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Inhibition of photophosphorylation by ribulose-1,5-bisphosphate carboxylase

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Purified ribulose-1,5-bisphosphate carboxylase inhibited coupled glycerate 3-phosphate, NADP and ferricyanide reduction in saturating light. Complete inhibition of coupled electron transport was achieved at an added carboxylase protein concentration of 5 mg · ml^{-1}. The rate of uncoupled electron transport was not inhibited by the carboxylase protein. Thus inhibition of photophosphorylation was indicated. Inhibition was promoted by high Mg^{2+} concentration and was not reversed in the light by subsequent additions of inorganic phosphate (P_i) or ADP. However, if P_i was added prior to ribulose-1,5-bisphosphate carboxylase then partial protection against the inhibition of coupled electron transport was observed. The apparent K_m P_i of photophosphorylation was considerably increased by increasing carboxylase concentration. This may contribute to a higher apparent K_m in vivo. When coupled electron flow associated with glycerate 3-phosphate-reduction was totally inhibited in the presence of carboxylase, the addition of ATP or an ATP-generating system facilitated electron flow but did not reverse inhibition of coupled electron transport. At limiting light intensities similar effects were produced by the addition of stromal protein (instead of purified carboxylase protein), but inhibition was not observed in saturating light. The inhibition of coupled electron transport was found with both the active and inactive forms of carboxylase but not with bovine serum albumin.

Introduction

To date, there have been three phases of chloroplast research, the last of which was initiated by the general availability of 'intact' chloroplasts which would support photosynthetic carbon metabolism [1]. More recently, increasing importance has been attached to relating results obtained with different in vitro systems (thylakoids, intact chloroplasts, the reconstituted chloroplast system, etc.) to one another and with events in the intact leaf. This has high-lighted a number of apparent anomalies. One such is that the isolated chloroplast will soon cease to assimilate carbon at rapid rates in the absence of added P_i [2] despite the apparent availability of ample P_i within the stroma. Leaves contain large amounts of P_i and show a significant capacity for rapid P_i uptake [3–5]. Photosynthesis in intact leaves can be readily influenced by feeding compounds (e.g., mannose and 2-deoxyglucose, etc.) which sequester cytosolic P_i [6,7] and even enhanced by petiolar P_i-feeding [8,9]. There is no doubt that the chloroplast is a P_i-consuming, triose phosphate-exporting organelle or that in order to synthesise triose phosphate from CO_2 and P_i, it must consume P_i in photophosphorylation [10]. However, in view of

Abbreviations: P_i, inorganic phosphate, Chl, chlorophyll; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

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the K_m of photophosphorylation for P_i determined in vitro (less than 600 μM [11]) and the high levels of P_i present in the chloroplast in vivo (up to 17 mM [12]) it seems unlikely that P_i could ever become limiting in the chloroplast stroma. An intrinsic difference between the isolated thylakoid and leaf systems is the presence of stromal components and possible effects of stromal proteins on electron transport and photophosphorylation are ignored.

Ribulose-1,5-bisphosphate carboxylase accounts for as much as half of the soluble protein in leaves of C_3 plants [13] and may be present in the chloroplast stroma at concentrations of up to 1.2 mM [14]. Therefore the enzyme itself must be considered as a kinetic component in the carboxylation reaction as it is present in vivo at concentrations similar to those of its substrates [15]. In addition to binding its substrate, ribulose 1,5-bisphosphate, ribulose-1,5-bisphosphate carboxylase is believed to bind a significant proportion of stromal metabolites (such as glycerate 3-phosphate, P_i, adenylates, NADP(H)) and may act as a metabolite 'buffer', binding and releasing metabolites according to the ribulose-1,5-bisphosphate concentration [16,17]. Ribulose-1,5-bisphosphate carboxylase also binds to the thylakoid membrane [16,18–20] with more than 30% of carboxylase present in the stroma may be associated with the thylakoid membrane, presumably due to electrostatic attraction [16,19]. There is also considerable immunological and microscopic evidence that ribulose-1,5-bisphosphate carboxylase and other enzymes of the carbon reduction cycle associate with the thylakoid membrane [21]. A possible consequence of interaction between the carboxylase and the thylakoid membrane could be a modification of fundamental kinetic parameters of photophosphorylation and electron transport. Accordingly, it was necessary to examine the effect of stromal protein and in particular ribulose-1,5-bisphosphate carboxylase on electron transport and photophosphorylation. This paper addresses this problem by measuring O_2 evolution in a reconstituted chloroplast system [22] using either glycerate 3-phosphate, $K_3Fe(CN)_6$ or NADP as electron acceptors. The effects of various concentrations of pure carboxylase and of stromal extracts on O_2 evolution are reported.

