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CONTROL OF CO_2 FIXATION

REGULATION OF SPINACH RIBULOSE-5-PHOSPHATE KINASE BY STROMAL METABOLITE LEVELS

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The effect of stromal metabolites on the light-activated form of ribulose-5-phosphate kinase was studied with the enzyme rapidly extracted from illuminated spinach chloroplasts. In some instances, the effect of metabolites on the dark-inactivated enzyme extracted from darkened chloroplasts was also investigated. (1) The light-activated form of the enzyme is competitively inhibited with respect to ribulose 5-phosphate by 6-phosphogluconate, ribulose 1,5-bisphosphate, 3-phosphoglycerate and phosphate. Also, fructose 1,6-bisphosphate is inhibitory. All these compounds, except ribulose 1,5-bisphosphate, show an increasing inhibitory effect at lower pH values. Therefore, in the presence of these inhibitors, ribulose-5-phosphate kinase becomes strongly pH dependent. These compounds also exert an inhibitory effect on the dark-inactivated enzyme. (2) The assay of stromal levels of 6-phosphogluconate showed that this compound increased dramatically during a light-dark transient. (3) The dark-inactivated form of ribulose-5-phosphate kinase is strongly inhibited by ADP, the inhibition being competitive with respect to ATP. (4) A simulation of stromal metabolite levels in the enzyme activity assay indicates that in illuminated chloroplasts ribulose-5-phosphate kinase attains only about 4% of its maximal activity. When the fully light-activated enzyme is assayed under conditions occurring in the stroma in the dark, the activity is further decreased by a factor of 20. The same assay with the dark-inactivated enzyme yields an activity of virtually zero. (5) These results demonstrate that in the chloroplasts ribulose-5-phosphate kinase can not only be very efficiently switched off in the dark, but also be subjected to fine control during the illuminated state through the action of stromal metabolites.

Introduction

The enzymes of the Calvin cycle are regulated by light in various ways. A light-dependent interconversion of enzymes is caused by reduction of thiol groups via thioredoxin and ferredoxin-thioredoxin reductase (for references see Ref. 1). The kinetics of the interconversion (half-times of about 0.5–3 min [2]) are influenced by stromal concentrations of H^+, Mg^{2+} and metabolites [2–5]. Furthermore, the catalytic activity of the interconverted forms of the enzymes is modulated by stromal solutes, like H^+ and metabolites [2,6]. The latter effects, which occur instantaneously, are the subject of the present report.

In earlier studies of stromal metabolite levels, it was shown that fructose- and sedoheptulose-1,7-

Abbreviations: Rbu-5-*P*, ribulose 5-phosphate; Rbu-1,5-P_2, ribulose 1,5-bisphosphate; Fru-1,6-P_2, fructose 1,6-bisphosphate; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Chl, chlorophyll.

bisphosphatases are potent regulatory steps of the CO_2-fixation cycle [7]. It has been clearly demonstrated recently that also ribulose-5-phosphate kinase is a regulatory enzyme of the Calvin cycle [8], but relatively little is known about the regulation of this enzyme. A light-dependent interconversion has been described [1,9], but the physiological function of this process remains a matter of debate [10]. It was proposed by Lavergne et al. [11] that ribulose-5-phosphate kinase was under the control of the energy charge of the adenylate system, and later shown by Laing et al. [2] that this enzyme could be regulated by the ATP/ADP ratio. Anderson [12] reported that ribulose-5-phosphate kinase was inhibited by 6-phosphogluconate and 3-phosphoglycerate, but speculated that the latter inhibition was caused by chelating the Mg^{2+} needed for enzyme activity.

We have reinvestigated the extent to which the catalytic activity of spinach chloroplast ribulose-5-phosphate kinase is modified by metabolites occurring in the stroma. As shown in the following, ribulose-5-phosphate kinase activity is strongly influenced by several stromal metabolites at concentrations resembling those found in the chloroplasts. The interaction between these metabolites, the stromal pH and Mg^{2+} and the light-dependent interconversion of the enzyme provide an exceptionally effective regulation mechanism both during light-dark transients and for the fine control of photosynthesis.

Materials and Methods

Spinach. Spinach (*Spinacea oleracea,* U.S. Hybrid 424, Ferry-Morse Co., Mountain View, CA, U.S.A.) was grown in hydroponic culture [13]. For the preparation of chloroplasts see Ref. 14.

Chloroplasts. The chloroplasts (30–50 μg Chl/ml) which showed a photosynthetic rate of 200–300 μmol O_2 evolved/mg Chl per h were suspended in a reaction medium containing 0.33 M sorbitol, 50 mM Hepes adjusted to pH 7.6 with KOH, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.5 mM K_2HPO_4, 5 mM $NaHCO_3$ and 0.04 mg/ml catalase from bovine liver (Boehringer, Mannheim), if not stated otherwise. The temperature was 20°C.

