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RATES AND PROPERTIES OF ENDOGENOUS CYCLIC PHOTOPHOSPHORYLATION OF ISOLATED INTACT CHLOROPLASTS MEASURED BY CO_2 FIXATION IN THE PRESENCE OF DIHYDROXYACETONE PHOSPHATE

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SUMMARY

1. Dihydroxyacetone phosphate in concentrations $\geqslant$ 2.5 mM completely inhibits CO_2-dependent O_2 evolution in isolated intact spinach chloroplasts. This inhibition is reversed by the addition of equimolar concentrations of P_i, but not by addition of 3-phosphoglycerate. In the absence of P_i, 3-phosphoglycerate and dihydroxyacetone phosphate, only about 20 % of the ^{14}C-labelled intermediates are found in the supernatant, whereas in the presence of each of these substances the percentage of labelled intermediates in the supernatant is increased up to 70–95 %. Based on these results the mechanism of the inhibition of O_2 evolution by dihydroxyacetone phosphate is discussed with respect to the function of the known phosphate translocator in the envelope of intact chloroplasts.

2. Although O_2 evolution is completely suppressed by dihydroxyacetone phosphate, CO_2 fixation takes place in air with rates of up to 65μ mol · mg^{-1} chlorophyll · h^{-1}. As non-cyclic electron transport apparently does not occur under these conditions, these rates must be due to endogenous pseudocyclic and/or cyclic photophosphorylation.

3. Under anaerobic conditions, the rates of CO_2 fixation in presence of dihydroxyacetone phosphate are low (2.5–7 μmol · mg^{-1} chlorophyll · h^{-1}), but they are strongly stimulated by addition of dichlorophenyl-dimethylurea (e.g. $2 \cdot 10^{-7}$ M) reaching values of up to 60 μmol · mg^{-1} chlorophyll · h^{-1}. As under these conditions the ATP necessary for CO_2 fixation can be formed by an endogenous cyclic photophosphorylation, the capacity of this process seems to be relatively high, so it might contribute significantly to the energy supply of the chloroplast. As dichlorophenyl-dimethylurea stimulates CO_2 fixation in presence of dihydroxyacetone phosphate under anaerobic but not under aerobic conditions, it is concluded that only under anaerobic conditions an "overreduction" of the cyclic electron transport system takes place, which is removed by dichlorophenyl-dimethylurea in suitable concentrations. At concentrations above $5 \cdot 10^{-7}$ M dichlorophenyl-dimethylurea inhibits dihydroxyacetone phosphate-dependent CO_2 fixation under anaerobic as well as under aerobic conditions in a similar way as normal CO_2 fixation. Therefore, we assume that a properly poised redox state of the electron transport chain is necessary for an optimal occurrence of endogenous cyclic photophosphorylation.

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

4. The inhibition of dichlorophenyl-dimethylurea-stimulated CO_2 fixation in presence of dihydroxyacetone phosphate by dibromothymoquinone under anaerobic conditions indicates that plastoquinone is an indispensible component of the endogenous cyclic electron pathway.

INTRODUCTION

Although there is now sufficient evidence [1–6] that an endogenous cyclic photophosphorylation takes place under suitable conditions in isolated intact chloroplasts as well as in vivo [7], the capacity and physiological significance of this process is still uncertain and controversal [8, 9]. In particular, the published rates of endogenous cyclic photophosphorylation of isolated intact chloroplasts are extremely low [10]. For example, the rates of light-dependent ^{32}P-incorporation are between 0.2 and 5 μmol · mg chlorophyll^{-1} · h^{-1}, whereas the rates of CO_2 fixation may reach 200 μmol · mg chlorophyll^{-1} · h^{-1} [10]. Even measurements of light-dependent changes of the ATP level in isolated intact chloroplasts do not yield higher rates of endogenous photophosphorylation than those measured by ^{32}P-incorporation [6, 11]. Although recent attempts in our laboratory to obtain higher rates of the different reactions of endogenous photophosphorylation in isolated intact chloroplasts by measuring ^{32}P-incorporation in presence of suitable phosphate acceptors were partially succesful [10], it seemed necessary to look for further methods of a quantitative measurement of endogenous photophosphorylation.

