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# REGULATION OF STROMAL SEDOHEPTULOSE-1,7-BISPHOSPHATASE ACTIVITY AND ITS ROLE IN CONTROLLING THE REDUCTIVE PENTOSE PHOSPHATE PATHWAY OF PHOTOSYNTHESIS

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**The activation and steady-state kinetics of a wheat sedoheptulose-bisphosphatase, shown to be located in the chloroplast, are examined. Enzyme activation in the presence of the reductant, dithiothreitol, is slow and modulated by the sedoheptulose 1,7-bisphosphate concentration. A mechanism for the regulation of enzyme activity is proposed. In this scheme, both the enzyme-substrate complex and the enzyme alone undergo a slow reductive activation and oxidative inactivation. The proportion of enzyme in the active form is governed by the reductant/oxidant ratio and the sedoheptulose 1,7-bisphosphate concentration. Control of sedoheptulose-bisphosphatase activity by these two factors is discussed in terms of regulation of the reductive pentose phosphate pathway of photosynthesis. Several observations made in previous studies using intact chloroplast systems are accounted for by the proposals outlined in this report.**

## Introduction

Light-mediated modulation of stromal sedoheptulose-bisphosphatase activity is one mechanism which ensures that $CO_2$ fixation by means of the reductive pentose phosphate pathway occurs only in the light. Experiments with isolated chloroplasts showed that, upon illumination, enzyme activity slowly increases from a level well below that required to support the subsequent maximum rate of photosynthesis to one that is ample [1,2]. In darkness, inactivation of sedoheptulose-bisphosphatase occurs within several minutes [1,2]. The activation mechanism is thought to involve a modification of the enzyme which is effected by reductants generated by photosynthetic electron transport [3,4]. A similar slow activation of highly purified sedoheptulose-bisphosphatase preparations occurs in the presence of the reductants, dithiothreitol and thioredoxin *f* [5–7]. The rate of this process depends upon both the reductant and sedoheptulose 1,7-bisphosphate concentration [1,5,7]. The former is an absolute requirement for enzyme activation. A preliminary kinetic analysis in the presence of substrate showed that sedoheptulose-bisphosphatase activation can be described as though it involves a single slow step between inactive and active enzyme forms [7]. However, a more detailed mechanism defining the way in which the reaction substrate and the reductant interact to regulate sedopheptulose-bisphosphatase has not been formulated.

The present study examines the activation and steady-state kinetics of a wheat sedoheptulose-bisphosphatase shown to be exclusively located in the chloroplast. A mechanism for the regulation of the

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Abbreviation: Tricine, *N*-tris(hydroxymethyl)methylglycine.

enzyme is proposed and discussed in terms of the regulation of the reductive pentose phosphate pathway of photosynthesis.

## Materials and Methods

### *Materials*

Wheat (*Triticum aestivum* L., cv Sappo) was grown in vermiculite under sunlight and supplementary incandescent lamps. Biochemicals and 6-phosphofructokinase were purchased from Sigma, Poole, Dorset, U.K., Sephadex products from Pharmacia, Hounslow, Middx., U.K., and other auxiliary enzymes from Boehringer, Lewes, East Sussex, U.K.

### *Methods*

*Fractionation of protoplasts.* Protoplasts were isolated from wheat leaves as previously described [8], and stored in the dark on ice for 3–4 h before use. Before fractionation, protoplasts were collected by centrifugation ($100 \times g$ for 2 min) and resuspended in a medium containing 0.4 M sorbitol, 1 mM $CaCl_2$, 30 mM Tricine-NaOH (pH 7.6), and 5 mM $NaHCO_3$ to give a final chlorophyll concentration of 100 $\mu g \cdot ml^{-1}$. Aliquots of 100 $\mu l$ were fractionated according to the procedure of Robinson and Walker [9]. Chloroplasts, extrachloroplast, and whole protoplast fractions were collected and stored at 4°C. Enzyme assays were performed as previously outlined [9] within 2 h of fractionation. Chlorophyll was determined by the method described by Arnon [10].

*Preparation of sedoheptulose-bisphosphatase.* Sedoheptulose-bisphosphatase was purified by the procedure outlined by Woodrow and Walker [7]. The enzyme was purified approx. 400-fold and had a final specific activity of 9 U/mg protein. The preparation was suitable for kinetic studies as it contained no enzyme activities which interfere with measurement of sedoheptulose-bisphosphatase activity by means of the continuous spectrophotometric assay [7]. The enzyme was stored at 2°C with half the original activity being lost in about 3 weeks. Protein was determined by the procedure of Lowry et al. [11].