Assay of ribulose-5-phosphate kinase activity. The radioactivity assay of ribulose-5-phosphate kinase by ^{14}C incorporation [2] was carried out with 50 μM Rbu-5-*P*, 0.2 mM ATP, 20 mM $MgCl_2$, 10 mM $NaH^{14}CO_3$, 100 mM Tris-HCl (pH 7.8) and 0.2% (v/v) Triton X-100, unless otherwise stated.

Assay of ribulose-5-phosphate kinase by photometric method. Chloroplasts were added to an assay mixture as above (200 μl) except that $NaH^{14}CO_3$ was omitted. After 30 s, 250 μl of 10% $HClO_4$ were added. Samples were stored for 12 h at −30°C, thawed, neutralized with KOH/triethanolamine, and the $KClO_4$ precipitate removed by centrifugation. The supernatant was immediately assayed for ADP in 100 mM Tris-HCl, pH 7.8, 0.2 mM NADH, 10 mM $MgCl_2$, 0.5 mM phospho*enol*pyruvate, and 0.01 mg/ml lactate dehydrogenase (Boehringer), the reaction started by addition of 0.01 mg/ml pyruvate kinase (Boehringer) and the absorbance change at 340 nm measured. Blank values were obtained in samples where Rbu-5-*P* was omitted from the assay mix.

Preparation of ribulose biphosphate. 4 mg ribose 5-phosphate were added to a reaction mixture containing the following reagents in a total volume of 4 ml: 20 mM Tris-imidazole, pH 7.8, 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.59 M creatine phosphate, 83 mM ATP, 1000 U ribosephosphate isomerase, 1000 U creatine kinase and 250 U ribulose-5-phosphate kinase. Before incubation the water was essentially freed of CO_2. After 7 h at room temperature under vacuum the enzymatic reaction was stopped by acidification with 0.24 M $HClO_4$. After centrifugation at 10000 × *g* and 4°C for 15 min, the supernatant was reneutralized and lyophilized. The sample was resuspended in a volume of 3 ml (20 mM Tris-imidazole, pH 8.0, 20 mM $MgCl_2$), applied to an anion-exchange column (15 × 1 cm, BioRad AG 1 × 8, 200–400 mesh, acetate form, BioRad, München, F.R.G.) previously equilibrated with water and eluted at 4°C with a linear gradient of HCl (0–0.5 M). At a flow rate of 15 ml/h, 5-ml fractions were collected. Each fraction was tested for Rbu-1,5-P_2 with the radioactive assay [15]. Rbu-1,5-P_2 was eluted between 0.20 and 0.24 M HCl with the peak at 0.22 M HCl. These fractions were pooled, reneutralized, lyophilized and resuspended in a volume of 4 ml water. Finally, the sample was photometrically assayed for Rbu-1,5-P_2 [16]. 1.28 mmol ribose

5-phosphate were enzymatically converted to 0.44 mmol Rbu-1,5-P_2.

Measurement of 6-phosphogluconate in a light-dark transient. For experimental details of silicone-oil centrifugation see Ref. 17. 6-Phosphogluconate was measured photometrically [18].

Results and Discussion

Kinetic properties of ribulose-5-phosphate kinase

Ribulose-5-phosphate kinase is subject to a light-dependent interconversion [1]. To activate the enzyme in situ, chloroplasts were preilluminated for 6–14 min, before injecting them into a hypotonic medium, which contained the detergent Triton X-100 as well as all the components of the enzyme assay. In the presence of the detergent the chloroplasts rupture immediately, exposing the released enzyme to the assay medium so that the assay is started at the same moment. It is terminated 30 s later by addition of perchloric acid (for details see Materials and Methods). This procedure minimizes a possible change of the activation state of the enzyme after extraction from the chloroplasts.

The data of Figs. 1–3 show that the enzyme activity has a hyperbolic saturation curve for its substrates Rbu-5-*P* and ATP. Evaluation of the data by Hill plots (not shown) clearly indicated that there is no cooperativity between the binding sites for ATP and Rbu-5-*P*. In this respect, the enzyme from spinach appears to be different from the ribulose-5-phosphate kinase in various micro-

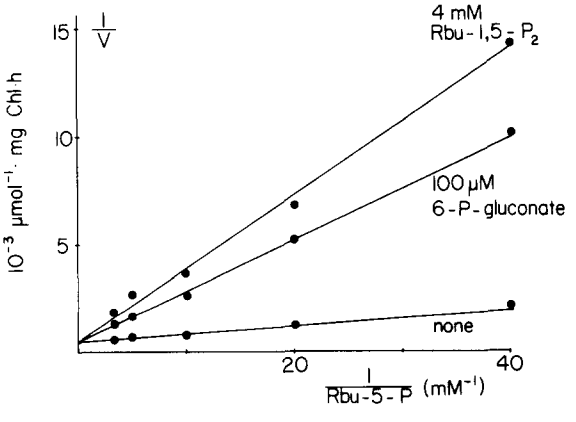

Fig. 1. Inhibition of the light-activated form of ribulose-5-phosphate kinase by 6-phosphogluconate and by Rbu-1,5-P_2.

