Biochimica et Biophysica Acta, 459 (1977) 337–346

BBA 47243

THE EFFECT OF DIHYDROXYACETONE PHOSPHATE AND 3-PHOSPHOGLYCERATE ON O_2 EVOLUTION AND ON THE LEVELS OF ATP, ADP AND P_i IN ISOLATED INTACT CHLOROPLASTS

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(Received July 26th, 1976)

SUMMARY

Addition of dihydroxyacetone phosphate (2.5 mM) or 3-phosphoglycerate (2.5 mM) to a suspension of isolated intact chloroplasts, which contains P_i only in low concentrations (0.2 mM) leads to a competitive inhibition of P_i uptake in the light. In consequence, the ATP/ADP ratio is strongly decreased. The rate of O_2 evolution is also reduced under these conditions, but the degree of inhibition is much higher after addition of dihydroxyacetone phosphate than after addition of 3-phosphoglycerate. Therefore, besides the competitive inhibition of P_i uptake, additional effects of dihydroxyacetone phosphate and 3-phosphoglycerate on O_2 evolution and CO_2 fixation of isolated intact chloroplasts must occur, which are discussed.

INTRODUCTION

The inner membrane of the chloroplast envelope contains a shuttle system, which mediates the mutual exchange of P_i, 3-phosphoglycerate and triose phosphates [1–5]. The actions of this shuttle influence photosynthesis in various ways; e.g. it is well known that addition of P_i in high concentrations inhibits CO_2 fixation as well as O_2 evolution [6, 7], presumably by reducing the level of 3-phosphoglycerate and triose phosphates inside the chloroplasts [1]. At low concentrations of P_i, addition of 3-phosphoglycerate in high concentrations is known to inhibit mainly CO_2 fixation with a much lesser effect on O_2 evolution and NADP reduction [8–12]. Recently we could show that dihydroxyacetone phosphate (2.5 mM) at low concentrations of P_i inhibits O_2 evolution completely while CO_2 fixation is not or only slightly influenced [13]. By investigating the effect of high concentrations of dihydroxyacetone phosphate and 3-phosphoglycerate on O_2 evolution and on the level of ATP, ADP and P_i in isolated intact chloroplasts, we tried to get more information on the mechanism(s), by which dihydroxyacetone phosphate and 3-phosphoglycerate act on photosynthesis.

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

MATERIALS AND METHODS

(1) Chloroplasts were isolated from freshly harvested spinach leaves (*Spinacea oleracea* var. "Atlanta") according to Cockburn et al. [7], with the following modifications: the grinding medium contained 0.33 M sorbitol, 0.05 M 2-(*N*-morpholino)-ethanesulfonic acid/NaOH(pH 6.5), 1 mM $MnCl_2$, 1 mM $MgCl_2$ and 2 mM EDTA. Instead of muslin, a pad of cotton wool sandwiched between two layers of nylon cloth was used for the filtration of the spinach homogenate, and the chloroplasts were washed once in the suspending medium, which contained 0.33 M sorbitol, 0.05 M HEPES/NaOH(pH 7.6), 1 mM $MnCl_2$, 1 mM $MgCl_2$ and 2 mM EDTA.

(2) The percentage of intact chloroplasts in the suspension was routinely controlled by comparing the rates of ferricyanide reduction of normal and of osmotically shocked chloroplast preparations [14]. Only those preparations which contained at least 70 % of intact chloroplasts were used in the experiments.

(3) O_2 evolution was measured polarographically with a Beckmann electrode in a temperature-controlled cuvette which was illuminated with a modified slide projector. The final volume of the reaction mixture was 2.5 ml and contained chloroplasts equivalent to 125 μg chlorophyll.