Materials and Methods

Plant material and intact chloroplast preparation

Spinacia oleracea L. cv virtuosa was grown hydroponically in a glasshouse and intact spinach chloroplasts were prepared from mature leaves as described previously [10]. For the reconstituted chloroplast system intact chloroplasts were lysed in the O_2 electrode vessel in 0.5 ml of a medium containing 10 mM $MgCl_2$/5 mM dithiothreitol in distilled water, and after 30 s in the dark assay medium was added to give a 1 ml final reaction mixture containing thylakoids and stromal protein (equivalent to 50 μg chlorophyll), plus 0.33 M sorbitol/1 mM EDTA/10 mM KCl/50 mM Hepes-KOH buffer (pH 7.9)/10 mM $MgCl_2$/4 mM ascorbate/2.5 mM dithiothreitol/200 units catalase.

Preparation of thylakoids and stromal proteins

Thylakoids and stromal proteins were prepared from intact chloroplasts as follows. Intact chloroplast pellets were lysed in 2 ml of a medium containing 10 mM Hepes/KOH (pH 7.6)/1 mM $MgCl_2$/5 mM dithiothreitol and centrifuged at $2000 \times g$ for 2 min. The supernatant (chloroplast stromal extract) was retained and passed through a PD 10 gel filtration column (Pharmacia Fine Chemicals, Uppsala) eluted with 100 mM Hepes/KOH buffer (pH 7.6) with or without 5 mM dithiothreitol. The dithiothreitol was omitted when ferricyanide reduction was to be measured. The thylakoid pellet was resuspended in 0.33 M sorbitol/2 mM EDTA/2 mM $MgCl_2$/50 mM Hepes-KOH buffer (pH 7.6) and stored on ice.

Assay conditions

For the assay of NADP reduction, 2 mM ADP/2 mM P_i/2 mM NADP/100 μg ferredoxin were included in the assay medium previously described. For glycerate 3-phosphate reduction, similar mixtures were used in which ADP and NADP were decreased to 0.2 mM, and glycerate 3-phosphate was 5 mM. For ferricyanide reduction the assay contained 1.5 mM ferricyanide/2 mM ADP/2 mM P_i/10 mM D,L-glyceraldehyde (dithiothreitol was omitted). To determine the P_i optimum of coupled electron transport, thylakoids were incubated for 90 s in the light at 100 μg

chlorophyll per ml in the presence of 2 mM ADP and 2 mM NADP to decrease the residual P_i content. Phosphate was added at the concentrations stated followed by the required amount of stromal protein or purified carboxylase and buffer.

The O_2 content in the reaction vessel was assayed polarographically at 25°C using a Clark type electrode (Hansatech Ltd, U.K.). Illumination was provided by a quartz halogen projector lamp (150 W) filtered through a Calflex C heat filter (Balzers, F.R.G.) and a red filter, giving 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ P.A.R. *.

Pure ribulose-1,5-bisphosphate carboxylase from wheat leaves [23] was the generous gift of the Department of Biochemistry, Rothamsted Experimental Station, U.K. Its activity was 1 μmol per mg protein per min (at 25°C) when activated prior to use by incubation for 40 min at 40°C in a medium containing 20 mM $MgCl_2$/10 mM $NaHCO_3$/0.33 M sorbitol/2 mM EDTA/10 mM KCl/50 mM Hepes/4 mM ascorbate/1 mM dithiothreitol. Bovine serum albumin was treated as for ribulose-1,5-bisphosphate carboxylase. Protein was estimated by the dye-binding protein assay (Bio-Rad laboratories, Munich, F.R.G.). Chlorophyll was estimated by the method of Arnon [24] and orthophosphate according to Taussky and Shorr [25].