*Determination of sedopheptulose-bisphosphatase activity.* The activity of the purified enzyme was measured at 20°C, using a continuous spectrophotometric assay [7]. The latter couples the formation of sedoheptulose 7-phosphate to the oxidation of NADH. The reaction was followed at 340 nm using a Pye-Unicam SP8-200 spectrophotometer. Enzyme activity in crude extracts was determined as previously described [1]. The standard continuous assay mixture contained, in a final volume of 1 ml, 50 mM Tricine-NaOH (pH 8.2), 10 mM $MgCl_2$, 20 mM KCl, 0.1 mM ATP, 1 mM phospho*enol*pyruvate, 0.15 mM NaDH, 20 mM dithiothreitol, 0.1 mM sedoheptulose 1,7-bisphosphate, 2 U pyruvate kinase, 2 U lactate dehydrogenase, 0.5 U 6-phosphofructokinase, and sedoheptulose-bisphosphatase solution. The latter was normally used to initiate the reaction. $Ca^{2+}$ was not added to the reaction mixtures, even though it may increase the rate of sedoheptulose-bisphosphatase activation [6]. Trace levels of $Ca^{2+}$ in the assay solutions were assumed to be constant since the activation kinetics of sedoheptulose, under specific conditions, did not alter significantly during the experiments.

*Analysis of reaction progress curves.* Progress curves (product formation with time) showed increasing slope from the initial state to the final steady state [7]. The instantaneous rates of product formation ($V_{obs}$) were measured by constructing tangents corresponding to various times. A plot of log ($V_f - V_{obs}$) was then made ($V_f$, final steady-state reaction velocity) in order to calculate the apparent rate constant for the transition between the initial and final reaction velocities ($\tau^{-1}$) (Fig. 2). When the initial reaction velocity is zero, this gives the induction or lag period. Plots of log ($V_f - V_{obs}$) showed an initial nonlinear phase [7] (Fig. 2) which was attributed to the coupled enzyme assay system. The activity of the latter was varied and the expected lag period at an infinite level of coupling enzyme calculated (Woodrow, I.E. and Manson, G., unpublished data). This corresponded to the $\tau$ value calculated using the semilogarithmic plot. Single first-order activation kinetics were also observed when $P_i$ cleavage was used as a measure of enzyme activity [7]. It is therefore concluded that the slow activation kinetics monitored by the continuous assay can be described as a single first-order process. $V_f$ was estimated from the final linear portion of the progress curves. $V_0$ (initial reaction velocity) was

estimated from the initial slope of the progress curves after a correction was made for the time required for the assay system to approach a steady state (Woodrow, I.E., unpublished data).

## Results

*Subcellular location of sedoheptulose-bisphosphatase*

The distribution of sedoheptulose-bisphosphatase and the marker enzymes in the chloroplast and extrachloroplast fractions is shown in Table I. The similar distribution of sedoheptulose-bisphosphatase and the chloroplast marker enzymes, ribulose-1,5-bisphosphate carboxylase and NADP-glyceraldehyde-3-phosphate dehydrogenase, indicates that the former enzyme is probably located in the chloroplast. These experiments examined $Mg^{2+}$- and dithiothreitol-dependent sedoheptulose-bisphosphatase activity [7]. The contamination of the chloroplast extract by nonchloroplast enzyme activity (indicated by phospho*enol*pyruvate carboxylase and cytochrome *c* oxidase activity) was relatively low and did not follow the distribution of sedopheptulose-bisphosphatase activity. That the activity of each enzyme was recovered almost completely indicates that enyzme inactivation during the experiments was insignificant and that any effectors present in the fractions did not differentially affect enzyme activity. Using this method, Robinson and Walker [9] measured a distribution of marker enzymes similar to that shown in Table I. When a preparation of intact chloroplasts was injected into an isotonic medium containing dithiothreitol, $Mg^{2+}$ and sedoheptulose 1,7-bisphosphate, insignificant (relative to that measured using ruptured chloroplasts) sedoheptulose-bisphosphatase activity was measured. These results lead to the conclusion that the sedoheptulose-bisphosphatase used in the current experiments is that which is located in the chloroplast.

