THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 259, No. 6, Issue of March 25, pp. 3791-3795, 1984
Printed in U.S.A.

Regulation of Stromal Sedoheptulose 1,7-Bisphosphatase Activity by pH and Mg^{2+} Concentration*

(Received for publication, May 31, 1983)

Ian E. Woodrow‡§, Denis J. Murphy¶, and Erwin Latzko‖

From the ‡Department of Environmental Biology, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra 2600, Australia, the ¶Agricultural Research Council Research Group on Photosynthesis, Botany Department, University of Sheffield, Sheffield S102TN, United Kingdom, and the ‖Botanical Institute, University of Münster, Schlossgarten 3, 4400 Münster, Federal Republic of Germany

A scheme is proposed for the regulation of stromal sedoheptulose 1,7-bisphosphatase activity which enlarges upon a previously elaborated mechanism (Woodrow, I. E., and Walker, D. A. (1983) *Biochim. Biophys. Acta* 722, 508–516). The latter involves oxidized (inactive) and reduced (active) enzyme forms. Both the free enzymes and the enzyme-substrate complexes undergo slow oxidation/reduction. This study examines the behavior of the system under pH and Mg^{2+} concentration regimes that are likely to occur in the chloroplast stroma. The control of enzyme activity by pH can be described in terms of each free enzyme and enzyme-substrate complex existing in protonated and nonprotonated forms. The molecular dissociation constants for each protonation reaction were calculated from kinetic data. Mg^{2+} concentration changes modulate these constants. Under conditions that are likely to obtain in the stroma in the dark, the model predicts that approximately 99.1% of the enzyme will be in the inactive forms. Such inactivation is important since it would prevent the reductive pentose phosphate pathway from operating in darkness.

Upon illumination the stromal pH increases by about one pH unit from a near neutral state in darkness (Heldt *et al.*, 1973). The mechanism is believed to involve the "pumping" of protons from the stroma into both the intrathylakoid space (Neumann and Jagendorf, 1964; Trebst, 1974) and the cytosol (Heber and Krause, 1971; Gimmler *et al.*, 1975). The process of altering the pH by using weak acids was employed to investigate the role of stromal pH changes in controlling the flux through the reductive pentose phosphate pathway of photosynthesis during illumination. Where the dissociated and nondissociated forms of weak acids could cross the chloroplast envelope, they constituted a proton shuttle (Purczeld *et al.*, 1978; Heber *et al.*, 1979) and effectively equilibrated the stroma with the surrounding medium (Heber *et al.*, 1979). Addition of weak acids to a medium containing isolated chloroplasts caused an inhibition of CO_2 fixation and an increase in both the fructose 1,6-bisphosphate and sedoheptulose 1,7-bisphosphate pool sizes (Hiller and Bassham, 1965; Pedersen *et al.*, 1966; Purczeld *et al.*, 1978; Enser and Heber, 1980; Flügge *et al.*, 1980). The rise in these metabolite levels and the concomitant decline in the carbon flux was interpreted to mean that the activities of fructose bisphosphatase and sedoheptulose bisphosphatase are sensitive to pH changes in the 7–8 range and that, under conditions of declining pH, these enzymes play a significant role in limiting the rate of CO_2 fixation.

The transfer of protons across the thylakoid membrane is believed to be electrically compensated for by a countertransfer of Mg^{2+} ions (Dilley and Vernon, 1965; Barber *et al.*, 1974; Hind *et al.*, 1974; Krause, 1974; Krause, 1977). Since the chloroplast envelope is relatively impermeable to Mg^{2+} (Pflüger, 1973; Gimmler *et al.*, 1975), this transfer results in an increase in the stromal Mg^{2+} concentration (Hind *et al.*, 1974; Chow *et al.*, 1976). Portis *et al.* (1977) lowered the stromal Mg^{2+} concentration of illuminated chloroplasts using a divalent ionophore. This effected a decline in the carbon flux and an increase in the sedoheptulose 1,7-bisphosphate and fructose 1,6-bisphosphate levels which were suggested to indicate that the bisphosphatases limited the carbon flux under conditions of declining Mg^{2+} concentration.

In the present report we enlarge upon a previously proposed model for the regulation of sedoheptulose 1,7-bisphosphatase (Woodrow and Walker, 1983) to include the role of stromal pH and Mg^{2+} concentration changes. This unified view of the relationship between enzyme kinetic parameters, the redox state of ferredoxin, and the stromal H^+, Mg^{2+}, and sedoheptulose 1,7-bisphosphate concentrations allows predictions to be made concerning the degree of activation of sedoheptulose 1,7-bisphosphatase under specific conditions. It is probable that enzyme activity in the chloroplast is, under most conditions, related to the light intensity since the latter controls all the factors which regulate sedoheptulose 1,7-bisphosphatase activity. In the extreme case (darkness), it is estimated that less than 1% of the total enzyme is in the active form. Inactivation of sedoheptulose 1,7-bisphosphatase in darkness, therefore, accounts for the cessation of CO_2 fixation by ribulose 1,5-bisphosphate carboxylase.