In this paper a new method of quantitatively investigating the rates of endogenous cyclic photophosphorylation of isolated intact chloroplasts by measuring $^{14}CO_2$ fixation in presence of dihydroxyacetone phosphate ($(OH)_2$acetone-P) is described.

METHODS

1. Chloroplasts were isolated from freshly harvested spinach leaves according to Cockburn et al. [12]. The percentage of intact chloroplasts in the suspension was routinely controlled by comparing the rates of ferricyanide reduction of normal and osmotically shocked chloroplast preparations [13]. A suspension of intact chloroplasts usually contained 70–95 % of chloroplasts with an integer envelope and fixed CO_2 with rates up to 170 μmol · mg chlorophyll^{-1} · h^{-1}.

2. Incorporation of $^{14}CO_2$ was carried out in glass centrifuge tubes (25 ml), which were fixed with a special holder in a Warburg apparatus. The tubes were illuminated with white-light (40 klux) from the bottom and the temperature of the water bath was kept at 18 °C. Our standard reaction mixture in a final volume of 1.5 ml was: 0.33 M sorbitol, 0.05 M HEPES-NaOH (pH 7.6), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.1 mM KH_2PO_4 and 10 mM $KHCO_3$ labelled with 20 μCi $NaH^{14}CO_3$. The reaction was usually started by adding 0.3 ml of the chloroplast suspension, containing 75 μg chlorophyll and stopped by the addition of 0.5 ml of trichloroacetic acid (20 %). The tubes were quickly stoppered. For the determination of the total fixed CO_2, 0.5 ml of the acidified suspension were dried briefly on planchets (⌀ 50 mm) fitted with lens tissue. The radioactivity was measured in a gas flow counter (Tracerlab). The total radioactivity added to the reaction mixture was determined by using a

complete reaction medium (chloroplasts included) which contained 0.5 ml NaOH (1 M) instead of trichloroacetic acid.

3. O_2 evolution was measured polarographically with a Beckmann O_2-electrode in a temperature controlled cuvette which was illuminated by a modified slide projector. The final volume of the reaction mixture was 2.0 ml, containing 100 μg chlorophyll. All other experimental conditions were the same as for the measurement of CO_2 fixation.

4. To determine the distribution of ^{14}C-labelled products between supernatant and chloroplast pellet, the chloroplast suspension was preilluminated for 2 min and CO_2 fixation was started by addition of ^{14}C-labelled $KHCO_3$ in the light. After 2 min the suspension was centrifuged with $2500 \times g$ at 0 °C for 1 min. Then 0.5 ml of the supernatant were immediately mixed with 0.2 ml of trichloroacetic acid (20 %) and 0.4 ml of trichloroacetic acid were added to the rest of the suspension containing the remaining supernatant (1.0 ml) and the complete pellet. 0.5 ml of both samples were dried on planchets and measured as described above. From the different ^{14}C-labelling of the clear supernatant and the acidified chloroplast suspension the amount of radioactivity in the pellet was calculated. When trichloroacetic acid was added before centrifuging the samples, very little radioactivity was found in the pellet.

5. In experiments under anaerobic conditions the tubes with the complete reaction mixture but without chloroplasts were gassed by a stream of N_2 while shaking in the Warburg apparatus for about 1 h before initiating the reaction. Only 5–10 % of the added bicarbonate was lost during this procedure. The freshly isolated chloroplasts were gassed separately for 10 min on a whirlmix at 0 °C.

6. Chlorophyll was determined according to Arnon [14].

RESULTS

O_2 evolution and CO_2 fixation in presence of $(OH)_2$acetone-P

Addition of $(OH)_2$acetone-P at concentrations of 2.5–5.0 mM completely inhibits O_2 evolution of isolated intact chloroplasts (Fig. 1a). The degree of inhibition was somewhat variable and may depend on pretreatment of chloroplasts, e.g. inhibition was sometimes complete only after 3 min of preillumination. Under the same conditions CO_2 fixation remains unaffected and occurs with rates between 30 to 60 μmol $CO_2 \cdot$ mg chlorophyll$^{-1} \cdot h^{-1}$ (Fig. 1b). Apparently CO_2 is fixed in the presence of $(OH)_2$acetone-P without reduced NADP and without ATP formed by a non-cyclic electron transport.