TABLE I

KINETIC CONSTANTS OF RIBULOSE-5-PHOSPHATE KINASE FROM SPINACH CHLOROPLASTS (MEAN VALUES)

	pH 8.0	pH 7.0
Light-activated enzyme		
V_{max} (µmol/mg Chl per h)	1907	1203
K_m (ATP) (µM)	53	41
K_m (Rbu-5-*P*) (µM)	48	34
Dark-inactivated enzyme		
V_{max} (µmol/mg Chl per h)	266	
K_m (ATP) (µM)	55	
K_m (Rbu-5-*P*) (µM)	41	

organisms, where sigmoidal saturation curves for ATP and Rbu-5-*P* have been found [19,20].

In Table I the kinetic constants obtained for ribulose-5-phosphate kinase in spinach have been placed together. Since these values were obtained with an unpurified system these will be approximations of the actual values, but the short assay time (30 s) would minimize errors. The data show that light activation of the enzyme results in an increase in V_{max}, whereas the K_m values are not markedly affected. It may be noted that the K_m value for Rbu-5-*P* reported here is below the previously published values for the isolated enzyme (0.2 mM in Ref. 12). This also holds true for the K_m value of ATP, for which values of 280 µM [21] and 650 µM [12,21] have been reported earlier. The enzyme seems to be specific for purine-

TABLE II

INHIBITORS OF RIBULOSE-5-PHOSPHATE KINASE

Inhibition type	Inhibitor	K_i (mM)
Competitive to Rbu-5-*P*	6-phosphogluconate	0.07 [a]
	Rbu-1,5-P_2	0.7 [a]
	3-phosphoglycerate	2 [a]
	phosphate	4 [a]
Competitive to ATP	ADP	0.04 [b]

[a] Data obtained with the light-activated enzyme.

[b] Only the dark-inactivated ribulose-5-phosphate kinase was inhibited competitively by ADP. The light-activated enzyme was only weakly affected by ADP with a mixed inhibition type.

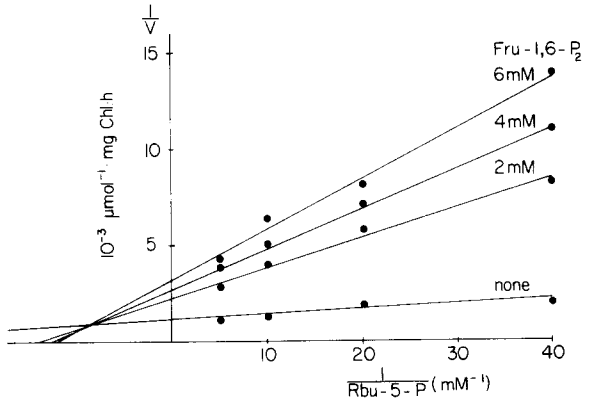

Fig. 2. Inhibition of ribulose-5-phosphate kinase by Fru-1,6-P_2.

nucleotide triphosphates. In experiments not shown here, we observed with 1 mM GTP 45%, but with 1 mM CTP only 4% of the maximal activity of ribulose-5-phosphate kinase found with ATP.

Inhibitors of ribulose-5-phosphate kinase

We investigated whether ribulose-5-phosphate kinase activity was affected by stromal metabolites when they were added at concentrations representative of those occurring in chloroplasts. None of the metabolites enhanced ribulose-5-phosphate kinase activity, but various compounds had an inhibitory effect, including ADP, 3-phosphoglycerate, 6-phosphogluconate, P_i, Rbu-1,5-P_2 and Fru-1,6-P_2 (Table II). We have previously reported that the inhibition by 3-phosphoglycerate was competitive to Rbu-5-*P* [22]. 6-Phosphogluconate, Rbu-1,5-P_2 (Fig. 1) and P_i (data not shown) also inhibit competitively with respect to Rbu-5-*P*, whereas the inhibition by Fru-1,6-P_2 (Fig. 2) is of a mixed type. All these substances, including 3-phosphoglycerate, inhibited the enzyme noncompetitively with respect to ATP. The inhibition by ADP was of a mixed type with respect to ATP in the light-activated enzyme (Fig. 3), whereas a competitive inhibition by ADP was found with the nonactivated form of the enzyme extracted from chloroplasts which had been kept in the dark (Fig. 4). As we reported previously [2], the dark-inactivated form of the enzyme is much more sensitive to inhibition by ADP than the light-activated form.