(4) For the measurement of ATP and ADP, 15 ml of a chloroplast suspension (containing 100 μg chlorophyll/ml) were incubated in a modified "lollipop", which allowed sampling of 1-ml aliquots at very short time intervals [15]. 0.5 ml of a neutralized $HClO_4$ extract were used for the measurement of ATP by the sensitive luciferin-luciferase assay [16, 17]. Another 0.5 ml aliquot of the same sample was used for the conversion of ADP to ATP by adding 0.1 ml phosphoenolpyruvate (in 0.133 M $MgSO_4$+0.266 M KCl) and 8 units of pyruvate kinase (in 0.25 M Tris · HCl, pH 7.4). All the samples were allowed to stand at 37 °C for 30 min before measuring the ATP content. The amount of ADP was calculated from the different content of ATP in the samples with and without phospho*enol*pyruvate plus pyruvate kinase.

(5) For the determination of P_i, the chloroplasts were incubated in 25-ml centrifuge tubes in a modified Warburg apparatus. Each tube contained chloroplasts equivalent to 150–250 μg chlorophyll in a final volume of 1 ml. After different times in the dark or in the light (see legends), the tubes were quickly centrifuged at 0 °C for 1 min at 2000 $\times g$. The supernatant was either discarded or used for the determination of P_i in the medium. The sediment was carefully rinsed twice with 0.5 ml of ice-cold suspending medium. In the most cases, the degree of intactness was not changed by this procedure. The washed sediment was resuspended in 1 ml of ice-cold water and 50 μl of this suspension were used for the measurement of the chlorophyll content, which was reduced to about 60–70 % of the initial value by the washing procedure. Immediately afterwards, 0.2 ml of trichloroacetic acid (20 %) were added to the rest of the suspension (and to the supernatant, if the P_i content of the medium was measured in parallel). The time from taking the tubes out of the water bath until adding trichloroacetic acid was approx. 100 s. After acidification, the samples were centrifuged for 10 min, and 1 ml of the clear supernatant was used for the colorimetric determination of P_i after isobutanol extraction according to Yanagita [18].

(6) The incubation conditions were the same in all types of experiments. The reaction medium was identical with the suspending medium, but contained in addition

10 mM $KHCO_3$. Recently, it has been shown [19, 20] that O_2 evolution of isolated intact chloroplasts sometimes can be partly inhibited by H_2O_2 formed under certain conditions in an endogenous Mehler reaction. Therefore the reaction medium always contained 2500 units of catalase. P_i, 3-phosphoglycerate or dihydroxyacetone phosphate were added as indicated in the legends of the figures and tables. The intensity of the saturating white light was 300 W/m^2 and the temperature was kept at 18 °C.

RESULTS

Addition of dihydroxyacetone phosphate (2.5 mM) to a suspension of isolated intact chloroplasts inhibits CO_2-dependent O_2 evolution completely, if P_i is present in the reaction medium only in low concentrations (0.1–0.2 mM, Fig. 1, compare ref. 13). This inhibition was recently shown to be reversed by higher concentrations of P_i, but not by 3-phosphoglycerate [13]. Therefore it might be assumed that dihydroxyacetone phosphate inhibits O_2 evolution due to a lack of P_i which is caused by a fast exchange of internal P_i against added dihydroxyacetone phosphate, facilitated by the phosphate translocator. If this assumption is correct, addition of 3-phosphoglycerate (2.5 mM) to a chloroplast suspension should also cause a lack of P_i due to a similar exchange reaction and therefore O_2 evolution should also be inhibited. While low concentrations of 3-phosphoglycerate (0.25 mM, not shown) do not inhibit CO_2-dependent O_2 evolution, but sometimes stimulate it, addition of 3-phosphoglycerate at higher concentrations (2.5 mM) inhibits O_2 evolution by about 50 % (Fig. 1). This inhibition of O_2 evolution by dihydroxyacetone phosphate or 3-phosphoglycerate is reversed after addition of P_i in higher concentrations (not shown, compare ref. 13). Thus it might be accepted that dihydroxyacetone phosphate and 3-phosphoglycerate produce a lack of P_i by an exchange reaction via the phosphate translocator. Such a lack of P_i might become rate limiting for photophosphorylation. Therefore we