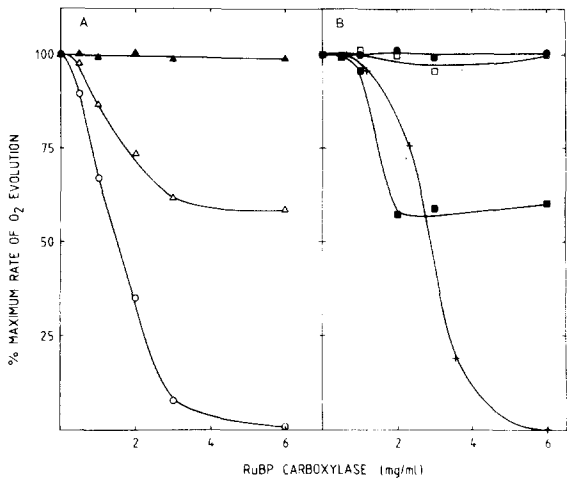

Fig. 1. (A) The effect of added ribulose-1,5-bisphosphate carboxylase (activated) on the rate of glycerate 3-phosphate reduction (○); NADP reduction in the presence of 10 mM NH_4Cl (▲) and in its absence (△). (B) The effect of added activated ribulose -1,5-bisphosphate carboxylase on the rate of $Fe(CN)_6$ reduction in the presence (□) and absence of NH_4Cl (ps) and of inactivated carboxylase on glycerate 3-phosphate reduction is also shown (+) as is the effect of added bovine serum albumin added at the same concentrations as and in place of the carboxylase (●). Rates are presented as percentages of the control rates in the absence of carboxylase. These control rates (in μmol O_2 per h per mg chlorophyll) are as follows: glycerate 3-phosphate reduction, 116; $Fe(CN)_6$, 172 and 187 plus NH_4Cl; NADP, 200 and 220 plus NH_4Cl. The results of $Fe(CN)_6$ reduction were obtained with a different chloroplast preparation and are not comparable with the other data as absolute rates.

Results

The effect of ribulose-1,5-bisphosphate carboxylase on electron transport

Fig. 1 shows the effect of the addition of activated pure ribulose-1,5-bisphosphate carboxylase on oxygen evolution in the reconstitued chloroplast system with $K_3Fe(CN)_6$ (Fig. 1B) or NADP (Fig. 1A) as terminal electron acceptors, or during glycerate 3-phosphate reduction (Fig. 1A). Electron flow to NADP was inhibited by 35–45% in the presence of 3 $mg \cdot ml^{-1}$ pure ribulose-1,5-bisphosphate carboxylase and ferricyanide reduction was inhibited 45% by 5 $mg \cdot ml^{-1}$ ribulose-1,5-bisphosphate carboxylase, while glycerate 3-phosphate-dependent oxygen evolution was totally inhibited by this amount of enzyme. Rates of electron transport uncoupled with NH_4Cl were unaffected by ribulose-1,5-bisphosphate carboxylase. The apparent anomaly in the degree of inhibition obtained with addition of similar amounts of ribulose-1,5-bisphosphate carboxylase to coupled NADP, ferricyanide and glycerate 3-phosphate reduction occurred because of differences in the amount of uncoupled electron transport which can proceed in the different systems. Ribulose-1,5-bisphosphate carboxylase inhibits only coupled electron flow. In this system NADP and ferricyanide-dependent O_2 evolution allow a degree of electron flow without concomitant ATP synthesis and this is reflected in the amount of O_2 evolution which occurred in the

* P.A.R., photosynthetically active radiation.

absence of added ADP or in the presence of inhibitory levels of added ribulose-1,5-bisphosphate carboxylase. The high sensitivity of glycerate 3-phosphate reduction to ribulose-1,5-bisphosphate carboxylase inhibition supports this hypothesis, since it requires comparable rates of photophosphorylation and electron transport. In this system NADP regeneration is dependent upon the phosphorylation of glycerate 3-phosphate which eliminates the contribution of uncoupled electron flow.

The addition of ribulose-1,5-bisphosphate carboxylase inhibited photophosphorylation but did not inhibit electron transport directly. This suggests inhibition of thylakoid ATPase. The absence of inhibition by equivalent amounts of bovine serum albumin suggests that the effect is not due to general non-specific protein binding (Fig. 1B).