EXPERIMENTAL PROCEDURES

Materials

Wheat (*Triticum aestivum* L. cv. Sappo) was grown in vermiculite under sunlight and supplementary incandescent lamps. Biochemicals were purchased from Sigma, Munich, F.R.G., auxiliary enzymes from

* This work was supported in part by the Deutsche Forschungs Gemeinschaft. Part of this work was done when I. E. W. and D. J. M. were employed at the Botanical Institute, Münster. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Recipient of a Queen Elizabeth II Postdoctoral Research Fellowship in support of this work. To whom correspondence should be sent.

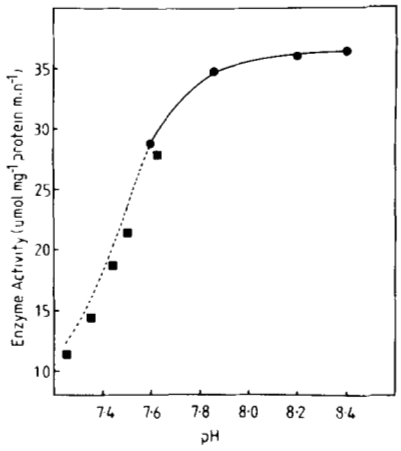

FIG. 1. **Relationship between enzyme activity and pH.** Measurements were made using Tricine (●——●) and Hepes (■——■) buffers. The *dotted section* represents the activities corrected for the inhibition observed in the presence of Hepes.

Boehringer Mannheim, F.R.G., and Sephadex gels from Pharmacia, Freiburg, F.R.G.

Methods

Preparation of Sedoheptulose 1,7-Bisphosphatase—Sedoheptulose 1,7-bisphosphatase was purified by the procedure outlined by Woodrow and Walker (1982). This preparation was loaded onto a column (2 cm^2 × 6 cm) of hydroxylapatite previously equilibrated with 50 mM Na acetate, pH 5.8, and 2 mM β-mercaptoethanol. Protein was eluted in a linear gradient of 0–300 mM K_3PO_4. Active fractions were combined and concentrated in an Amicon ultrafiltration cell fitted with a PM-10 membrane. The enzyme was estimated to be about 90% pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and had a final specific activity of 42 units mg^{-1} protein (bovine serum albumin standard). Enzyme was stored in 20% glycerol and 50 mM Tricine-NaOH[1] (pH 8.0) in liquid nitrogen. Protein was determined by the procedure of Lowry *et al.* (1951).

Determination of Sedoheptulose 1,7-Bisphosphatase Activity—Enzyme activity was measured at 20 °C using a continuous spectrophotometric assay which couples the formation of sedoheptulose 7-phosphate to the oxidation of NADH (Woodrow and Walker, 1982). The reaction was followed at 340 nm using a Pye-Unicam SP-1800 spectrophotometer. The standard reaction mixture contained, in a final volume of 1 ml: 50 mM buffer; 10 mM $MgCl_2$; 20 mM KCl; 0.1 mM ATP; 1 mM phosphoenolpyruvate; 0.15 mM NADH; 20 mM dithiothreitol; 0.1 mM sedoheptulose 1,7-bisphosphate; 2 units of pyruvate kinase; 2 units of lactate dehydrogenase; 0.5 unit of 6-phosphofructokinase; and sedoheptulose bisphosphatase solution. The latter was normally used to initiate the reaction. Calcium was not added to the reaction mixture although it may increase the rate of enzyme activation (Wolosiuk *et al.*, 1982). The activation kinetics did not vary, under standard conditions, throughout the experiments. Two buffers were used in the experiments: Tricine (pH 7.7–8.4) and Hepes (pH 7.25–7.8). The reaction velocities were standardized around those obtained with Tricine buffer (Fig. 1).

Analysis of Reaction Progress Curves—Reaction progress curves (product formation with time) were analyzed by plotting log ($V_f - V_{obs}$) *versus* time, where V_f is the final steady state reaction velocity and V_{obs} the instantaneous rate of product formation. These plots yielded the apparent rate constants for enzyme activation (Woodrow and Walker, 1983). Activation was, in all cases, described by a single first order rate constant. The contribution of the coupled enzyme system to the apparent activation kinetics was discussed in a previous study (Woodrow and Walker, 1983).