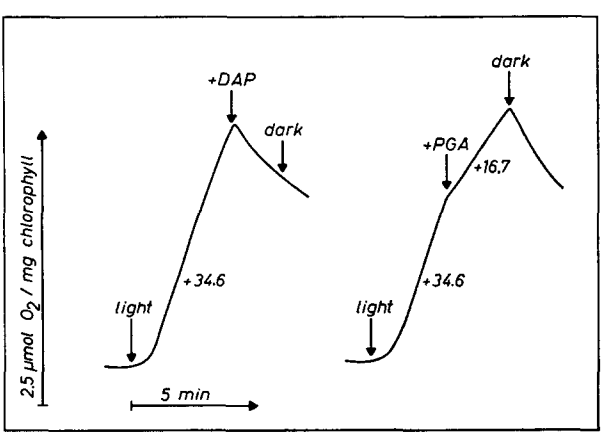

Fig. 1. Effect of dihydroxyacetone phosphate (DAP) and 3-phosphoglycerate (PGA) on O_2 evolution of isolated intact chloroplasts. The numbers near the traces indicate the rates of O_2 evolution in µmol/mg chlorophyll per h. These rates are not corrected for the portion of broken chloroplasts in the suspension. Dihydroxyacetone phosphate and 3-phosphoglycerate were added as indicated in a volume of 100 µl to give a final concentration of 2.5 mM. For further details see Materials and Methods.

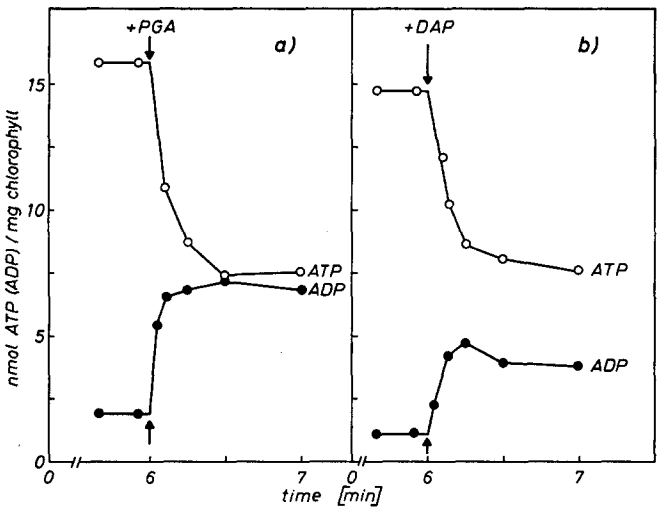

Fig. 2. The level of ATP and ADP in the light as influenced by addition of 3-phosphoglycerate and dihydroxyacetone phosphate. 3-Phosphoglycerate (PGA) and dihydroxyacetone phosphate (DAP) were added as indicated in a volume of 0.5 ml to give a final concentration of 2.5 mM. For further details see Materials and Methods.

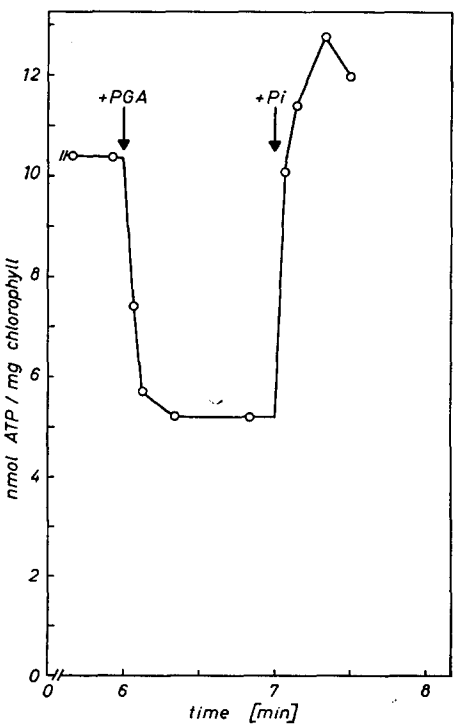

Fig. 3. Reversal of the 3-phosphoglycerate-induced decrease of the ATP level in the light by addition of P_i. 0.5 ml 3-phosphoglycerate and 0.1 ml P_i were added as indicated to give a final concentration of 2.5 mM.