The effect of ribulose-1,5-bisphosphate carboxylase activation state on the inhibition of photophosphorylation is shown in Fig. 1B where the inhibition of glycerate 3-phosphate reduction by fully activated carboxylase was compared to that with inactivated carboxylase (incubated in the absence of Mg^{2+} and CO_2, but in the presence of 5 mM ribulose-1,5-bisphosphate [26]). Both forms of the enzyme inhibited glycerate 3-phosphate reduction (as did not-activated enzyme, i.e., incubated without ribulose-1,5-bisphosphate, $MgCl_2$ or $NaHCO_3$). It can be seen by comparison of the data in Fig. 1A and 1B that more inactivated carboxylase was required to achieve the same degree of inhibition of glycerate 3-phosphate reduction than the active form. This was usually the case but was not reproducible in all experiments, possibly due to varying degrees of reactivation of the deactivated form of the carboxylase in the assay buffer (which contained $MgCl_2$ and CO_2).

To verify these observations and confirm that the inhibition of photophosphorylation was not an artefact generated by the ribulose-1,5-bisphosphate carboxylase preparation, glycerate 3-phosphate reduction was examined in the reconstituted chloroplast system adding various amounts of freshly prepared chloroplast stromal protein from which low-molecular-weight compounds had been removed by gel filtration (Fig. 2). The proportion of ribulose-1,5-bisphosphate carboxylase in the

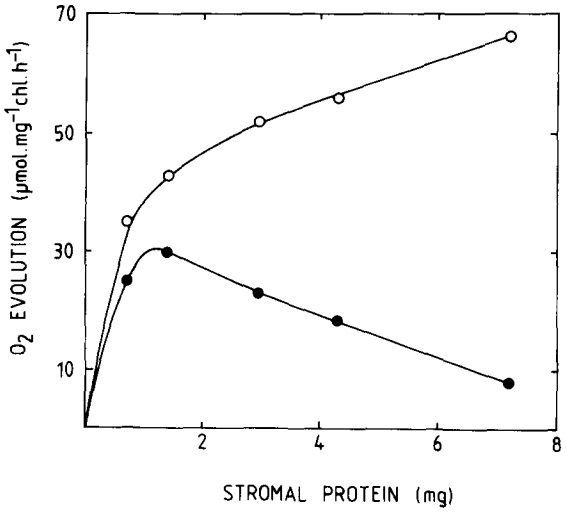

Fig. 2. The effect of added stromal protein on the rate of glycerate 3-phosphate reduction at saturating (○) and limiting (●) light intensity (500 and 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ P.A.R.).

stromal protein was approx. 65%. As expected, the addition of stromal protein to the reconstituted chloroplast system resulted in a stimulation in the rate of O_2 evolution at high light, since glycerate 3-phosphate reduction was normally limited by the amounts of glycerate 3-phosphate kinase and NADP-glyceraldehyde-3-phosphate dehydrogenase present. However, in low light the rate at which ATP can be supplied to phosphorylate glycerate 3-phosphate limits NADP reduction (see Ref. 27). Hence, when the experiment was performed at limiting light intensities (Fig. 2), an optimal rate of O_2 evolution was reached at quite a low portein concentrations and then the reaction was progressively inhibited until the addition of 7 $mg \cdot ml^{-1}$ stromal protein gave about 70% inhibition.

When the amount of thylakoid membrane was varied with a constant level of added ribulose-1,5-bisphosphate carboxylase the inhibition of the rate of coupled glycerate 3-phosphate-dependent O_2 evolution relative to controls (without added enzyme) decreased with the increasing thylakoid concentration (Fig. 3). With a membrane concentration of 35 $\mu g \cdot ml^{-1}$ chlorophyll the rate of glycerate 3-phosphate-dependent O_2 evolution was completely inhibited by the addition of 3 $mg \cdot ml^{-1}$ enzyme protein, while at 85 $mg \cdot ml^{-1}$ chlorophyll