TABLE I

DISTRIBUTION OF ACTIVITIES OF SEDOHEPTULOSE-BISPHOSPHATASE AND MARKER ENZYMES FOLLOWING FRACTIONATION OF WHEAT PROTOPLASTS

| | Percentage activity of (pellet + supernatant) | | |
|---|---|---|---|
| | Whole extract | Pellet | Supernatant |
| Sedoheptulose-bisphosphatase | 97 | 82 | 18 |
| NADP-glyceraldehyde-3-phosphate dehydrogenase | 98.5 | 84 | 16 |
| Ribulose-1,5-bisphosphate carboxylase | 101 | 86 | 14 |
| Phospho*enol*pyruvate carboxylase | 98 | 6 | 94 |
| Cytochrome *c* oxidase | 96 | 22 | 78 |
| Chlorophyll | 102 | 92 | 8 |

*Kinetic and regulatory properties of sedoheptulose-bisphosphatase*

When sedoheptulose-bisphosphatase was preincubated with 10 mM dithiothreitol, activity increased slowly. Aliquots of enzyme were removed from the incubation mixture at regular time intervals and added to a continuous spectrophotometric assay mixture such that the final dithiothreitol concentration was also 10 mM. As the duration of

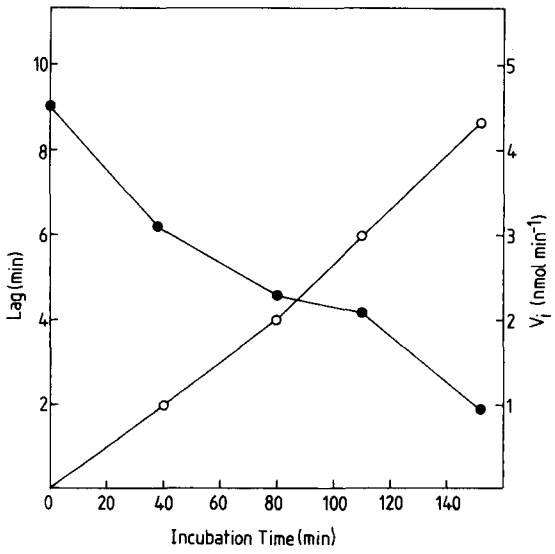


Fig. 1. Increase in the intial sedoheptulose-bisphosphatase reaction velocity ($V_i$) (○——○) and decrease in the measured activation lag (●——●) observed when enzyme was preincubated with 10 mM dithiothreitol. At the indicated time intervals an aliquot of enzyme was removed from the incubation mixture and added to a continuous spectrophotometric assay mixture.

enzyme preincubation increased, the initial reaction velocity measured by the continuous assay also increased (Fig. 1). A more rapid activation process was observed during the continuous assay and the lag or induction period describing this activation decreased as the time of incubation increased (Fig. 1). The rate constant describing this more rapid activation was constant (Fig. 2). The relatively rapid activation observed in the presence of $Mg^{2+}$ and sedoheptulose 1,7-bisphosphate in addition to dithiothreitol is consistent with other studies [1,5,7].

