pyrophosphorylase, is necessary to regulate the transition between steady states with different fluxes through the two principle export pathways. Inorganic phosphate may well be the common denominator in the regulation of such transitions [14].

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