Titration of Sedoheptulose 1,7-Bisphosphate—The sodium salt of sedoheptulose 1,7-bisphosphate (0.53 mM) was titrated under nitrogen (20 °C) by successive additions of 2 μl of 0.5 N HCl. The pH was measured after each addition with a Radiometer (Copenhagen) pH meter 26 system. The ionic strength was maintained at 0.08 M by the addition of tetraethylammonium bromide. The interaction between the latter, sedoheptulose 1,7-bisphosphate and Mg^{2+} is assumed to be negligible. The pH meter was calibrated at 20 °C using 50 mM potassium hydrogen phthalate (pH 4.0) and 50 mM sodium borate (pH 9.22). The titration was repeated at three levels of $MgCl_2$.

[1] The abbreviations used are: Tricine, *N*-tris(hydroxymethyl)-methylglycine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

RESULTS

Titration of Sedoheptulose 1,7-Bisphosphate—The titration curve of sedoheptulose 1,7-bisphosphate over the pH range 4 to 8 shows a single inflection of about pH 6.42. pK_a values of the third (pK_{a_1}) and fourth (pK_{a_2}) dissociation constants for sedoheptulose 1,7-bisphosphoric acid at an ionic strength of 0.08 M of 6.02 and 6.82, respectively, were estimated from the pH and slope at the midpoint (Table I) (Martell and Calvin, 1952). The separation of these values is only slightly greater than that expected for identical phosphate groups (Martell and Calvin, 1952).

The ionic species most probably present in the current experiments are related by the following equilibria.

$$\begin{array}{ccccc} H_2SBP^{2-} & \overset{pK_{a_1}}{\rightleftharpoons} & HSBP^{3-} & \overset{pK_{a_2}}{\rightleftharpoons} & SBP^{4-} \\ \Updownarrow K_1 & & \Updownarrow K_2 & & \Updownarrow K_3 \\ MgH_2SBP & \overset{pK_{m_1}}{\rightleftharpoons} & MgHSBP^{-} & \overset{pK_{m_2}}{\rightleftharpoons} & MgSBP^{2-} \end{array}$$

The stability constants of the magnesium sedoheptulose 1,7-bisphosphatase complexes are given by K_1, K_2, and K_3. pK_{m_1} and pK_{m_2} are pK values of the dissociation constants for the MgH_2SBP and $MgHSBP^-$ species, respectively.

In the absence of Mg^{2+}, the amount of sedoheptulose 1,7-bisphosphate in the tetraanionic state over the pH range used in the kinetic experiments is shown in Table II. Titrations in the presence of 2, 10, and 20 mM $MgCl_2$ were also performed to evaluate the other equilibrium constants (Table I). Approximate values for K_2 and K_3 of 159 M^{-1} and 587 M^{-1}, respectively, were calculated using the method described by O'Sullivan and Perrin (1964). The pK_a values of the dissociation constants K_{m_1} and K_{m_2} are approximately 5.43 and 6.24, respectively. Over the pH range used in the kinetic experiments and in the presence of 10 mM Mg^{2+}, most of the sedoheptulose 1,7-bisphosphate occurs in the $MgSBP^{2-}$ form (Table II). It is, therefore, probable that the latter is a substrate for the catalytic reaction. Nevertheless, formation of species such as Mg_2SBP and $Mg(SBP)_2^{6-}$ cannot be excluded, especially at higher Mg^{2+} levels.

Regulation of Sedoheptulose 1,7-Bisphosphatase Activity—The dependence of the steady state reaction velocity (V_f) upon the proton concentration is consistent with the simple dibasic acid model of Michaelis and Davidsohn (1911). The data were analyzed by plotting the logarithms of the kinetic constants against pH (Dixon, 1953). Fig. 3 shows a plot of log V *versus* pH. V was calculated from Lineweaver-Burk plots and represents the maximum reaction velocity at given H^+ and Mg^{2+} concentrations. Over the pH range likely to occur in the chloroplast stroma, the data are consistent with the occurrence of a single ionization step. The second step of the

TABLE I

Parameters from the pH titration of sedoheptulose 1,7-bisphosphate at various $MgCl_2$ concentrations

[$MgCl_2$]	Midpoint pH	Slope[a]
mM		
0	6.42	−0.98
2	6.25	−0.93
10	6.03	−0.87
20	5.78	−0.80

[a] At the midpoint of the titration curve.