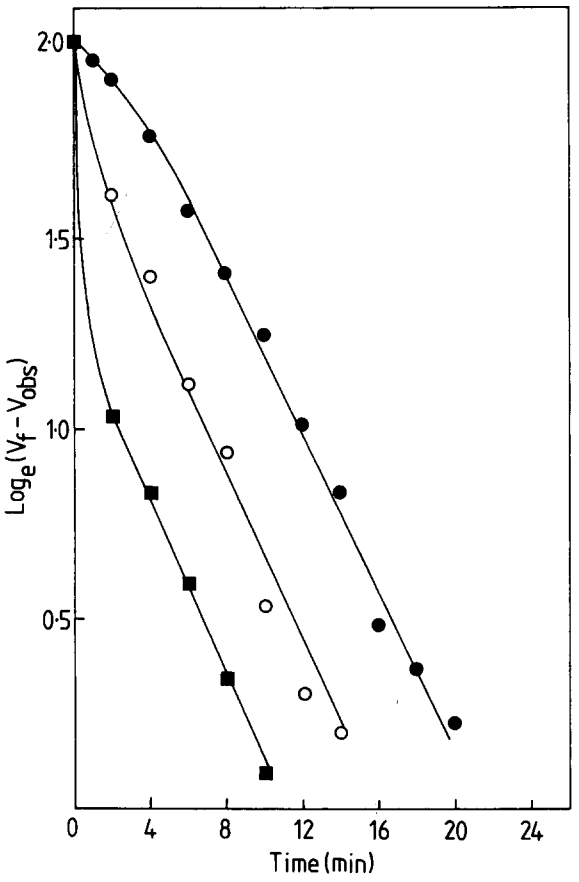


Fig. 2. Semilogarithimic plot of $\log_e(V_f - V_{obs})$ vs. time for sedoheptulose-bisphosphatase activated as described in the legend to Fig. 1. Reactions were initiated with enzyme that had been preincubated with 10 mM dithiothreitol for 0 (●———●), 80 (○———○) and 152 min (□———□). $V_f$ is the final steady-state reaction velocity and $V_{obs}$ the instantaneous reaction velocity. The slope of the linear sections gives the apparent rate constants for enzyme activation.

Sedoheptulose-bisphosphatase activation depends on the presence of a reducing agent such as dithiothreitol [7]. The relationship between $\tau$ and dithiothreitol concentration was shown to have the form of a rectangular hyperbola with $\tau$ approaching zero at infinite dithiothreitol concentration [7]. Considering that sedoheptulose-bisphosphatase activation can be described as though it involves a single slow step between inactive and active forms [7], the simplest model consistent with the linear relationship between $\tau^{-1}$ and dithiothreitol concentration is:

$$E + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E' + O \qquad (1)$$

where R is a reducing agent (dithiothreitol in the present experiments), O the oxidised product, E the inactive enzyme form, E′ the active enzyme form, and $k_1$ and $k_{-1}$ the rate constants for activation and inactivation, respectively. If the concentration of R is much greater than that of E, and the level of O remains essentially constant, then activation can be described by:

$$\tau^{-1} = k_1[R] + k_{-1}[O] \qquad (2)$$

In the present experiments these assumptions are valid. Therefore, under standard conditions the concentration of O can be assumed to be essentially zero throughout the activation process. This was confirmed in experiments showing that when the sedoheptulose-bisphosphatase reaction velocity approaches a steady state ($V_f$), essentially all of the enzyme is in the active form [12]. Under the present conditions, Eqn. 1 anticipates the linear relationship between $\tau^{-1}$ and dithiothreitol concentration. At very low dithiothreitol levels, a small deviation from linearity occurred [7]. This probably reflects the relatively slow inactivation of the reduced enzyme which can be observed in the absence of reductant under aerobic conditions (Woodrow, I.E., unpublished data). The apparent rate constant for enzyme activation ($k_1$) is 0.0313 $min^{-1} \cdot mM^{-1}$.

It was not possible to measure accurately the relationship between $\tau$ and dithiothreitol concentration in the absence of sedophetulose 1,7-bisphosphate because enzyme activation was too slow

to be directly monitored by the continuous assay. Therefore, enzyme activation was examined at various substrate levels (Fig. 3). These data show that, at a constant level of dithiothreitol, the rate of enzyme activation can also be modulated by the sedoheptulose-1,7-bisphosphatase concentration. The model described in Eqn. 1 was extended to accommodate the role of the reaction substrate in enzyme activation (Fig. 4). In this scheme both the enzyme-substrate complex and the enzyme alone can undergo activation/inactivation. Substrate binds to both enzyme forms. It is assumed that the enzyme is maintained in the active form after product release, and that the latter is irreversible. The slowest relaxation observed in this system would be that due to activation of the enzyme and enzyme-substrate complexes as long as the activation process is slow compared to substrate binding

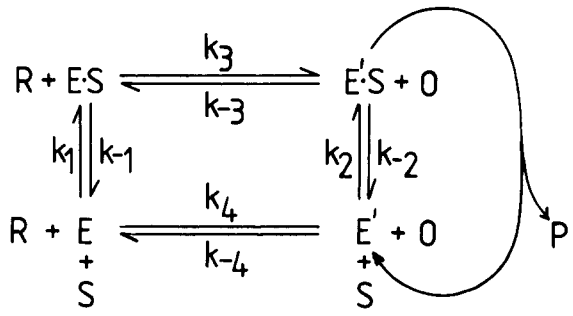


Fig. 4. Mechanism for regulation of sedoheptulose-bisphosphatase activity. Both the enzyme-substrate complex (E·S) and the enzyme alone (E) undergo slow reductive activation to the active (primed) forms. R and O are the reductant and oxidant, respectively. $k_i$ is the rate constant for a given step. Only the active enzyme form is capable of catalysing the conversion of substrate (S) to product (P).