examined the effect of 3-phosphoglycerate and dihydroxyacetone phosphate on the steady-state ATP level of chloroplasts in the light. As shown in Fig. 2, addition of 3-phosphoglycerate (2.5 mM) as well as of dihydroxyacetone phosphate (2.5 mM) to illuminated chloroplasts causes a strong decrease of the ATP level (as also shown by Cockburn [21]) with a concomitant increase in the level of ADP. The much lower decrease of the ATP level after addition of 3-phosphoglycerate (2 mM) described by Heber [22] is presumably due to the higher concentration of P_i (0.8 mM) used in these experiments (compare discussion).

The decrease of the [ATP]/[ADP] ratio shown in Fig. 2 is completely reversed by addition of P_i in higher concentrations (2.5 mM, Fig. 3). Therefore we conclude that the [ATP]/[ADP] ratio in the light depends strongly on the availability of P_i. This interpretation is confirmed by the results given in Fig. 4a. If a suspension of intact chloroplasts is illuminated in the presence of P_i and CO_2, the level of ATP increases quickly until it reaches a steady state, which remains high over a period of up

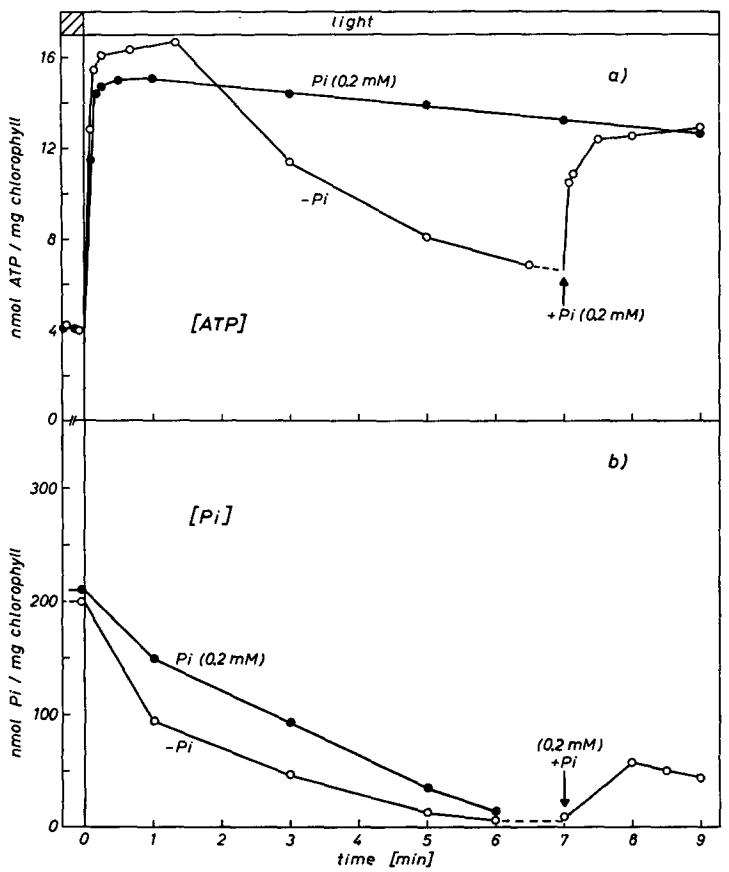

Fig. 4. Light-induced changes in the level of internal ATP (a) and P_i (b) with and without P_i (0.2 mM) in the reaction medium. Where indicated, 100 μl P_i was added to the P_i-free suspension (○—○) to give a final concentration of 0.2 mM. ATP and P_i were measured with different chloroplast preparations, as described in Materials and Methods.