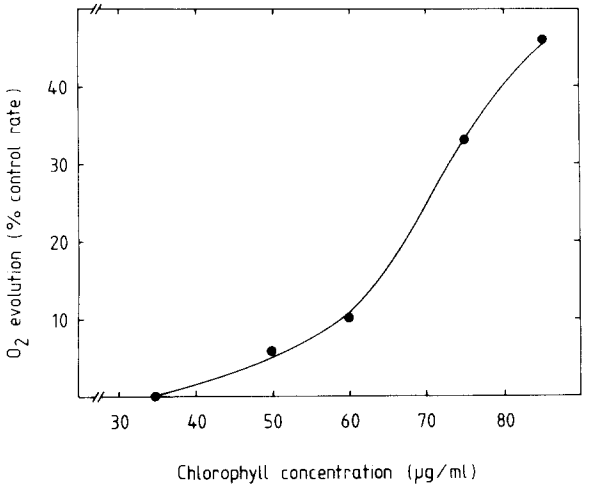

Fig. 3. Response of the inhibition of coupled glycerate 3-phosphate reduction by 3 $mg \cdot ml^{-1}$ ribulose-1,5-bisphosphate carboxylase to increasing thylakoid membrane concentration. Control rates of O_2 evolution ranged from 81 μmol per mg Chl per h (at 35 $\mu g\ Chl \cdot ml^{-1}$) to 67 μmol per mg Chl per h (at 85 $\mu g\ Chl \cdot ml^{-1}$).

the same amount of enzyme protein inhibited glycerate 3-phosphate reduction by only 55%.

Mg^{2+} dependence of inhibition of photophosphorylation by ribulose-1,5-bisphosphate carboxylase

Because of its electrostatic nature, the association of ribulose-1,5-bisphosphate carboxylase with the thylakoid membrane depends upon the surrounding cation concentration [19]. Fig. 4 shows that inhibition of glycerate 3-phosphate reduction by ribulose-1,5-bisphosphate carboxylse is highly dependent on $MgCl_2$ concentration. In the absence of added ribulose-1,5-bisphosphate carboxylase the maximum rate of glycerate 3-phosphate reduction was obtained between 4 and 5 mM $MgCl_2$. It then remained relatively constant with increasing $MgCl_2$ concentrations, so that only a 5% decrease in rate was found at 20 mM. However, in the presence of 2.5 mg purified ribulose-1,5-bisphosphate carboxylase, the Mg^{2+} optimum was reduced to 2 to 3 mM with a subsequent decline to 55% of the maximum rate at 20 mM MCl_2. This suggested that the inhibition of coupled electron transport by ribulose-1,5-bisphosphate carboxylase has a similar cation requirement to the binding phenomena previously reported [19].

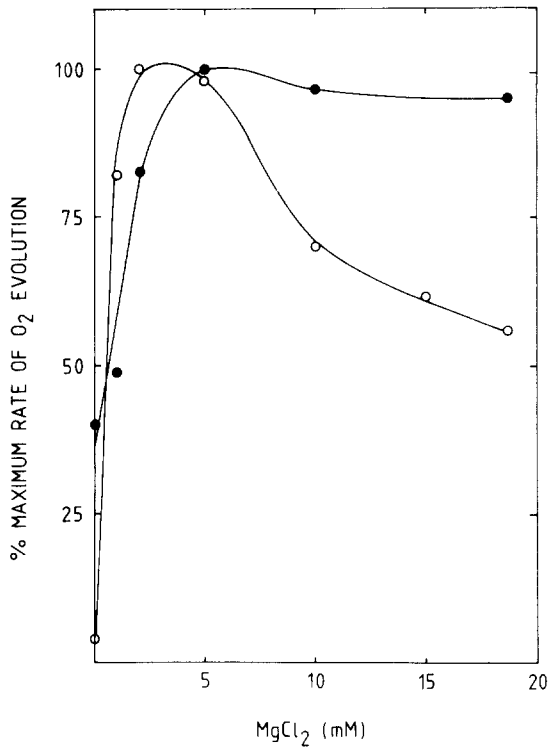

Fig. 4. The response of glycerate 3-phosphate reduction to $MgCl_2$ concentration in the presence (○) and absence (●) of 3 mg/ml ribulose-1,5-bisphosphate carboxylase (non-activated to avoid carry over of Mg^{2+}). Control maximum rates in μmol $O_2 \cdot h^{-1}$ per mg chlorophyll were 127 and 145, respectively.