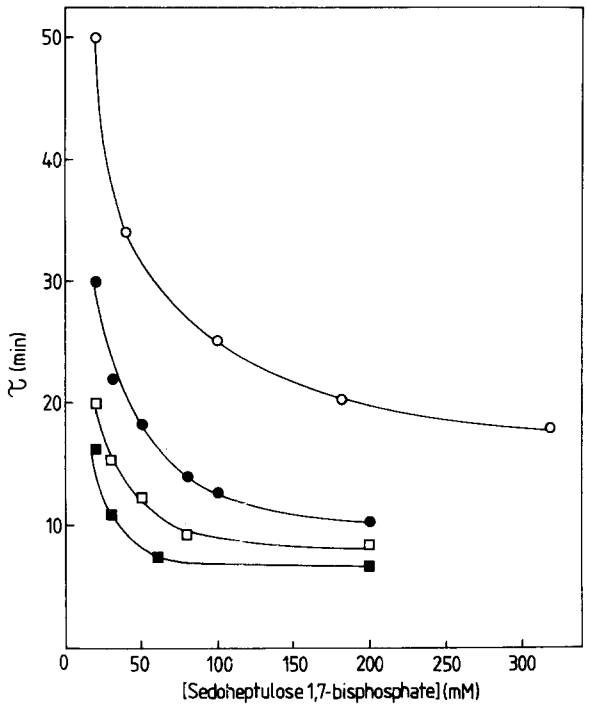


Fig. 3. Relationship between sedoheptulose-bisphosphatase activation lag ($\tau$) and the sedoheptulose 1,7-bisphosphate concentration at several levels of dithiothreitol. At any substrate concentration, plots of $1/\tau$ vs. the dithiothreitol concentration were linear (data not shown). The slope of these plots was dependent upon the substrate concentration. Dithiothreitol concentrations of 5 (○——○), 10 (●——●), 15 (□——□) and 20 mM (■——■) were used.

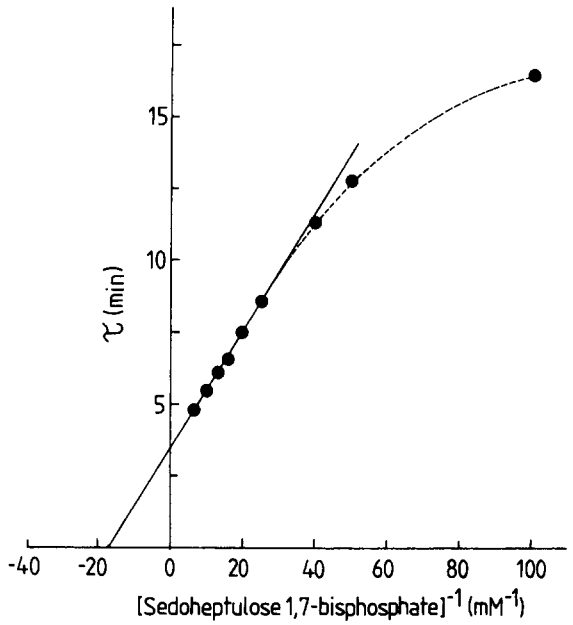


Fig. 5. Relationship between the sedoheptulose-bisphosphatase activation lag ($\tau$) and the inverse of the sedoheptulose 1,7-bisphosphate concentration. The ordinate intercept is a function of the dithiothreitol concentration and gives the rate constant for activation of the enzyme-substrate complex. The abscissa intercept gives the equilibrium constant for substrate binding to the inactive enzyme form. The dotted line shows the deviation from linearity at low substrate levels which reflects the increasing contribution of the activation of the enzyme alone to the overall activation.