It has been shown earlier by Anderson [12] that 6-phosphogluconate competitively inhibited ribulose-5-phosphate kinase activity, although the extent of inhibition was much lower than in our experiments. A weak inhibition by 3-phosphoglycerate, observed by Anderson [12], was explained as a competition between the 3-phosphoglycerate and the substrates of ribulose-5-phosphate kinase for Mg^{2+} required for the catalytic activity of this enzyme. As shown elsewhere [22], this is not the cause of the inhibition by 3-phosphoglycerate observed in our experiments. Differences between the results of these earlier studies and those presented here may be due to the fact that the studies by Anderson were performed at unphysiologically high concentrations of ATP and Rbu-5-*P* and also at alkaline pH (see below).

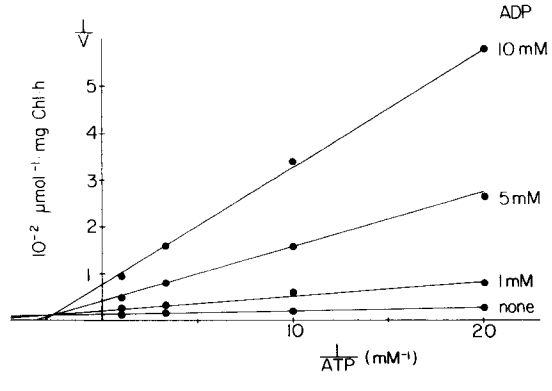

Fig. 3. Inhibition of the light-activated form of ribulose-5-phosphate kinase by ADP.

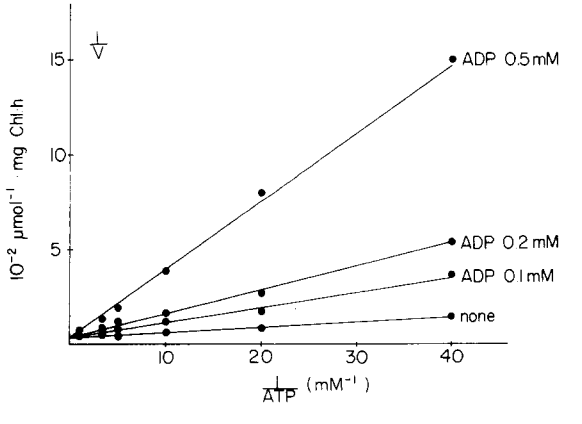

Fig. 4. Inhibition of the dark-inactivated form of ribulose-5-phosphate kinase by ADP.

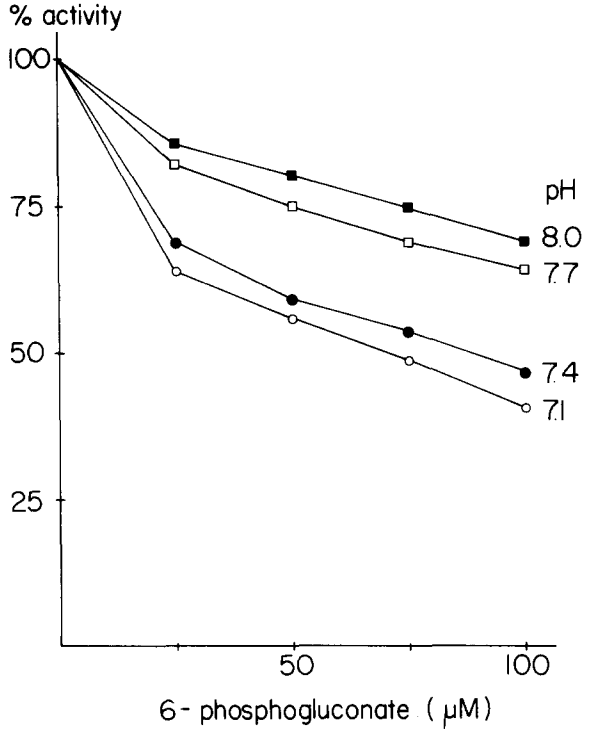

Fig. 5. pH Dependence of the inhibition of the light-activated form of ribulose-5-phosphate kinase by 6-phosphogluconate. The absolute activities of ribulose-5-phosphate kinase in the absence of 6-phosphogluconate were, in μmol/mg Chl per h: pH 7.1, 590; pH 7.4, 850; pH 7.7, 860; pH 8.0, 900.