Reversal of ribulose-1,5-bisphosphate carboxylase inhibition

The inhibition of glycerate 3-phosphate reduction by ribulose-1,5-bisphosphate carboxylase could not be reversed by adding high concentrations of ADP, P_i, NADP or glycerate 3-phosphate during illumination. The O_2 electrode traces in Fig. 5 show that, after glycerate 3-phosphate addition, a linear rate of O_2 evolution is obtained which declines within 1–2 min in the presence of ribulose-1,5-bisphosphate carboxylase, particularly at low P_i concentrations (Fig. 5B and E). This decline in rate may be due to photoinhibition during the initial period of illumination in the absence of an electron acceptor [28]. The addition of saturating NADP or an ATP-generating system (creatine phosphate and creatine phosphokinase) seemingly restored O_2 evolution but only to the

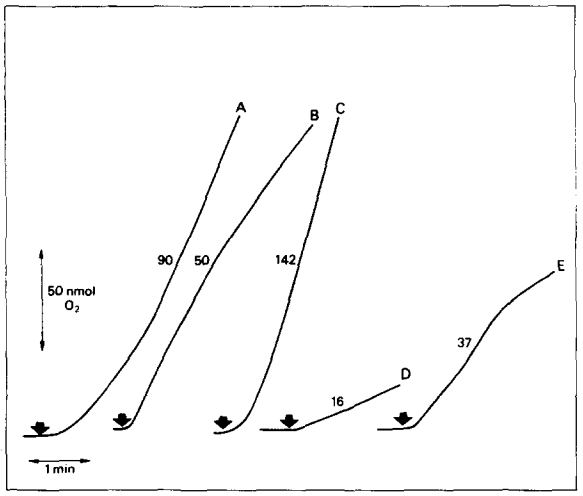

Fig. 5. Representative O_2 electrode traces of the effect on glycerate 3-phosphate reduction of adding ribulose-1,5-bisphosphate carboxylase (2.5 mg/ml). Trace A was obtained with 10 mM P_i and carboxylase (added in the light after P_i). Trace B had 2 mM P_i present along with carboxylase, added in the light after P_i, while trace C was obtained at 10 mM P_i in the absence of ribulsoe-1,5-bisphosphate carboxylase. Traces D and E were carried out with a different chloroplast preparation and with ribulose-1,5-bisphosphate carboxylase added before 2 mM P_i either prior to illumination (trace D) or after 2 min illumination (trace E). The numbers on the traces given the rate of O_2 evolution in μmol $O_2 \cdot h^{-1}$ per mg chlorophyll. The standard reconstituted chloroplast system was used with 0.4 mg stromal protein added.

basal non-phosphorylating rate of NADP reduction seen in the absence of ADP and P_i. This is consistent with the view that ribulose-1,5-bisphosphate carboxylase affects the operation of thylakoid ATPase.

When ribulose-1,5-bisphosphate carboxylase was added (during illumination) after the addition of P_i, partial protection of the rate glycerate 3-phosphate-dependent O_2 evolution against inhibition was afforded (Fig. 5). Traces D and E compare inhibition of glycerate 3-phosphate reduction by ribulose-1,5-bisphosphate carboxylase added prior to illumination (trace D) and after 2 min illumination (trace E). In both cases, 2 mM P_i was added after 2 min in the light. In the latter case, inhibition was less severe. Traces A and B were obtained by allowing NADP to become exhausted in the absence of added ribulose-1,5-bisphosphate carboxylase then P_i (2 or 10 mM) and glycerate 3-phosphate were added, and subsequently ribulose-1,5-bisphosphate carboxylase. Under these conditions, 10 mM P_i was sufficient to maintain 62% of the control rate of O_2 evolution. At higher P_i concentrations, the kinetics of O_2 evolution also changed. Higher P_i concentrations prevented the decline in rate observed after 1–2 min at lower P_i concentrations (traces A and B).