One possible mechanism for the inhibition of O_2 evolution by $(OH)_2$acetone-P might be due to a fast counter exchange of 3-P-glycerate against $(OH)_2$acetone-P (see discussion). In this case the inhibition should be reversed by addition of 3-P-glycerate. However, as shown in Fig. 2, 3-P-glycerate is ineffective in reversing the inhibition of O_2 evolution by $(OH)_2$acetone-P, whereas P_i in concentrations of about 2.5 mM abolishes the inhibition completely.

Distribution of ^{14}C-labelled products between supernatant and chloroplast pellet

In order to test whether the inhibition of O_2 evolution by $(OH)_2$acetone-P is due to a fast exchange of 3-P-glycerate for $(OH)_2$acetone-P, we measured the distribution of ^{14}C-labelled products between the chloroplast pellet and the supernatant under

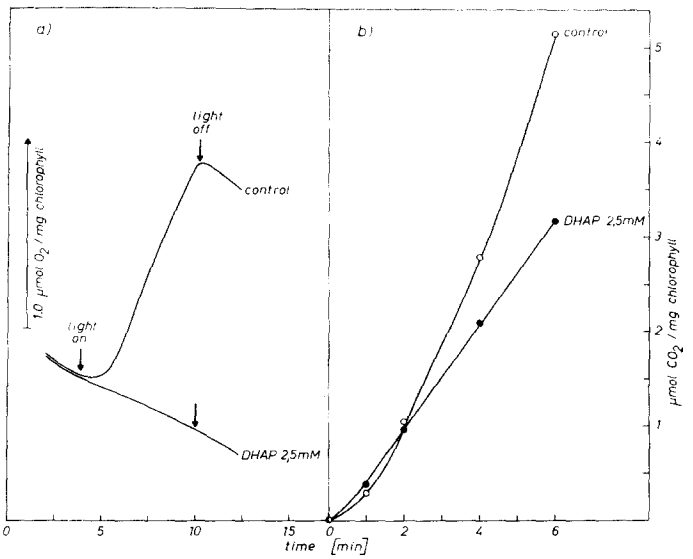

Fig. 1. Effect of 2.5 mM $(OH)_2$acetone-P (a) on O_2 evolution and (b) CO_2 fixation (b). $(OH)_2$ acetone-P was added in the dark period before illumination. For details see methods. DHAP, $(OH)_2$ acetone-P;

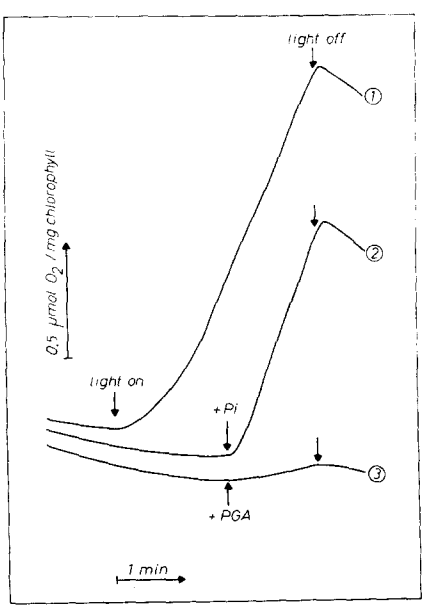

Fig. 2. Influence of P_i and 3-P-glycerate (PGA) on $(OH)_2$acetone-P-inhibited O_2 evolution. P_i and 3-P-glycerate were added in a volume of 20 µl up to a final concentration of 2.5 mM. Trace 1, control; trace 2 and 3, O_2 evolution in presence of 2.5 mM $(OH)_2$acetone-P.

TABLE I

INFLUENCE OF P_i, 3-P-GLYCERATE AND $(OH)_2$ACETONE-P ON THE DISTRIBUTION OF ^{14}C-LABELLED PRODUCTS BETWEEN SUPERNATANT AND CHLOROPLAST PELLET

For details see Methods.