TABLE II

Dependence of the proportion of two ionic species of sedoheptulose 1,7-bisphosphate on pH

pH	SBP^{4-a}	$MgSBP^{2-b}$
	%	%
7.3	77	92
7.5	82	95
7.7	88	97
8.0	94	98
8.4	97	99

[a] Calculations were made assuming pK_a values for the third and fourth dissociation constants of 6.02 and 6.82, respectively, and the absence of Mg^{2+}.

[b] Calculations were made assuming pK_a values for MgH_2SBP and $MgHSBP^-$ dissociation constants of 5.43 and 6.24, respectively, and the presence of 10 mM Mg^{2+}.

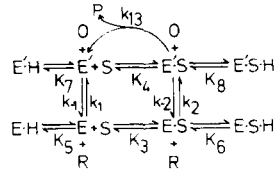

FIG. 2. **Mechanism for the regulation of sedoheptulose 1,7-bisphosphatase activity.** The primed and unprimed species represent reduced and oxidized enzyme forms, respectively. *R* and *O* are the reductant and oxidant, respectively. k_i is the rate constant for a given step. K_5, K_6, K_7, and K_8 are the molecular dissociation constants which describe the protonation of the enzyme forms. K_3, K_4, K_{11}, and K_{12} describe substrate binding to the enzyme forms. It is assumed that only the reduced nonprotonated enzyme form (E') is capable of catalyzing the conversion of substrate (S) to product (P).

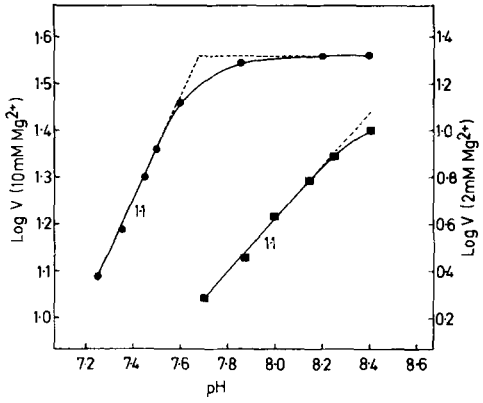

FIG. 3. **Relationship between $\log_{10}$ V and pH at 2 mM (■——■) and 10 mM (●——●) Mg^{2+}.** V (maximum reaction velocity) values were calculated from Lineweaver-Burk plots at the indicated H^+ and Mg^{2+} concentrations. The reaction velocity under a given set of conditions is defined as the steady state reaction velocity (V_f) after activation. The intercept of the linear portions of the graphs gives the molecular dissociation constant for protonation at the active enzyme-substrate complex ($E' \cdot S$). The slopes of the linear portions, which occur at the lower pH values, are printed next to the lines.

Michaelis and Davidsohn (1911) model is negligible over this pH range. Since most of the substrate exists as a single species ($MgSBP^{2-}$) over this pH range, changes in apparent kinetic properties of sedoheptulose 1,7-bisphosphatase do not appear to be due to substrate effects. The plot of log V *versus* pH was made using both 10 and 2 mM Mg^{2+}, and molecular dissociation pK values of 7.67 and at least 8.3, respectively, were recorded for the active enzyme-substrate complex. The plot of log (V/K_m) *versus* pH shown in Fig. 4 gives a pK value for ionization of the free active enzyme of 7.82 (10 mM Mg^{2+}). In both graphs, the slope of the linear portions at the lower pH values is about 1. This probably indicates that one protonation is required to convert the enzyme form predominant over these pH ranges into the active form (Dixon, 1953).

The activation of sedoheptulose 1,7-bisphosphatase was studied under several H^+ regimes at constant concentrations of substrate, reductant, and Mg^{2+} (Fig. 5). Plots of τ (τ^{-1} is the apparent rate constant for enzyme activation) *versus* the H^+ concentration yielded pK_6 (Fig. 2) values of 8.49 and 8.8 for 10 mM Mg^{2+} and 2 mM Mg^{2+}, respectively. The linear relationship between τ and the H^+ concentration is consistent with the models shown in Fig. 2. The K_6 value represents the molecular dissociation constant for the inactive enzyme-substrate complex. Graphs of τ *versus* [sedoheptulose 1,7-bisphosphate]$^{-1}$ were made at various pH values. The inverse of the abscissa intercepts of these plots were then plotted against the inverse of the H^+ concentration (Fig. 6). The linear portion of this graph gave K_3 and pK_5 values of 69.2 μM and 8.02, respectively (Fig. 2). This method of analyzing the