[13,14]. Frieden [15] confirmed for a system essentially the same as that shown in Fig. 4 that the two slow activation steps can be described by a single relaxation. Making the same assumptions as used in the derivation of Eqn. 1, the activation of sedoheptulose-bisphosphatase is described by:

$$\tau^{-1} = \frac{k_3[R][S] + k_4[R]K_1}{K_1 + [S]} + \frac{k_{-3}[O][S] + k_{-4}[O]K_2}{K_2 + [S]} \quad (3)$$

where $K_1$ and $K_2$ are given by $k_1/k_{-1}$ and $k_2/k_{-2}$ (Fig. 4), respectively. It is assumed that the level of O remains zero throughout the activation process. Therefore, the second term in Eqn. 3 can be neglected. Examining the first term in Eqn. 3, it is evident that as the substrate concentration increases, the $E \cdot S \rightarrow E' \cdot S$ step tends to dominate the activation process. If $k_3[S] \gg k_2K_1$, Eqn. 3 reduces to:

$$\tau = K_1/k_3[R][S] + 1/k_3[R] \quad (4)$$

This equation is essentially the same as that derived by Eigen [16] for a sequence involving rapid ligand binding followed by a slow process such as a conformational change.

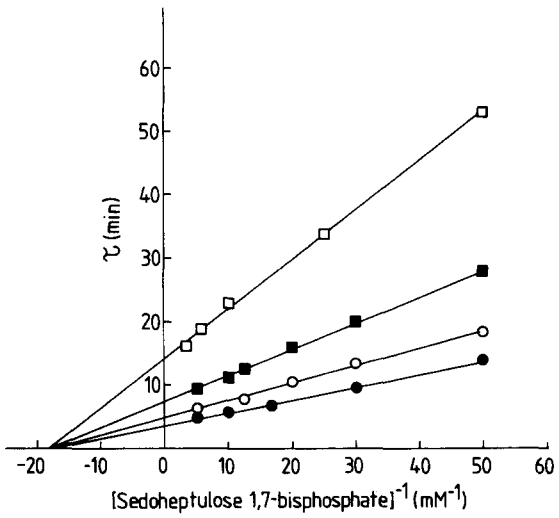


Fig. 6. Relationship between the sedoheptulose-bisphosphatase activation lag ($\tau$) and the inverse of the sedoheptulose 1,7-bisphosphate concentration. Conditions were the same as those outlined in the legend to Fig. 5 except that 5 (□——□), 10 (■——■), 15 (○——○) and 20 mM (●——●) dithiothreitol was used. The affinity of the inactive enzyme for substrate is unaffected by dithiothreitol.

The relationship between $\tau$ and $1/[S]$ for sedoheptulose-bisphosphatase activation is shown in Fig. 5. Consistent with the assumptions underlying Eqn. 4, a linear relationship occurs at higher substrate concentrations. However, significant activation via the $E \rightarrow E'$ step is probably reflected by the deviation from linearity at low substrate levels. The linear regions were examined under various dithiothreitol regimes (Fig. 6). As the dithiothreitol level was raised, the ordinate intercept also increased at a rate proportional to $1/[R]$. These intercepts gave a $k_3$ value of about 0.0138 min$^{-1}$ · mM$^{-1}$. The abscissa intercept was independent of the dithiothreitol concentration and gave a $K_1$ value of 0.069 mM. This $K_1$ value is altered by the presence of $P_i$, a competitive inhibitor (with respect to sedoheptulose 1,7-bisphosphate) of sedoheptulose-bisphosphatase activity. This indicates that $P_i$ also competitively inhibits substrate binding to the inactive enzyme form [12].

## Discussion

Frieden [17] stressed that, in order to distinguish between possible mechanisms, a slow transition should be investigated at several ligand concentrations. In this sense the current mechanism for sedoheptulose-bisphosphatase is well founded. However, it awaits a direct demonstration or enzyme reduction or modification in the presence of a reducing agent. Furthermore, although activation of sedoheptulose-bisphosphatase in the presence of an oxidant has been demonstrated [7], a redox couple (denoted by R and O) has not been shown to be effective in enzyme activation/inactivation. Soulie et al. [18] showed that full activation of stromal fructose-bisphosphatase occurs in the presence of excess dithiothreitol or reduced thioredoxin *f*, with a reversal of this process occurring when oxidised thioredoxin *f* is used. On the basis of these experiments, they suggested that a scheme similar to Eqn. 1 could also describe fructose-bisphosphatase activation/inactivation, with O and R being oxidised and reduced thioredoxin *f*, respectively. Maize chloroplast sedoheptulose-bisphosphatase is also activated by reduced thioredoxin *f* [5]. It therefore seems probable that inactivation of this

enzyme is also effected by oxidised thioredoxin *f*.