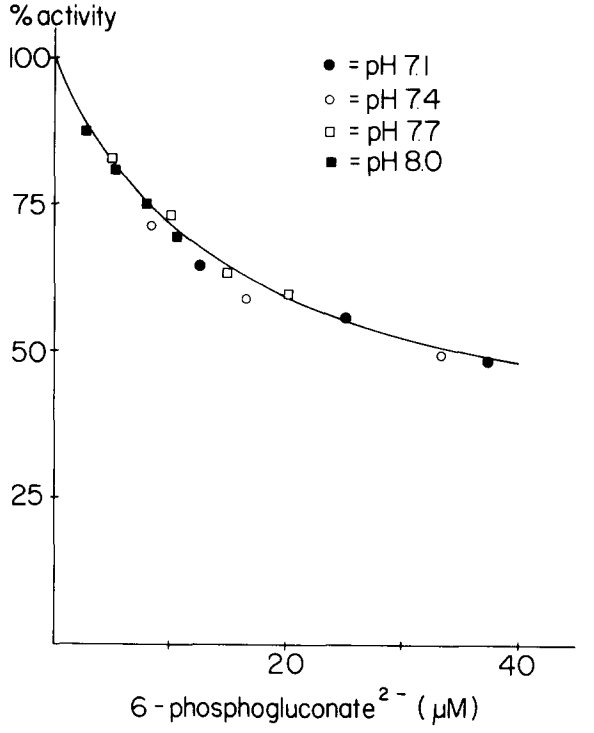

Fig. 6. Inhibition of the light-activated form of ribulose-5-phosphate kinase by 6-phosphogluconate^{2-}. The values are calculated from the experiment of Fig. 5. The absolute activities of ribulose-5-phosphate kinase in the absence of 6-phosphogluconate are given in the legend to Fig. 5. The calculation used the formula [6-phosphogluconate^{2-}]/([6-phosphogluconate^{2-}]+[6-phosphogluconate^{3-}]) = $[H^+]/[H^+]+10^{-pK}$ (pK = 7.1).

Furthermore, K_m values for Rbu-5-*P* observed in these earlier studies were 4-times higher than in our experiments with the enzyme activated in situ. Because of that, the K_i values for 6-phosphogluconate were apparently shifted to higher values, and the inhibition by other metabolites probably became difficult to measure.

pH dependence of the inhibition

The pH in the stroma is variable, being about 7 in the dark and 8 in the light [17], and within this pH range both phosphate and the various phosphate esters occur in two forms ($ROPO_3H^-$, $ROPO_3^{2-}$). It is likely that only one of these forms would inhibit ribulose-5-phosphate kinase and it has indeed already been shown that for 3-phosphoglycerate the inhibiting ion species is 3-phosphoglycerate^{2-} [22]. 3-Phosphoglycerate became an increasingly effective inhibitor as the assay pH was decreased. This is due to an increasing proportion being present as the inhibitory form 3-phosphoglycerate^{2-} (instead of 3-phosphoglycerate^{3-}). In further studies we found that 6-phosphogluconate also inhibited more effectively at lower pH (Fig. 5). When the extent of the inhibition was related to the concentration of 6-phosphogluconate^{2-} (instead of total 6-phosphogluconate) the values from the different pH values all lay on one curve, showing that 6-phosphogluconate^{2-} was indeed the inhibitory form (Fig. 6). From similar experiments it was shown that the inhibitory forms of P_i and Fru-1,6-P_2 are P_i^{1-} and Fru-1,6-P_2^{2-}, respectively (Figs. 7 and 8). It may be noted that the inhibition by Rbu-1,5-P_2 did not follow this pattern and that no marked pH dependence of the inhibition by ADP was found (data not shown).

In contrast to fructose-1,6-bisphosphatase and

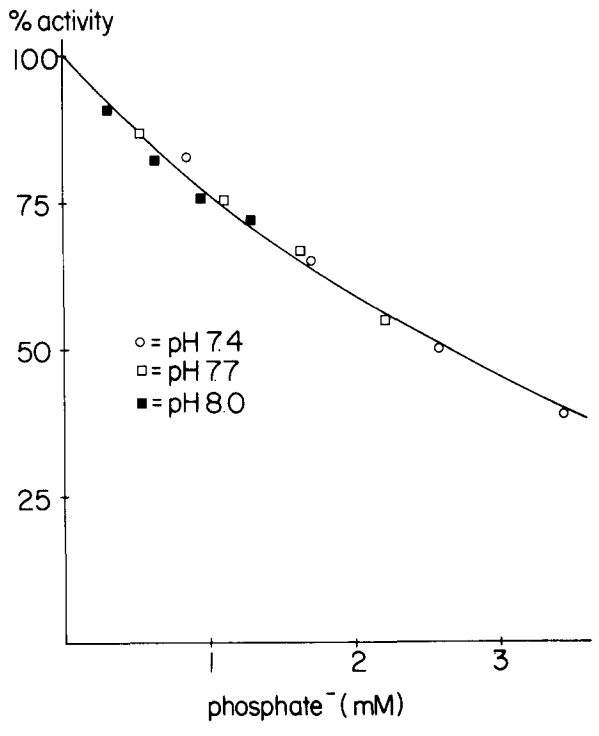

Fig. 7. Inhibition of the light-activated form of ribulose-5-phosphate kinase by phosphate^{-}. The absolute activities of ribulose-5-phosphate kinase in the absence of phosphate were, in μmol/mg Chl per h: pH 7.4, 880; pH 7.7, 980; pH 8.0, 1060. For calculation see legend to Fig. 6 (pK = 7.2).