Additions	CO_2-fixation (μmol · mg^{-1} chlorophyll · h^{-1})	Total activity in the supernatant (%)	Total activity in the pellet (%)
–	51	19	81
P_i, 0.25 mM	95	69	31
3-P-glycerate, 0.25 mM	64	71	29
$(OH)_2$ acetone-P, 0.25 mM	98	73	27
$(OH)_2$ acetone-P, 2.50 mM	106	95	5
$(OH)_2$ acetone-P, 2.5 mM + P_i, 2.5 mM	171	89	11
$(OH)_2$ acetone-P, 2.5 mM + 3-P-glycerate 2.5 mM	67	95	5

different conditions (Table I). The results show that under normal conditions, i.e. without addition of either P_i, $(OH)_2$acetone-P or 3-P-glycerate to the reaction medium, about 80 % of the total ^{14}C-labelled products remain in the pellet. At a concentration of $(OH)_2$acetone-P which was shown to inhibit O_2 evolution completely (2.5 mM), 95 % of the ^{14}C-labelled products were present in the supernatant. These results indicate an almost complete transport of ^{14}C-labelled products out of the chloroplast when $(OH)_2$acetone-P at this high concentration is added. We were not able to examine the effect of either P_i or 3-P-glycerate under similar conditions, because both substances inhibit CO_2 fixation of isolated intact chloroplasts at relatively high concentrations [10, 12, 15]. If, however, $(OH)_2$acetone-P is added together with P_i or together with 3-P-glycerate, the total CO_2 fixation is stimulated rather than inhibited (Table I). Therefore, it is possible to investigate the distribution of ^{14}C-labelled products in presence of these substances. If $(OH)_2$acetone-P is added together with 3-P-glycerate, O_2 evolution remains inhibited (Fig. 2) and the distribution of ^{14}C-labelled products is the same as that when only $(OH)_2$acetone-P is present (Table I). If the inhibition of O_2 evolution were due exclusively to a fast exchange of ^{14}C-labelled products for $(OH)_2$acetone-P, one might expect a considerably lower transport of ^{14}C-labelled products out of the chloroplast when the inhibition of O_2 evolution is reversed, i.e. when $(OH)_2$acetone-P is added together with P_i. But as shown in Table I, this is not the case.

Measurement of endogenous cyclic photophosphorylation

Independently of a final interpretation for the inhibition of O_2 evolution by $(OH)_2$acetone-P our results show that CO_2 fixation is able to occur without NADPH and ATP formed by a non-cyclic electron transport, because the loss of reducing equivalents is apparently substituted by $(OH)_2$acetone-P. Under these conditions ATP necessary only for the phosphoribulokinase reaction must be supplied by a pseudo-cyclic or cyclic photophosphorylation (see discussion). Under anaerobic

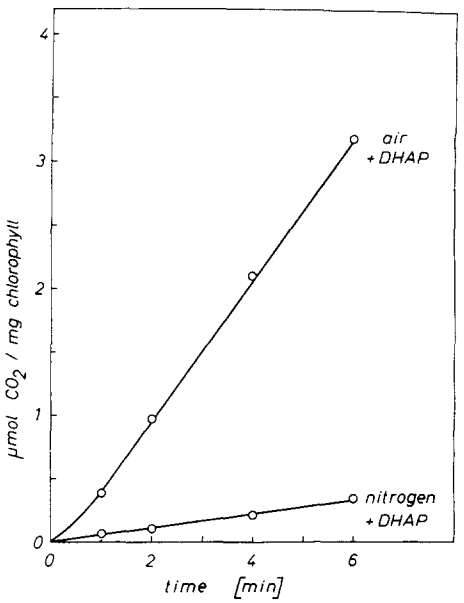

Fig. 3. Time course of CO_2 fixation in presence of 2.5 mM $(OH)_2$acetone-P (DHAP) under aerobic and anaerobic conditions. For details see Methods.

conditions only the latter can take place. Since $(OH)_2$acetone-P prevents O_2 evolution, anaerobic conditions can be maintained even in presence of CO_2. Thus, CO_2 fixation in an atmosphere of N_2 should permit a quantitative measurement of endogenous cyclic photophosphorylation. Fig. 3 shows the time course of CO_2 fixation in presence of 2.5 mM $(OH)_2$acetone-P in air and in N_2. Whereas the rates of this CO_2 fixation in air reach approximately the rates of normal CO_2 fixation without addition of $(OH)_2$acetone-P (e.g. 20–60 μmol · mg chlorophyll^{-1} · h^{-1}), the rates of $(OH)_2$-acetone-P-dependent CO_2 fixation under anaerobic conditions are very low (2.5–7 μmol · mg chlorophyll^{-1} · h^{-1}). It will be shown later that these rates of endogenous cyclic photophosphorylation, indicated by $(OH)_2$acetone-P-dependent CO_2 fixation are limited under these conditions but may be greatly increased.