The properties of the proposed mechanism for the regulation of sedoheptulose-bisphosphatase (Fig. 4) can be better illustrated by assuming that all steps prior to product release are in equilibrium and that the time of measurement is long relative to the time required for adjustment to equilibrium. The proportion of enzyme in the active state is given by:

$$\frac{[E']+[E'\cdot S]}{[E_0]} = \left[\frac{K_2[O](1+K_1[S])}{K_3K_1[R](1+K_2[S])}+1\right]^{-1} \qquad (7)$$

where $K_i = k_i/k_{-i}$, $[E_0]$ is the total enzyme concentration, and [S] the concentration of sedoheptulose 1,7-bisphosphate. If the affinity of both enzyme forms for the substrate is identical (i.e., $K_1 = K_2$), the distribution between active and inactive states will be governed purely by the R/O ratio. However, the active sedoheptulose-bisphosphatase binds substrate more strongly than the inactive form (Fig. 7) [7,12]. Consequently, given an increase in substrate concentration, there will be a shift in enzyme from the inactive to the active form. The rate of this transition will be determined by the substrate concentration and, if conditions in the present experiments approximate those occurring in vivo, the transition will be slow. The relationship between such slow transitions and the substrate or ligand concentration in similar mechanisms had been discussed in detail by other workers [15,19–21].

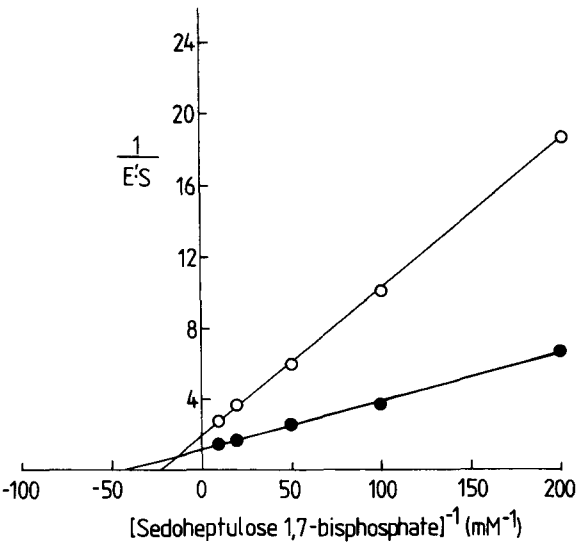


Fig. 7. Theoretical relationship between the inverse of the proportion of sedoheptulose-bisphosphatase in the E'·S form (see Fig. 4) and the inverse of the substrate concentration. Points were calculated from Eqn. 8 and $K_3$ was assumed to be 1. The ordinate intercept gives the apparent $K_m$ of the system (Fig. 4) for substrate. When reductant/oxidant ratios of 5 and 1 were used, the apparent $K_m$ was 22 and 44 $\mu$M, respectively.

According to the analysis made, which assumed equilibrium conditions, sedoheptulose-bisphosphatase activity is regulated at two basic levels: by the reductant/oxidant ratio, and by the substrate concentration. Changes in the former result in slow modifications of enzyme activity. However, changes in the latter have more complex effects. Thus, when the substrate concentration is not too 'saturating' for the active enzyme, an increase in the former would effect both a rapid and slow increase in the rate of sedoheptulose 1,7-bisphosphate hydrolysis. The rapid response would be that of the active enzyme to the higher substrate level, while the slow response would be due to the slow shift of enzyme from he inactive to the active form. The relative contribution of each type of response to the final reaction velocity can be analysed by considering the amount of enzyme in the E' · S state. Making the assumptions underlying Eqn. 7:

$$[E'\cdot S] = \frac{K_3K_1[R][S]}{[O](1+K_1[S])+[R]K_3K_1\left[\frac{1+K_2[S]}{K_2}\right]} \qquad (8)$$