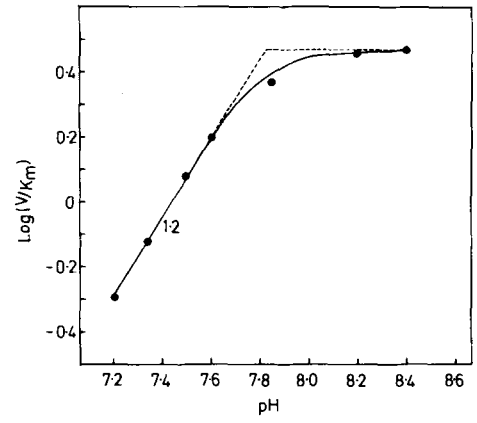

FIG. 4. **Relationship between log (V/K_m) and pH in the presence of 10 mM Mg^{2+}.** V (maximum reaction velocity) and K_m (Michaelis constant) values were calculated from Lineweaver-Burk plots at the indicated pH values. The intercept of the linear portions of the graph gives the molecular dissociation constant for protonation of the active enzyme (E'). The slope of the linear portion, which occurs at lower pH values, is shown on the graph.

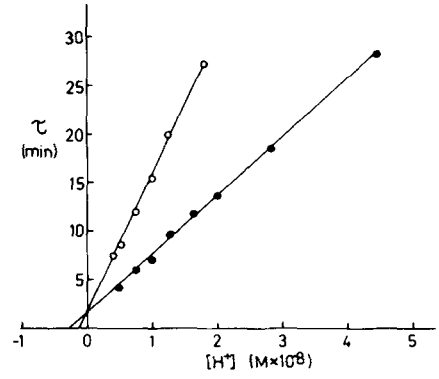

FIG. 5. **Relationship between the induction period for enzyme activation (τ) and the proton concentration at 2 mM (○——○) and 10 mM (○——○) Mg^{2+}.** The τ values reflect activation of the inactive enzyme substrate complex ($E \cdot S$). τ values at an infinite substrate concentration (when all the inactive enzyme is in the $E \cdot S$ form) were estimated from plots of τ *versus* [sedoheptulose 1,7-bisphosphate]$^{-1}$ (Woodrow and Walker, 1983). The abscissa intercepts give the molecular dissociation constants for protonation of the inactive enzyme-substrate complex.

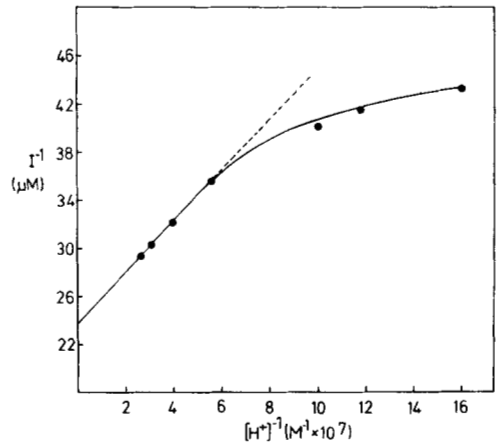

FIG. 6. **Relationship between the inverse of the proton concentration and the inverse of the abscissa intercepts of a series of τ *versus* [sedoheptulose 1,7-bisphosphate]$^{-1}$ plots.** The interpretation of these graphs is discussed under "Results." The ordinate intercept and slope of the linear section of this graph allow the calculation of molecular dissociation constant for protonation of the inactive enzyme (E) and the dissociation constant for substrate binding to the inactive enzyme.

results is discussed in the next section.

Analysis of Sedoheptulose 1,7-Bisphosphatase Activation Kinetics—The data presented in this study indicate that enzyme species undergo protonation/deprotonation reactions which can be described by the models presented in Fig. 2. The nonprotonated reduced form is the active species. The basis of this model was deduced by Woodrow and Walker (1983) who examined the catalytic and relaxation properties of the system under various substrate and reductant regimes. These measurements were made at a constant pH, and the apparent K_m and K_D for substrate binding to the inactive enzyme form were functions of K_7, K_4, K_8, and k_{13}, and K_5 K_3, and K_6, respectively.

In the present analysis, the protonation/deprotonation and substrate-binding reactions are assumed to be much faster than the oxidation/reduction reactions. This is a reasonable assumption because the latter have half-times of the order of minutes (Woodrow and Walker, 1983; Woodrow *et al.*, 1983). It is also assumed that the overall reaction is irreversible, the substrate level remains constant, and there is no product inhibition. Under these conditions, the slowest relaxation will be due to the oxidation/reduction of the enzyme and enzyme-substrate complex and can be described by a single rate constant. The relationship between the latter and the catalytic reaction velocity was derived by Frieden (1970) and is given by

$$v_t = v_f + (v_0 - v_f)e^{-kt} \tag{1}$$

where v_t is the reaction velocity at time t (which is relative to the change in conditions that initiates the slow relaxation), v_f the final reaction velocity at $t = \infty$, v_0 the initial reaction velocity, and k the apparent rate constant describing the transition to the new steady state.