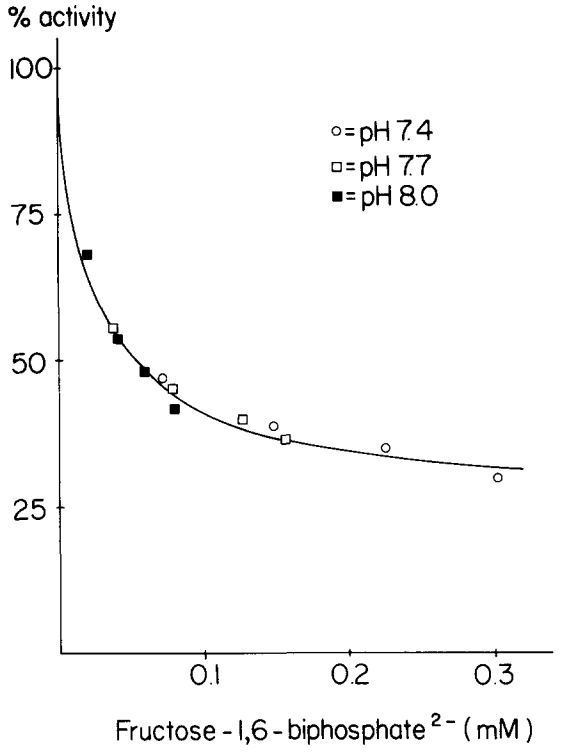

Fig. 8. Inhibition of the light-activated form of ribulose-5-phosphate kinase by fructose 1,6-bisphosphate^{2-}. The absolute activities of ribulose-5-phosphate kinase in the absence of fructose 1,6-bisphosphate were, in μmol/mg Chl per h: pH 7.4, 790; pH 7.7, 910; pH 8.0, 970. For calculation see legend to Fig. 6 (pK = 7.1).

sedoheptulose-1,7-bisphosphatase, which have a strong pH dependence [2,6], the pH sensitivity of ribulose-5-phosphate kinase is rather low and could not contribute significantly to an inhibition of the enzyme when the stromal pH decreases from pH 8 in the light to pH 7 in the dark. However, the activity of ribulose-5-phosphate kinase is markedly influenced by pH in the presence of inhibitors like 3-phosphoglycerate, 6-phosphogluconate, P_i or Fru-1,6-P_2, as the inhibition by these compounds is itself pH dependent. This is illustrated in the experiment of Fig. 9 with 3-phosphoglycerate as an example. In the presence of 5 mM 3-phosphoglycerate, a concentration which can be actually found in the stroma, a change of pH from 7.0 to 7.9 increased the catalytic activity of the light-activated ribulose-5-phosphate kinase by a factor of 6.3.

Simulation of 'light' and 'dark' conditions

As ADP, 3-phosphoglycerate, Fru-1,6P_2, Rbu-1,5-P_2, 6-phosphogluconate and P_i all occur in the stroma in inhibitory concentrations they will exert a combined effect on the enzyme activity. Thus, the extent to which ribulose-5-phosphate kinase may be controlled in vivo is dependent not only on the concentrations of the individual compounds, but also on the interactions between them. Whereas the inhibition by ADP was found to be additive to that by each one of the rest of the inhibitory compounds (Ref. 22 and unpublished data), no such pronounced additivity was found for the inhibition by P_i, Fru-1,6-P_2, 3-phosphoglycerate and 6-phosphogluconate. This is not unexpected, since the latter compounds all appeared to affect the Rbu-5-P binding in some way, in contrast to ADP which interacts with the ATP site (see Table

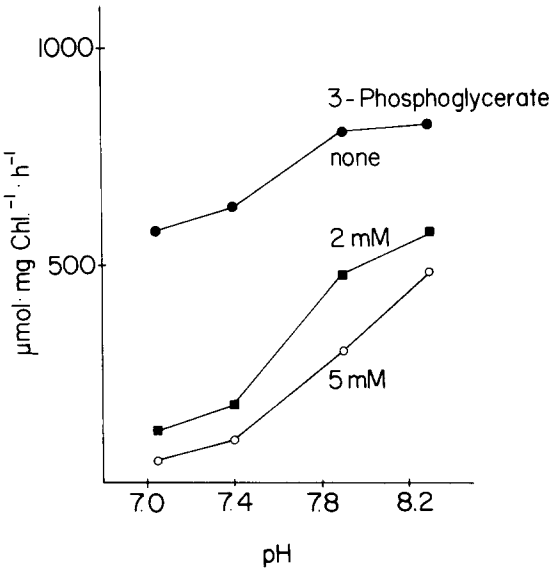

Fig. 9. pH Dependence of the light-activated form of ribulose-5-phosphate kinase in the presence and absence of 3-phosphoglycerate.

II). In fact, the pattern seems to be quite complicated, as the inhibitors not only competed with each other but sometimes even relieved the inhibition by other compounds. For example 2 mM P_i not only relieved the inhibition due to low 3-phosphoglycerate or Fru-1,6-P_2 but also raised the activity above that found in the absence of Fru-1,6-P_2 or 3-phosphoglycerate (unpublished data). The mechanism of this effect is still unclear, and studies with the purified enzyme will be required to elucidate this matter. It is therefore at present impossible to predict the inhibition by a combination of inhibitors from an evaluation of their individual effects. Instead, we used an alternative approach to assess the effect of the inhibitors in vivo, in which the stromal metabolite levels occurring in light and dark were simulated in the enzyme assay.