When the level of O is zero, as in the current experiments, this equation reduces to:

$$\frac{[E'\cdot S]}{[E_0]} = \frac{1}{1+1/K_2[S]} \qquad (9)$$

This equation is consistent with the hyperbolic relationship between $V_f$ and [S] observed in a previous study [7]. When R/O is constant, it is possible to 'substrate-saturate' the system such that an increase in substrate concentration does not result in a significant shift in enzyme from the inactive to the active form. Examining the steady-state rate of substrate hydrolysis, the apparent $K_m$ of the system for the substrate is governed by the R/O ratio. For example, at R/O = 1 and 5 the

apparent $K_m$ of the system is 44 and 22 $\mu$M, respectively (Fig. 7). In these calculations a $K_3$ value of 1 was assumed. This, however, does not affect the interpretation made above. As the R/O ratio tends to infinity, the apparent $K_m$ of the system and the $K_m$ of the active enzyme converge. The difference between the two $K_m$ values reflects the capacity of the system for a substrate-induced slow shift in enzyme from one form to the other. Thus, at certain lower R/O values the slow response would be primarily responsible for reaction velocity increases, while at higher R/O values most of the enzyme would be in the active form and the slow response would be relatively unimportant.

The control over sedoheptulose-bisphosphatase activity exerted by the R/O ratio accounts for the light-mediated activation and dark inactivation of the enzyme [1,2]. In darkness, the low R/O level would probably maintain most of the enzyme in the inactive form. Upon illumination, an increase in the R/O ratio would effect a slow increase in enzyme activity. The final amount enzyme in the active form ultimately depends on the R/O ratio, but is also controlled by the stromal $H^+$ and $Mg^{2+}$ concentrations (Woodrow, I.E., Murphy, D.J. and Latzko, E., unpublished results) together with the sedoheptulose 1,7-bisphosphate level. Such changes in the reductant level were observed in isolated chloroplasts where the proportion of reduced thioredoxin *m* varies between 75 and 25% in the light and dark, respectively [22]. Leegood and Walker [23–25] also used isolated chloroplasts to show that the activity of fructose-bisphosphatase – which seems to be regulated by a mechanism similar to that controlling sedoheptulose-bisphosphatase [7,18,26,27] – can be modulated by addition of various electron acceptors and donors. The latter presumably alter the level of the reduced effector which reduces fructose-bisphosphatase.

The dependence of the rate and degree of sedoheptulose-bisphosphatase activation on the R/O ratio and the substrate concentration has been demonstrated in several previous studies using isolated chloroplasts. Modulation of the size of the sedoheptulose 1,7-bisphosphate pool within the chloroplast was reflected by changes in the rate of sedoheptulose-bisphosphatase activation which are anticipated by the present model [1,2]. When chloroplasts were incubated in the absence of bicarbonate with triose phosphate as a carbon source, the degree of enzyme activation was relatively high [1]. This probably resulted from the higher level of reductant available for enzyme activation under these conditions [24].

Woodrow and Walker [1] observed that, after illumination of chloroplasts, sedoheptulose-bisphosphatase activity oscillated before attaining a stable level. Such oscillations during a transition to a new steady state are characteristic of this sort of system (Fig. 4) (Woodrow, I.E., unpublished data), and may also reflect fluctuations in the sedoheptulose 1,7-bisphosphate pool size [28,29]. The amplitude of the oscillations was greatly reduced at higher R/O levels [1]. This response is also anticipated by the present model for the regulation of sedoheptulose-bisphosphatase.

Frieden [15] discussed the ways in which a slow change in enzyme kinetic properties in response to a change in ligand concentration could contribute to controlling the flux through a range of metabolic sequences by 'damping effects'. Walker et al. [30] monitored rapidly damped oscillations in the rate of $O_2$ evolution and $CO_2$ fixation following reillumination of leaves after a dark interval. It is possible that the mechanism proposed for sedoheptulose-bisphosphatase also serves to damp such flux oscillations in the reductive pentose phosphate pathway.

The analysis of the proposed mechanism for sedoheptulose-bisphosphatase regulation involved the assumption that all the reaction steps prior to product release are close to equilibrium. This allows the provision of an approximate description of the system. Nevertheless, if the system is described using steady-state equations, complex expressions involving $[S]^2$ terms occur. Under some conditions these terms could become predominant in the reaction velocity expression. It has therefore been suggested that cooperativity may sometimes be a kinetic rather than a binding phenomenon [15,20,31–34]. The current mechanism contains two different paths which contribute to overall reaction and, thus, could generate kinetic cooperativity. Confirmation of this possibility awaits the elucidation of all the rate constants and verification of a rate equation.

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