If it is assumed that the concentration of reductant is much greater than that of the enzyme and that the level of reductant (R) and oxidant (O) remains essentially constant, then the rate constant for activation/inactivation is given by

$$k = \frac{K_5K_6[R](k_1K_3 + [S]k_2)}{K_3K_6([H^+] + K_5) + K_5[S](K_6 + [H^+])} + \frac{K_7K_8[0](k_{-1}K_4 + [S]k_{-2})}{K_4K_8(K_7 + [H^+]) + K_7[S]([H^+] + K_8)}. \tag{2}$$

If enzyme activation is examined and the amount of oxidant is assumed to remain essentially zero, then the slow relaxation can be described by

$$\tau = \frac{1}{k} = \frac{K_3([H^+] + K_5)}{K_5[R](k_1K_3 + [S]k_2)} + \frac{[S](K_6 + [H^+])}{K_6[R](k_1K_3 + [S]k_2)}. \tag{3}$$

As the concentration of substrate is increased and $[S]k_2 \gg k_1k_3$, τ becomes a linear function of $1/[S]$ (assuming a constant H^+ and R concentration),

$$\tau \simeq \frac{K_3([H^+] + K_5)}{[R]K_5k_2}\frac{1}{[S]} + \frac{K_6 + [H^+]}{K_6[R]k_2}. \tag{4}$$

At very large substrate levels,

$$\tau \simeq \frac{1}{[R]k_2} + \frac{[H^+]}{K_6[R]k_2}. \tag{5}$$

A graph of τ *versus* $[H^+]$, under these conditions, intercepts the ordinate axis at $[H^+] = -K_6$.

Plots of τ *versus* $1/[S]$ intercept the abscissa at

$$I = \frac{-1}{[S]} = \frac{K_5(K_6 + [H^+])}{K_6K_3(K_5 + [H^+])}. \tag{6}$$

And if $[H^+] \gg K_6$, $1/I$ becomes a linear function of $1/[H^+]$,

$$\frac{1}{I} \simeq \frac{K_6K_3}{K_5} + \frac{K_6K_3}{[H^+]}. \tag{7}$$

Equations 6 and 7 allow the determination of the molecular dissociation constants (K_5 and K_6) and substrate-binding constant (K_3) for the inactive enzyme form.

The concentration of individual enzyme species can be described by assuming that all the steps prior to product release are at equilibrium and that the time of measurement is long compared to the time required for adjustment to equilibrium. The concentrations of the species shown in Fig. 2 are given by

$$\frac{[E' \cdot S]}{E_0} = \frac{[S]A}{K_4} \tag{8}$$

$$\frac{[E' \cdot S] + [E']}{E_0} = \frac{(K_4 + [S])A}{K_4} \tag{9}$$

$$\frac{[E' \cdot H \cdot S] + [E' \cdot H]}{E_0} = \frac{A[H^+]}{K_7} + \frac{A[S][H^+]}{K_4K_8} \tag{10}$$

$$\frac{[E \cdot S] + [E]}{E_0} = \frac{A[O]}{[R]}\left[K_1 + \frac{K_2[S]}{K_4}\right] \tag{11}$$

$$\frac{[E \cdot H \cdot S] + [E \cdot H]}{E_0} = \frac{A[H^+][O]}{[R]}\left[\frac{K_1}{K_5} + \frac{K_2[S]}{K_6K_4}\right] \tag{12}$$

where

$$A = \left[1 + \frac{[H^+]}{K_7} + \frac{[H][S]}{K_4K_8} + \frac{[S]}{K_4} + \frac{[0]}{[R]}\left(K_1 + K_2[S] + \frac{[H^+]K_1}{K_5} + \frac{[H^+][S]K_2}{K_6K_4}\right)\right]^{-1} \tag{13}$$

and E_0 is the total enzyme concentration.

DISCUSSION

The simplest model that is consistent with the measured dependence of the apparent K_m, V_{max}, and τ values on proton concentration is shown in Fig. 2. This mechanism is based upon one previously suggested to account for the effect of reductant, oxidant, and substrate on enzyme activity (Woodrow and Walker, 1983; Woodrow *et al.*, 1983).

These mechanisms are based on kinetic evidence and, therefore, may represent simplifications of the actual mechanisms.