The effect of DCMU on endogenous cyclic photophosphorylation

Cyclic photophosphorylation of broken chloroplasts with artificial cofactors e.g. with phenazine methosulfate is insensitive to DCMU in concentrations up to 10^{-5} M. Endogenous cyclic photophosphorylation of isolated intact chloroplasts, however, has been recently shown to be nearly completely inhibited by DCMU in concentrations lower than 10^{-5} M in an atmosphere of N_2 and to be much more sensitive to DCMU in far red light under aerobic conditions [6]. Therefore, it was of special interest to examine the effect of DCMU on endogenous cyclic photophosphorylation with the aid of $(OH)_2$acetone-P-dependent CO_2 fixation. As shown in Fig. 4, DCMU inhibits normal CO_2 fixation and $(OH)_2$acetone-P-dependent CO_2 fixation under aerobic conditions to the same extent. In N_2, however, $(OH)_2$acetone-P-dependent CO_2 fixation shows quite a different response to DCMU as it is stimulated

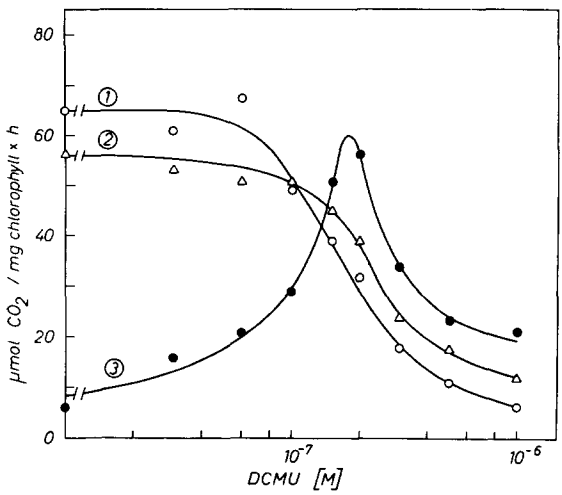

Fig. 4. Rates of CO_2 fixation as influenced by different concentrations of DCMU. 1, control; 2, with 2.5 mM $(OH)_2$acetone-P in air; 3, with 2.5 mM $(OH)_2$acetone-P in N_2.

with increasing concentrations of the inhibitor to a rate similar to the rate of normal CO_2 fixation, e.g. 60 μmol · mg chlorophyll^{-1} · h^{-1} (Fig. 4).

The effect of dibromothymoquinone on endogenous cyclic photophosphorylation

According to Trebst and co-workers [16–18], dibromothymoquinone interrupts the electron transport chain between the two photosystems by acting as an antagonist of plastoquinone. By introducing this inhibitor, the role of plastoquinone in different types of electron transport with artificial electron carriers was comprehensively investigated [16–18]. Nevertheless, its function in the cyclic electron pathway in vivo is still in question. On one hand, dibromothymoquinone was found to inhibit cyclic photophosphorylation in the green alga *Ankistrodesmus braunii* [11], but on the other hand the redox kinetics of cytochrome b_{563}, a component of the cyclic electron pathway in cells of the red alga *Porphyridium* was uneffected by the inhibitor [19, 20]. The experiments of Forti and Rosa [3] suggest that plastoquinone is not involved in the endogenous cyclic photophosphorylation of isolated chloroplasts. Therefore, dibromothymoquinone should not inhibit $(OH)_2$acetone-P-dependent CO_2 fixation at least under anaerobic conditions. In order to examine this prediction, we compared the effect of dibromothymoquinone on normal and $(OH)_2$acetone-P-dependent CO_2 fixation. As seen in Fig. 5, dibromothymoquinone inhibits both types of CO_2 fixation under aerobic conditions to nearly the same extent. In N_2, $(OH)_2$acetone-P-dependent CO_2 fixation seems to be relatively insensitive to dibromothymoquinone, but in contrast to the effect of DCMU, dibromothymoquinone never caused a stimulation of this reaction. As the rate of $(OH)_2$acetone-P-dependent CO_2 fixation is very low under anaerobic conditions, however, it seemed more favourable to study the effect of dibromothymoquinone on $(OH)_2$acetone-P-dependent CO_2 fixation in the presence of $2 \cdot 10^{-7}$ M DCMU. Under these conditions, the rates of normal CO_2 fixation and of $(OH)_2$acetone-P-dependent CO_2 fixation are rather similar (Fig. 4) and thus the effect of dibromothymoquinone on both types of CO_2 fixation is more comparable. Fig. 5