TABLE I

DECREASE OF THE INTERNAL LEVEL OF P_i IN THE DARK AFTER ADDITION OF 3-PHOSPHOGLYCERATE OR DIHYDROXYACETONE PHOSPHATE

0.1 ml of chloroplasts (228 μg chlorophyll) was added to 0.9 ml of the reaction medium with or without 3-phosphoglycerate respectively dihydroxyacetone phosphate and kept in the dark for 3 min. For further details, see Materials and Methods.

Conditions	[P_i] in the chloroplasts (nmol/mg chlorophyll)
Control	265
+ 3-phosphoglycerate (2.5 mM)	95
+ dihydroxyacetone phosphate (2.5 mM)	65

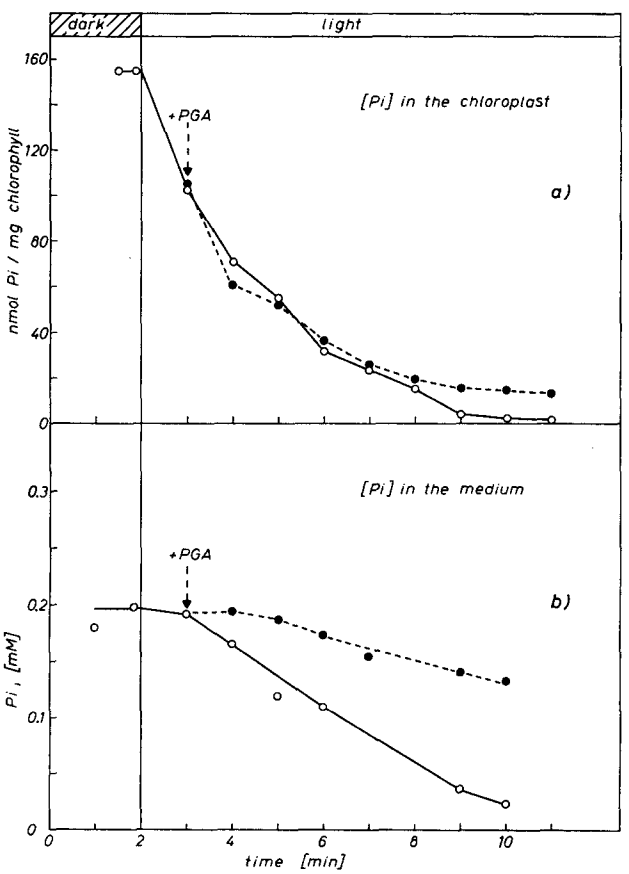

Fig. 5. The effect of light and 3-phosphoglycerate (PGA) on the level of P_i in the chloroplasts (a) and in the reaction medium (b). At the beginning of the light phase the samples contained 0.2 mM P_i. Where indicated, 50 μl of 3-phosphoglycerate were added to give a final concentration of 2.5 mM. (○—○, control; ●- - -●, level of P_i after addition of 3-phosphoglycerate). For further details see Materials and Methods.

to 10 min. If P_i is omitted from the medium, however, the level of ATP decreases after about 1 min until it reaches nearly the dark level. If P_i is added at this state, the level of ATP rises again to the level of the suspension which contains P_i.

The level of internal P_i, which varies in the dark between 150 and 350 nmol/mg chlorophyll, drops down after illumination until it also reaches a steady state, which under our conditions was near to zero (Fig. 4b). Surprisingly, this decrease of the internal P_i level is largely independent of the presence of P_i in the reaction medium at least at the low concentrations of P_i (0.2 mM) used in our experiments. If the level of internal P_i has reached its very low steady state, addition of P_i (0.2 mM) to the reaction medium causes a small and transitory rise in the level of internal P_i (Fig. 4b), which is much lower than the response of the ATP level shown in Fig. 4a. Obviously, the [ATP]/[ADP] ratio inside the chloroplasts can be high in the light even at low internal P_i concentrations, if sufficient P_i is present in the surrounding medium.

If 3-phosphoglycerate or dihydroxyacetone phosphate are added to a chloroplast suspension in the dark, the high level of internal P_i is strongly decreased (Table I), indicating a fast exchange of internal P_i against added 3-phosphoglycerate or dihydroxyacetone phosphate. Therefore it should be expected that the rate of the light-induced decrease of the internal P_i level is accelerated and its steady-state level is lowered by addition of 3-phosphoglycerate. However, as shown in Fig. 5a, this is not the case.