Values for stromal metabolite levels occurring during a light-dark transient are available from measurements with isolated chloroplasts [8]. In these measurements, ADP was found to increase from 0.1 mM in the light to 0.6 mM after 1 min of darkness, with the phosphate level increasing simultaneously from 5 mM (light) to 12 mM (dark). In isolated chloroplasts, the concentrations of 3-phosphoglycerate (3 mM) and Fru-1,6-P_2 (0.5 mM) are not markedly changed between illumination and darkness. For Rbu-1,5-P_2, light levels of 2–4 mM have been found with saturating CO_2, and higher levels under CO_2-limiting conditions, whereas on darkening the Rbu-1,5-P_2 decreases to about 0.2 mM. As there are no published data on the stromal levels of 6-phosphogluconate, a strong inhibitor of ribulose-5-phosphate kinase, we measured the stromal levels of this compound during a light-dark transition. In the experiment of Fig. 10, 6-phosphogluconate is shown to rise within 2 min from 40 μM in the light to 300 μM in the dark. In other experiments dark levels around 200 μM were found. This dramatic increase in the 6-phosphogluconate level upon darkening with its strong inhibitory effect on ribulose-5-phosphate kinase,

TABLE III

INHIBITION OF RIBULOSE-5-PHOSPHATE KINASE IN A SIMULATED LIGHT-DARK TRANSITION

Assay: Rbu-5-*P*, 50 μM; ATP, 0.2 mM.

	pH	Additions (mM)					Rbu-5-P kinase (μmol/mg Chl per h)
		ADP	Fru-1,6-P_2	3-Phospho-glycerate	P_i	6-Phospho-gluconate	
Light-activated enzyme							
Standard assay	7.9	0	0	0	0	0	2330
Simulation light	7.9	0.1	0.5	3	5	0.04	87
Simulation dark	7.1	0.6	0.5	3	12	0.18	4
Dark-inactivated enzyme							
Standard assay	7.1	0	0	0	0	0	370
Simulation dark	7.1	0.6	0.5	3	12	0.18	< 1

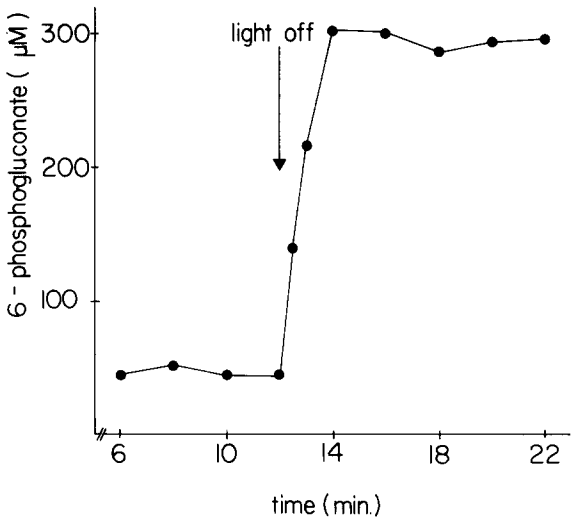

Fig. 10. Changes in 6-phosphogluconate after darkening of spinach chloroplasts which had been illuminated for 12 min. The results are based on a stromal space of 25 µl/mg Chl.

suggests that this compound is an important regulatory factor. The rise in 6-phosphogluconate is probably due to a rapid dark activation of glucose-6-phosphate dehydrogenase [23].

Table III shows the results of an experiment where ribulose-5-phosphate kinase was assayed in the presence of metabolite concentrations resembling those found in the stroma in the light and dark. At pH 7.9, which is found for the stroma of illuminated chloroplasts, the maximal activity of ribulose-5-phosphate kinase is very high. In the presence of physiological concentrations of stromal metabolites found in the light, the ribulose-5-phosphate kinase activity decreases to about 4% of its maximal activity, and is now well in the range of the overall rate of CO_2 fixation. At pH 7.1 in the presence of metabolite concentrations which are typical for chloroplasts kept in the dark, ribulose-5-phosphate kinase activity is further decreased to about 0.2% of the maximal activity, which corresponds to 5% of the activity observed in the simulation of the physiological conditions in the light. It may be noted that this 20-fold decrease in enzyme activity between the simulated metabolic conditions light and dark does not involve a reversal of the light-dependent interconversion of the enzyme. When the latter effect is included as well, by exposing the chloroplasts to darkness before extracting the ribulose-5-phosphate kinase, the enzyme activity assayed under simulated dark conditions becomes virtually zero. Although the changes in metabolite levels in isolated chloroplasts observed during a light-dark transient are not fully representative of the corresponding changes of stromal metabolites in situ [24], these simulations of light and dark conditions clearly demonstrate the large extent to which ribulose-5-phosphate kinase activity can be modulated by the presence of stromal metabolites.