Description of the effect of pH as a single protonation reaction is undoubtedly a simplification (Tipton and Dixon, 1980). The mechanism could also conceivably involve oxidation/reduction of the protonated enzyme forms. In this case, activation would also be described by a single first order rate constant. However, to be consistent with the present data the rate constants for reduction of the $E{\cdot}H$ and $E{\cdot}S{\cdot}H$ forms must be much smaller than k_1 and k_2. It is also possible that the protonated enzyme forms bind substrate. Although this leads to different paths by which the active enzyme-substrate complex may be formed, the interpretation will not be affected if all the steps prior to product release are close to equilibrium. If the latter is not the case, substrate binding to the protonated enzyme forms would result in extremely complicated kinetics (Laidler, 1955; Peller and Alberty, 1963; Stewart and Lee, 1967; Kaplan and Laidler, 1967). The inclusion of more than one enzyme-substrate intermediate is also a possibility. However, the number of these intermediates is unimportant in terms of describing the behavior of the system since the constants obtained from the pH dependence of V are average values weighted in favor of the predominant complex (Tipton and Dixon, 1980).

The proton concentration has a multiple role in the present model; it controls the apparent K_m[2] and V_{max} values as well as the total amount of enzyme in the reduced form. Variations in pH will cause a net shift of enzyme from the active to the inactive forms or vice versa. This phenomenon is caused by the differences between pK_5 and pK_6, and pK_7 and pK_8. The dependence of the concentration of the various enzyme species upon the proton, reductant, oxidant, and substrate concentrations is described in Equations 8–12. By altering the apparent pK_8 and pK_6 (and almost certainly pK_5 and pK_7) values, Mg^{2+} also controls the distribution of enzyme between protonated and nonprotonated and oxidized and reduced forms (Table III). Low levels of Mg^{2+} may also affect the system by reducing the proportion of substrate in the $MgSBP^{2-}$ form. Effects of H^+ and Mg^{2+} consistent with the present proposals were also observed by Laing *et al.* (1981) using a chloroplast extract. The pH for optimal enzyme activity was shifted by changing the Mg^{2+} concentration. A similar effect was observed by Minot *et al.* (1982) for stromal fructose 1,6-bisphosphatase. These authors also used a single protonation step to describe the effect of the H^+ concentration on enzyme activation.

In the chloroplast, the kinetic properties of sedoheptulose 1,7-bisphosphatase appear to be ultimately controlled by the light intensity. Both the stromal H^+ and Mg^{2+} concentrations are linked to the rate of photosynthetic electron transport. And the R/O ratio, which is used to describe the relationship between the amount of reductant available for enzyme activation and the position of the equilibrium between active and inactive enzyme forms, appears to be linked to the redox state of the ferredoxin pool (Woodrow and Walker, 1983). In this way, the light intensity could control the amount of enzyme in the active form and the apparent K_m and V_{max} values. It is conceivable that at nonsaturating light intensities, changes in the flux through the reductive pentose phosphate pathway may be paralleled by similar changes in sedoheptulose 1,7-bisphosphatase activity. In darkness, inactivation of this enzyme is necessary to prevent futile cycling of metabolites and would contribute to the cessation of CO_2 fixation. Table III shows the anticipated distribution of enzyme species under conditions which approximate those obtaining in the chloroplast stroma in the light and the dark. Assuming a constant O/R ratio, the pH and Mg^{2+} changes alone account for a 17-fold reduction in the proportion of enzyme in the active form. This change is magnified by a concomitant change in the redox state, with 99.1% of the enzyme in inactive forms with an O/R ratio of 5. This represents a 60-fold inactivation of sedoheptulose 1,7-bisphosphatase in darkness.

TABLE III

Variation in the proportions of enzyme species under conditions approximating those obtaining in the light and dark in chloroplasts

Conditions	$E' + E'{\cdot}S$[a]	$E'{\cdot}H + E'{\cdot}S{\cdot}H$	$E + E{\cdot}S$	$E{\cdot}H + E{\cdot}S{\cdot}H$
	%			
10 mM Mg^{2+}; pH 8; $O/R = 1$	35.4	20.0	19.8	24.7
10 mM Mg^{2+}; pH 8; $O/R = 0.2$	55.0	31.1	6.1	2.8
2 mM Mg^{2+}; pH 7; $O/R = 1$	2.1	65.0	1.2	31.6
2 mM Mg^{2+}; pH 7; $O/R = 5$	0.9	28.1	2.6	68.4

[a] The abbreviation of enzyme forms is described in the legend to Fig. 1. Calculations of the proportions were made according to the equations described under "Results." In the calculations it was assumed for convenience that $K_1 = 1$ and [sedoheptulose 1,7-bisphosphate] = 8.1 μM.

Acknowledgments—We would like to thank Drs. Y. A. M. Holtum and U. Shyminder for their valuable assistance.