Starting with a concentration of 0.2 mM, the level of P_i in the medium is also decreased upon illumination (Fig. 5b), indicating an uptake of P_i from the medium. The rate of this light-dependent P_i uptake is considerably higher (11.5 μmol/mg chlorophyll per h) than the rate of the light-induced decrease of the internal level of P_i (3 μmol/mg chlorophyll per h, Fig. 5a). By addition of 3-phosphoglycerate (2.5 mM), the rate of this light-dependent P_i uptake is decreased to about 30 % of the control rate (Fig. 5b). Therefore it is concluded that the decrease of the [ATP]/[ADP] ratio after addition of 3-phosphoglycerate or dihydroxyacetone phosphate (Fig. 2) is mainly due to an inhibition of P_i uptake and not to a fast exchange of internal P_i against external 3-phosphoglycerate or dihydroxyacetone phosphate.

DISCUSSION

If P_i is omitted from a suspension of isolated intact chloroplasts in the light, O_2 evolution ceases after few minutes [6, 23, 24]. Our experiments show that under these conditions the level of internal ATP is decreased to the dark level. Addition of P_i restarts O_2 evolution and reverses the decrease of the ATP level. A differential inhibition of O_2 evolution and a decrease of the [ATP]/[ADP] ratio can be induced also by addition of high concentrations of 3-phosphoglycerate or dihydroxyacetone phosphate to a reaction medium containing only low concentrations of P_i. The finding of Heber [22] that addition of 3-phosphoglycerate at saturating light intensities only slightly decreases the level of ATP, is not contradictory to our results. Under the described conditions [22] the ratio of [3-phosphoglycerate]/[P_i] was 2 mM/0.8 mM = 2.5, while under our conditions this ratio is much higher (2.5 mM/0.2 mM = 12.5). Therefore the effect of 3-phosphoglycerate necessarily must be much stronger under our conditions. From the experiments of Heldt et al. [1, 4], two possible reasons for the effect of high concentrations of external 3-phosphoglycerate or dihydroxyacetone

phosphate on the ratio of [ATP]/[ADP] can be postulated. At high ratios of [3-phosphoglycerate]/[P_i] or [dihydroxyacetone phosphate]/[P_i] in the reaction medium a fast countertransfer of internal P_i against external 3-phosphoglycerate or dihydroxyacetone phosphate might strongly reduce the internal [P_i]. Furthermore, under these conditions a competitive inhibition of P_i uptake into the chloroplasts should be expected. At constant phosphorylation potential [ATP]/[ADP] × [P_i], reduction of internal [P_i] by a fast countertransfer should lead to the observed decrease of [ATP]/[ADP]. In the dark we found indeed a rapid release of internal P_i after addition of 3-phosphoglycerate or dihydroxyacetone phosphate (Table I). In the light, the internal [P_i] decreases to a very low steady-state level, regardless whether P_i at low concentrations is present in the medium or not. In contrast, the level of ATP remains high even at low internal [P_i], but only if some P_i is present in the reaction medium. Addition of 3-phosphoglycerate has no effect neither on this light-induced decrease of internal [P_i] nor on the low steady-state level of internal P_i. Therefore it is concluded that the reduction of [ATP]/[ADP] after addition of 3-phosphoglycerate or dihydroxyacetone phosphate in the light is not caused by an additional release of P_i or by the low internal level of P_i per se, but by the competitive inhibition of P_i uptake demonstrated in Fig. 5b. As the internal level of P_i remains constantly low in the light also after addition of 3-phosphoglycerate or dihydroxyacetone phosphate, the reduction of [ATP]/[ADP] indicates a decrease of the phosphorylation potential [ATP]/[ADP] × [P_i]. This decrease might strongly influence the activity of the phosphoribulokinase as well as of the phosphoglycerate kinase, which both compete for ATP. Recently it was shown [25, 26] that phosphoglycerate kinase is strongly inhibited by ADP. Therefore it is assumed that the increased level of internal ADP observed after addition of 3-phosphoglycerate or dihydroxyacetone phosphate might especially reduce the activity of phosphoglycerate kinase, leading to an inhibition of O_2 evolution.