On the regulation of ribulose-5-phosphate kinase in chloroplasts

In earlier studies we have observed that when isolated chloroplasts are darkened the CO_2 fixation ceases within about 15 s and the Rbu-1,5-P_2 level fell immediately, although the stromal pentose phosphate levels increased and there was still free ATP in the stroma [8]. Such results indicate that in these intact chloroplasts ribulose-5-phosphate kinase was in fact markedly inhibited within a few seconds. These findings stimulated further investigations of the light activation and dark inactivation of this enzyme in situ. We found that on illumination the enzyme was 9-fold activated with a half-time of about 30 s [2], and an even larger activation of the enzyme with a similar half-time was also found in whole leaves [25]. The corresponding inactivation of ribulose-5-phosphate kinase upon darkening, however, was a relatively slow process with a half-time of about 100 s. This slow process could not account for the very rapid inhibition of ribulose-5-phosphate kinase which was revealed by the changes in the stromal metabolite levels during a light-dark transient.

We can now explain this very rapid inhibition of ribulose-5-phosphate kinase in situ after darkening as the result of the inhibitory effects of stromal metabolites, especially ADP, 6-phosphogluconate and 3-phosphoglycerate. ADP and 6-phosphogluconate are present at low levels in the light and increase to a large extent after chloroplasts are darkened. 3-Phosphoglycerate is present at high levels in the light and dark, and the decrease in stromal pH after darkening will markedly increase the extent to which it inhibits ribulose-5-phosphate kinase. Also, the inhibition by 6-phosphogluconate is further increased by acidification of the stroma.

The combination of light-dependent interconversion of the enzyme, and the modulation of its catalytical activity by metabolites allows a total inactivation of this enzyme in the dark. In this respect it is noteworthy that the inhibitory effect of ADP increases drastically when ribulose-5-phosphate kinase is converted to the dark-inactivated form [2]. Attention may be also drawn to the fact that 6-phosphogluconate, the product of the light-inactivated enzyme glucose-6-phosphate dehydrogenase, inhibits the light-activated ribulose-5-phosphate kinase. This interaction may be of importance for a coordination of the reductive and oxidative pentose phosphate pathways.

Since the extractable maximal activity of ribulose-5-phosphate kinase in chloroplasts (approx. 2000 μmol/mg Chl per h) is more than an order of magnitude higher than the rate of CO_2 fixation, it had been doubted in the past whether this enzyme would be capable of regulating the flow of carbon in the Calvin cycle [10]. Our data show that under physiological conditions the actual rate of catalysis of this enzyme is only about 4% of the maximal activity and thus resembles the overall rate of CO_2 fixation. This clearly demonstrates that in the light this enzyme can be rate limiting and thus a potential site for a fine control of the Calvin cycle. Such a fine control, for instance, may be important for adjusting the rate of the conversion of Rbu-5-*P* to Rbu-1,5-P_2 to the rate of the conversion of 3-phosphoglycerate to 1,3-bisphosphoglycerate. If both reactions were freely competing for the stromal ATP, ribulose-5-phosphate kinase would attract the ATP more strongly than the phosphoglycerate kinase reaction because of its high maximal activity, its low K_m for ATP and the irreversibility of the reaction. Especially during decreased ATP production, which could result from low light intensity, an unregulated competition of the two enzymes for the ATP would have the consequence that the conversion of 3-phosphoglycerate to triose phosphates would be decreased, and the flow of carbon through the Calvin cycle become unbalanced. It may be envisaged that a feedback inhibition of ribulose-5-phosphate kinase by its primary products ADP and Rbu-1,5-P_2 and its secondary product 3-phosphoglycerate represents a very efficient mechanism in order to avoid an excessive rate of ribulose-5-phosphate kinase activity.

A further role of the inhibitors of ribulose-5-phosphate kinase in the fine control of this enzyme in the light is a matter of speculation. It seems feasible, that under CO_2-limiting conditions, where the level of Rbu-1,5-P_2 will be increased and those of 3-phosphoglycerate and Fru-1,6-P_2 decreased, Rbu-1,5-P_2 may adjust the rate of ribulose-5-phosphate kinase to the decreased flow of carbon through the Calvin cycle. The physiological function of the inhibition of ribulose-5-phosphate kinase by Fru-1,6-P_2, sedoheptulose 1,7-biphosphate (unpublished data) and phosphate is not clear. Inactivation of fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase would lead to an increase in the levels of Fru-1,6-P_2 and sedoheptulose 1,7-bisphosphate and these could in turn exert an inhibitory effect of ribulose-5-phosphate kinase. This may provide a mechanism for a coordination of the activities of these different regulatory enzymes of the Calvin cycle.

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