Effects of ribulose-1,5-bisphosphate carboxylase on the K_m of photophosphorylation for P_i

Data such as that shown in Fig. 5 implicate P_i as a protecting agent against ribulose-1,5-bisphosphae carboxylase inhibition of photophosphorylation. The P_i concentrations (mM) required for this protective action are much higher than the levels (μM) normally saturating for photophosphorylation [11]. It is possible that in the presence of ribulose-1,5-bisphosphate carboxylase, the protective effect may be superimposed on the P_i requirement of the ATPase alone, resulting in a higher apparent K_m P_i for this process. This possibility was explored by examining the P_i requirement for coupled NADP reduction with iso-

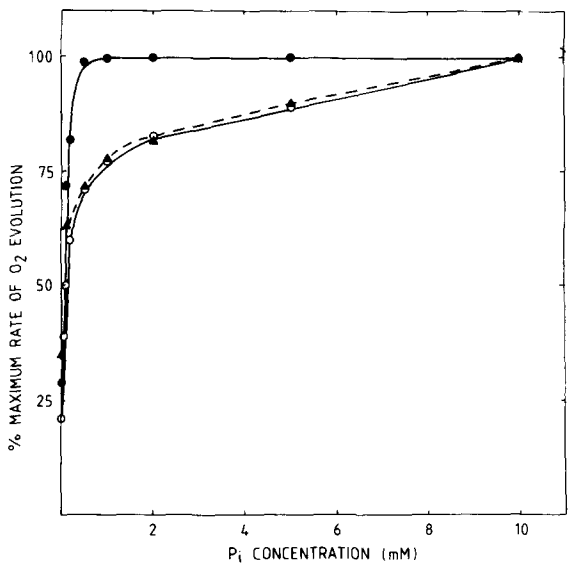

Fig. 6. The response of coupled NADP reduction to P_i in the presence of added ribulose-1,5-bisphosphate carboxylase (○) or stromal protein (▲) or in the absence of added protein (●). The concentration of added ribulose-1,5-bisphosphate carboxylase was 3 mg/ml and stromal protein added gave a final concentration of 0.4 mg/ml.

lated thylakoids in the presence and absence of stromal protein (Fig. 6). The stromal protein was added following a 2 min incubation of the thylakoids in the reaction medium in the light to reduce residual P_i (as in Fig. 5). Fig. 6 shows that the presence of a stromal protein concentration of 4 mg/ml considerably increased the P_i optimum for photophosphorylation (the $K_{1/2}$ increased from 50 to 130 μM). Apart from this effect at low P_i, the rate of coupled NADP reduction increased 25% between 1 and 10 mM P_i in the treated sample, while the control showed no response over this P_i range. A similar response was seen when purified ribulose-1,5-bisphosphate carboxylase was added (Fig. 6).

Discussion

The data shown here indicate that ribulose-1,5-bisphosphate carboxylase acts as a classical energy-transfer inhibitor of photophosphorylation [29]. Inhibitors of this type inhibit photophosphorylation without uncoupling, often via direct blockage of the thylakoid ATPase [29]. The inhibition of glycerate 3-phosphate reduction and the inhibition of coupled, but not uncoupled electron flow (Fig. 1) are consistent with this hypothesis. The dependence of the inhibition on Mg^{2+} concentration suggests that inhibition of photophosphorylation is associated with binding of the ribulose-1,5-bisphosphate carboxylase to the thylakoid membrane [16,19]. The binding is caused by electrostatic attraction between the negative charge on the enzyme protein (at pH 8.0) and Mg^{2+} present at the thylakoid membrane surface and would promote binding of the enzyme to stroma-exposed thylakoids [19].