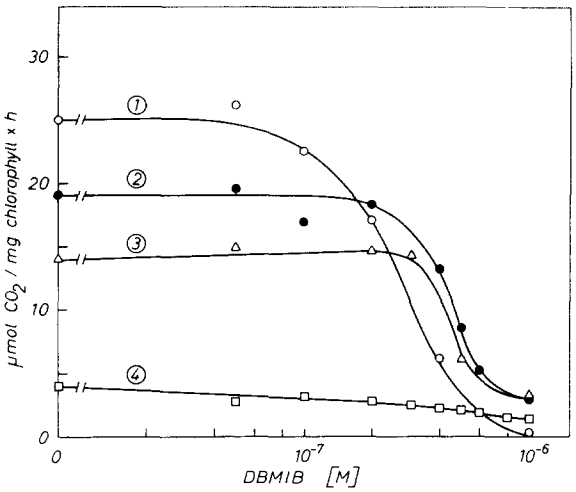

Fig. 5. Rates of CO_2 fixation as influenced by different concentrations of dibromothymoquinone. 1, control; 2, with 2.5 mM $(OH)_2$acetone-P in air; 3, with 2.5 mM $(OH)_2$acetone-P and $2 \cdot 10^{-7}$ M DCMU in N_2; 4, with 2.5 mM $(OH)_2$acetone-P in N_2.

shows that the DCMU-stimulated reaction is inhibited by dibromothymoquinone nearly to the same extent as normal CO_2 fixation.

DISCUSSION

The inhibition of O_2 evolution by $(OH)_2$acetone-P

It has been demonstrated [21–23] that the inner envelope of intact chloroplasts contains a shuttle system, which mediates the counter exchange of P_i, 3-P-glycerate and triose phosphates between the stroma region of the chloroplast and the cytoplasm or the surrounding medium [24]. On the basis of these results the well known inhibition of photosynthetic O_2 evolution and CO_2 fixation of isolated intact chloroplasts by high concentrations of P_i [12] might be explained by the transport of 3-P-glycerate and the triose phosphates out of the chloroplast if P_i is present in the reaction medium. In consequence, the Calvin cycle is depleted of intermediates and breaks down [21]. It might be supposed that the inhibition of CO_2-dependent O_2 evolution in presence of high concentrations of $(OH)_2$acetone-P in the reaction medium is caused by a similar transport of 3-P-glycerate, glyceraldehyde-3-P and P_i out of the chloroplast. In contrast to the inhibition of photosynthesis by P_i, this export of intermediates (mainly 3-P-glycerate?) does not lead to a breakdown of the Calvin cycle because the lost intermediates are compensated for by the import of $(OH)_2$-acetone-P. A model for this concept is given in Fig. 6. It is supported especially by the finding that in presence of $(OH)_2$acetone-P about 95 % of the ^{14}C-labelled intermediates are transported out of the chloroplast. If, however, P_i and $(OH)_2$acetone-P as well as $(OH)_2$acetone-P and 3-P-glycerate compete for the same carrier [23], the addition of P_i together with $(OH)_2$acetone-P should result in an equally complete transport of intermediates out of the chloroplast and therefore should lead to a total inhibition of O_2 evolution. On the other hand, addition of 3-P-glycerate