The described competitive inhibition of P_i uptake by 3-phosphoglycerate or dihydroxyacetone phosphate, however, cannot be the only mechanism by which both compounds act on photosynthesis. As we could see in Fig. 1 as well as from other investigations [8–10, 13], there are also some striking differences between the effect of 3-phosphoglycerate and dihydroxyacetone phosphate: (i) at high external [3-phosphoglycerate]/[P_i] CO_2 fixation is much stronger inhibited than O_2 evolution [8–10], and (ii) high external [dihydroxyacetone phosphate]/[P_i] inhibits O_2 evolution completely with no or a much lesser effect on CO_2 fixation [13]. It should be expected that at high ratios of [triose phosphate]/[P_i] in the chloroplasts in the light addition of 3-phosphoglycerate leads to a fast exchange of external 3-phosphoglycerate against internal triose phosphates [8, 10]. This loss of triose phosphates might cause a reduced supply of ribulose 1,5-diphosphate and consequently CO_2 fixation must be much stronger inhibited than O_2 evolution [12]. Furthermore it is known that 3-phosphoglycerate is a non-competitive inhibitor of ribulose-1,5-diphosphate carboxylase with respect to ribulose 1,5-diphosphate and a competitive inhibitor with respect to HCO_3^- [27]. This might be another reason for the comparatively strong inhibition of CO_2 fixation by 3-phosphoglycerate. On the other hand, on addition of dihydroxyacetone phosphate the chloroplasts should have enough substrate for the formation of ribulose 1,5-diphosphate and thus CO_2 should be fixed at least as far as a minimal supply of P_i is warranted. Therefore dihydroxyacetone phosphate would cause no or only a slight inhibition of CO_2 fixation, as indeed observed [13]. Recently it has been shown

[28, 29] that isolated intact chloroplasts are able to fix CO_2 even in the dark, if dihydroxyacetone phosphate and oxaloacetate are added. Therefore dihydroxyacetone phosphate might be able in high concentrations to drive 3-phosphoglycerate reduction into the reverse direction leading to an accumulation of NADPH (if no oxaloacetate is present to reoxidize NADPH) and consequently to an inhibition of O_2 evolution. Addition of 3-phosphoglycerate in equally high concentrations should then reverse this inhibition of O_2 evolution. However, from the results shown above it is clear that addition of 3-phosphoglycerate together with dihydroxyacetone phosphate (both 2.5 mM), which would increase the ratio of [3-phosphoglycerate+dihydroxyacetone phosphate]/[P_i] to a value of 25, must lead to an even stronger competitive inhibition of P_i uptake. Consequently the inhibition of O_2 evolution by dihydroxyacetone phosphate cannot be reversed by addition of 3-phosphoglycerate, as already shown [13].

From these results and considerations it is concluded that at low P_i in the reaction medium, 3-phosphoglycerate or dihydroxyacetone phosphate added in high concentrations will influence photosynthesis by two different mechanisms. One common effect of 3-phosphoglycerate and dihydroxyacetone phosphate is a competitive inhibition of P_i uptake, leading to a reduction of the [ATP]/[ADP] ratio and to a partial inhibition of O_2 evolution. Furthermore, dihydroxyacetone phosphate and 3-phosphoglycerate have additional effects on O_2 evolution and CO_2 fixation, and that in a different way. While dihydroxyacetone phosphate inhibits mainly O_2 evolution but not CO_2 fixation [13], presumably by preventing the reoxidation of NADPH, 3-phosphoglycerate blocks CO_2 fixation much stronger than O_2 evolution [8–10] by reduction of the internal level of triose phosphates and/or by diminishing the activity of ribulose-1,5-diphosphate carboxylase.