REFERENCES

Barber, J., Mills, J. & Nicolson, J. (1974) *FEBS Lett.* **49,** 106–110

Chow, W. S., Wagner, G. & Hope, A. B. (1976) *Aust. J. Plant Physiol.* **3,** 853–861

Dilley, R. A. & Vernon, L. P. (1965) *Arch. Biochem. Biophys.* **3,** 365–375

Dixon, M. (1953) *Biochem. J.* **55,** 161–170

Enser, U. & Heber, U. (1980) *Biochim. Biophys. Acta* **592,** 577–591

Flügge, U. I., Freisl, M. & Heldt, H. W. (1980) *Planta* **149,** 48–51

Frieden, C. (1970) *J. Biol. Chem.* **245,** 5788–5799

Gimmler, H., Schäfer, G. & Heber, U. (1975) *Proc. Int. Congr. Photosynth. Res.* **2,** 1381–1392

Heber, U. & Krause, G. H. (1971) in *Photosynthesis & Photorespiration* (Hatch, M. D., Osmond, C. B., & Slatyer, R. O., eds) pp. 218–223, Wiley-Interscience, New York

Heber, U., Enser, U., Weis, E., Ziem, U. & Giersch, C. (1979) in *Covalent and Non-covalent Modulation of Protein Function* (Atkinson, D. E. & Fox, C. E., eds) pp. 113–139, Academic Press, New York

Heldt, H. W., Werdan, K., Milovancev, M. & Geller, G. (1973) *Biochim. Biophys. Acta* **314,** 224–241

Hiller, R. G. & Bassham, J. A. (1965) *Biochim. Biophys. Acta* **109,** 607–610

Hind, G., Nakatani, H. Y. & Izawa, S. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71,** 1484–1488

Kaplan, H. & Laidler, K. J. (1967) *Can. J. Biochem.* **45,** 539–547

Krause, G. H. (1974) *Biochim. Biophys. Acta* **333,** 301–313

Krause, G. H. (1977) *Biochim. Biophys. Acta* **460,** 500–510

Laidler, K. J. (1955) *Trans. Faraday Soc.* **51,** 528–535

Laing, W. A., Stitt, M. & Heldt, H. W. (1981) *Biochim. Biophys. Acta* **637,** 348–359

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275

Martell, A. E. & Calvin, M. (1952) *Chemistry of the Metal Chelate Compounds,* Prentice-Hall, Inc. New York

Michaelis, L. & Davidsohn, H. (1911) *Biochem. Z.* **35,** 386–412

Minot, R., Meunier, J.-C., Buc, J. & Ricard, J. (1982) *FEBS Lett.* **142,** 118–120

Neumann, J. & Jagendorf, A. T. (1964) *Arch. Biochem. Biophys.* **107,** 109–119

O'Sullivan, W. J. & Perrin, D. D. (1964) *Biochemistry* **3,** 18–26

Pedersen, T. A., Kirk, M. & Bassham, J. A. (1966) *Physiol. Plant.* **19,** 219–231

Peller, L. & Alberty, R. A. (1963) *J. Am. Chem. Soc.* **81,** 5709–5713

Pflüger, H. (1973) *Z. Naturforsch. Teil C Biochem. Biophys. Biol. Virol.* **28,** 779–780

Portis, A. R., Jr., Chon, C. J., Mosbach, A. & Heldt, H. W. (1977) *Biochim. Biophys. Acta* **461,** 313–325

Purczeld, P., Chon, C. J., Portis, A. R., Jr., Heldt, H. W. & Heber, U. (1978) *Biochim. Biophys. Acta* **501,** 488–498

Stewart, J. A. & Lee, H. S. (1967) *J. Phys. Chem.* **71,** 3888–3891

Tipton, K. F. & Dixon, H. B. F. (1980) *Methods Enzymol.* **63,** 182–234

Trebst, A. (1974) *Annu. Rev. Plant Physiol.* **25,** 423–458

Wolosiuk, R. A., Hertig, C. M., Nishizawa, A. N. & Buchanan, B. B. (1982) *FEBS Lett.* **140,** 31–35

Woodrow, I. E. & Walker, D. A. (1982) *Arch. Biochem. Biophys.* **216,** 416–422

Woodrow, I. E. & Walker, D. A. (1983) *Biochim. Biophys. Acta* **722,** 508–516

Woodrow, I. E., Murphy, D. J. & Walker, D. A. (1983) *Eur. J. Biochem.* **132,** 121–123

[2] Although the active enzyme exhibits Michaelis-Menten substrate kinetics (Woodrow and Walker, 1982), this study does not exclude the possibility that sedoheptulose 1,7-bisphosphatase shows a kinetic cooperativity when held in an equilibrium between inactive and active forms (Woodrow and Walker, 1983).