To assess the physiological significance of these processes it is necessary to relate the experimental conditions to those within the intact chloroplast. The concentration of ribulose-1,5-bisphosphate carboxylase occurring in vivo may approach 648 $mg \cdot ml^{-1}$ or $1 \cdot 2$ mM (assuming a stromal protein content of 18 $mg \cdot mg^{-1}$ chlorophyll of which 90% is ribulose-1,5-bisphosphate carboxylase, and a stromal volume of 25 $\mu l \cdot mg^{-1}$ chlorophyll). Thus, the concentration of ribulose-1,5-bisphosphate carboxylase used in our experiments was two orders of magnitude less than that in vivo. However, on a chlorophyll basis, the amount of carboxylase used here was 10–20-fold higher than in vivo. The relative importance of these values depends upon the nature of the interaction between carboxylase and the thylakoid membrane. In view of the difficulty of reversing the inhibition of photophosphorylation by ribulose-1,5-bisphosphate carboxylase, it seems likely that this binding is 'tight', or that the inhibition of photophosphorylation is not readily reversible regardless of removal of bound carboxylase. Thus, the amount of ribulose-1,5-bisphosphate carboxylase available for binding relative to the area of thylakoid membrane may be of primary importance. This is supported by Fig. 3 which shows a sigmoidal response of carboxylase inhibition of glycerate 3-phosphate reduction to the concentration of chlorophyll included in the assay, resulting in less inhibition at higher thylakoid concentrations. If this is the case, it is unlikely that in vivo levels of ribulose-1,5-bisphosphate carboxylase would have more than a minimal effect on photophosphorylation under normal conditions. Indeed, electron microscopy of immunogold-labelled sections of barley leaves has shown that ribulose-1,5-bisphosphate carboxylase is uniformly distributed throughout the chloroplast stroma with little on the thylakoid membranes [30]. However, it may be that the absolute carboxylase concentration present is also of importance. In support of this point, Mori et al. [20] observed that the proportion of ribulose-1,5-bisphosphate carboxylase bound to the thylakoid was almost linearly related to the concentration of carboxylase present (between 0 and 40 $mg \cdot ml^{-1}$). In view of this, it would be difficult to account for high rates of photosynthesis in vivo unless the screening effect of P_i (Figs. 5 and 6) is sufficient to prevent inhibition of photophosphorylation.

Inhibition of photophosphorylation by ribulose-1,5-bisphosphate carboxylase may be of particular importance during water stress. During hyperosmotic stress the chloroplasts of higher plants shrink considerably, resulting in a several-fold decrease in stromal volume [31,32]. This reduction in volume results in a corresponding increase in protein concentration and cation levels in the stroma. Increased stromal cation concentrations have been implicated as the cause of inhibi-

tion of photosynthesis [31]. However, inhibition of photophosphorylation by ribulose-1,5-bisphosphate carboxylase would also become highly significant. Indeed, there is considerable evidence that a principal site of inhibition of photosynthesis by water stress is photophosphorylation [33–36]. Whole-chain electron transport in intact cells is unaffected by water stress despite a gross effect on ATP supply [35]. In addition, in isolated cell systems [35], no uncoupling of electron transport has been observed during water stress and in intact leaves water stress does not result in any decrease in the ΔpH [37]. These observations point towards a blockage of the thylakoid ATPase during water stress which could be attributed to the inhibitory effect of ribulose-1,5-bisphosphate carboxylase reported here.

An important physiological implication of ribulose-1,5-bisphosphate carboxylase interacting with the chloroplast thylakoid is the potential shielding of the ATPase from its substrates. The potential for ribulose-1,5-bisphosphate carboxylase to alter the apparent K_m of photophosphorylation for P_i is clearly shown in Fig. 6. It is possible then that the inhibition of photophosphorylation by ribulose-1,5-bisphosphate carboxylase seen here is due to binding of the protein in the region of the ATPase, reducing accessibility of ADP and P_i. This is suggested by the observation of partial protection of photophosphorylation from ribulose-1,5-bisphosphate carboxylase inhibition by high concentrations of P_i (Figs. 5 and 6). However, this protection was only afforded by P_i added prior to the treatment of thylakoids with carboxylase and no restoration of photophosphorylation was evident when P_i and ADP concentrations were increased following inhibition. Thus, it seems likely that the protective effect of P_i was due to anionic screening of the thylakoids, preventing cation-dependent binding of ribulose-1,5-bisphosphate carboxylase to the membranes.

The relationship between the binding of ribulose-1,5-bisphosphate carboxylase the thylakoid and the observed inhibition of coupled electron flow may also be related to recent observations which suggest that ribulose-1,5-bisphosphate carboxylase activation in leaves is controlled primarily by light intensity and not CO_2 concentration [38] via a mechanism requiring high ΔpH [39] and the presence of two stromal polypeptides [40]. Finally, these results may explain the fact that the chloroplast behaves as though it were P_i-deficient in concentrations of P_i considerably higher than those which would affect photophosphorylation in isolated thylakoids. If this is the case they are of considerable significance in their relation to the manner in which cytosolic P_i concentration influences the rate of photosynthesis and the distribution of photosynthetic product between sucrose and starch [4–9].

Acknowledgements

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