ACKNOWLEDGEMENT

We are grateful for the skilled technical assistance of Mrs. Spidola Neimanis.

REFERENCES

1 Heldt, H. W. and Rapley, L. (1970) FEBS Lett. 10, 143–148
2 Heldt, H. W. and Sauer, F. (1971) Biochim. Biophys. Acta 234, 83–91
3 Heldt, H. W., Sauer, E. and Rapley, L. (1972) in Proceedings of the 2nd International Congress on Photosynthesis Research 2, (Forti, G., Avron, M. and Melandri, A., eds.), pp. 1345–1355, Junk, The Hague
4 Werdan, K. and Heldt, H. W. (1972) in Proceedings of the 2nd International Congress on Photosynthesis Research 2, (Forti, G., Avron, M. and Melandri, A., eds.), pp. 1337–1344, Junk, The Hague
5 Heber, U. (1974) Ann. Rev. Plant Physiol. 339–421
6 Cockburn, W., Baldry, C. W. and Walker, D. A. (1967) Biochim. Biophys. Acta 143, 614–624
7 Cockburn, W., Baldry, C. W. and Walker, D. A. (1968) Biochem. J. 107, 89–95
8 Bamberger, E. S., Ehrlich, B. A. and Gibbs, M. (1974) in Proceedings of the 3rd International Congress on Photosynthesis Research 2, (Avron, M., ed.), pp. 1349–1362, Elsevier, Amsterdam
9 Kaiser, W. and Urbach, W. (1974) Ber. Dtsch. Bot. Ges. 87, 145–153
10 Bamberger, E. S., Ehrlich, B. A. and Gibbs, M. (1975) Plant. Physiol. 55, 1023–1030
11 Bamberger, E. S. and Avron, M. (1975) Plant Physiol. 56, 481–485
12 Anderson, W. R. and Gibbs, M. (1975) Biochem. Biophys. Res. Commun. 62, 953–956
13 Kaiser, W. and Urbach, W. (1976) Biochim. Biophys. Acta 423, 91–102
14 Heber, U. and Santarius, K. A. (1970) Z. Naturforsch. 25b, 718–728

15 Bornefeld, T., Kaiser, W. and Kleinschnitz, W. (1975) Z. Naturforsch. 30c, 680
16 Welsch, F. and Smith, L. (1969) Biochemistry 8, 3403–3408
17 Strehler, B. L. (1970) in Methoden der enzymatischen Analyse II (Bergmeyer, H. U., ed.), pp. 2036–2050, Chemie-Verlag, Weinheim
18 Yanagita, T. (1964) J. Biochem. Tokyo 55, 260–268
19 Egneus, H., Heber, U., Matthiesen, U. and Kirk, M. (1975) Biochim. Biophys. Acta 408, 252–268
20 Kaiser, W. (1976) Biochim. Biophys. Acta 440, 476–482
21 Cockburn, W. (1974) J. Exp. Bot. 25, 111–120
22 Heber, U. (1973) Biochim. Biophys. Acta 305, 140–152
23 Cockburn, W., Baldry, C. W. and Walker, D. A. (1967) Biochim. Biophys. Acta 131, 594–596
24 Walker, D. A. (1973) New Phytol. 72, 209–235
25 Lilley, R. McC. and Walker, D. A. (1974) Biochim. Biophys. Acta 368, 269–278
26 Slabas, A. R. and Walker, D. A. (1976) Biochem. J. 154, 185–192
27 Wishnik, M. and Lane, M. D. (1971) in Methods in Enzymology (San Pietro, A., ed.), Vol. XXIII, pp. 570–577, Academic Press, New York
28 Heldt, H. W., Fliege, R., Lehner, K., Milonvancev, M. and Werdan, K. (1974) in Proceedings of the 3rd International Congress on Photosynthesis Research 2, (M. Avron, ed.), pp. 1369–1379, Elsevier, Amsterdam
29 Werdan, K., Heldt, H. W. and Milonvancec, M. (1975) Biochim. Biophys. Acta 396, 276–292