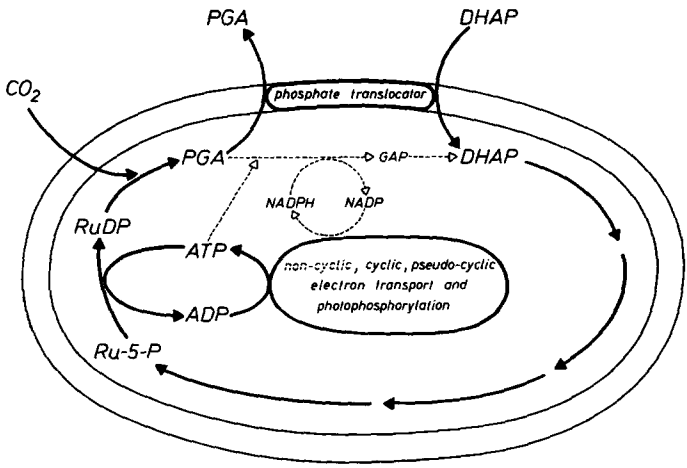

Fig. 6. A proposed model for the inhibition of O_2 evolution by $(OH)_2$acetone-P. The reactions symbolized by dotted arrows do not occur if $(OH)_2$acetone-P is present. Further explanations in the text. DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-P; PGA, 3-P-glycerate; RuDP, ribulose-1,5-diphosphate; Ru-5-P, ribulose-5-phosphate.

should at least partially reverse the inhibition of O_2 evolution by compensating the loss of endogenous 3-P-glycerate. But our results show that the inhibition of O_2 evolution by $(OH)_2$acetone-P is not reversed by addition of 3-P-glycerate, but only by addition of P_i. Therefore, besides a possible counter exchange of 3-P-glycerate and $(OH)_2$acetone-P, an additional explanation for the effect of $(OH)_2$-acetone-P should be taken into account. Recently McLilley and Walker [25] found that ADP inhibits the light dependent reduction of 3-P-glycerate in a reconstituted chloroplast system. If the addition of $(OH)_2$acetone-P in high concentrations caused a fast counter exchange of P_i, the internal concentration of P_i might become rate limiting for photophosphorylation. In consequence the level of ATP in the chloroplast would decrease and the level of ADP would increase. The resulting high level of endogenous ADP might then cause an inhibition of 3-P-glycerate reduction as described [25]. Addition of 3-P-glycerate would then result in a further export of P_i, a further increase of the ADP level and an unchanged inhibition of O_2 evolution. Addition of P_i, however, would increase the level of ATP by stimulating photophosphorylation with a concommitant decrease of the ADP level and thus O_2 evolution should be stimulated. Indeed, preliminary experiments give evidence that addition of $(OH)_2$acetone-P to a suspension of isolated intact chloroplasts causes a decrease of the ATP level and an increase of the ADP level. Therefore, we assume that perhaps both of the proposed mechanisms are involved in the inhibition of O_2 evolution by $(OH)_2$acetone-P.

Measurement of endogenous cyclic photophosphorylation by $(OH)_2$acetone-P-dependent CO_2 fixation

In the presence of $(OH)_2$acetone-P, CO_2 fixation occurs apparently without reduction of 3-P-glycerate, because this reaction is restricted by a fast transport of 3-P-glycerate out of the chloroplast and/or a high level of ADP. In this system, the

ATP for CO_2 fixation might be supplied by different reactions. Recently it was shown [26, 27] that isolated chloroplasts were able to fix CO_2 in the dark, if $(OH)_2$acetone-P and oxaloacetate were added in relatively high concentrations (5 mM). According to a hypothetical scheme proposed by Heldt and co-workers [26, 27], part of the $(OH)_2$acetone-P added might be oxidized to 3-P-glycerate yielding ATP and NADPH in a back-reaction of normal 3-P-glycerate reduction and the remainder might be transformed to ribulose monophosphate. The ATP might be utilized for the formation of ribulose diphosphate from ribulose monophosphate. However, this system seems to work only if the NADPH formed is reoxidized by addition of oxaloacetate. The malate formed by this reaction might leave the stroma in exchange for oxaloacetate via the dicarboxylate translocator [22, 26, 27]. As our system contains no oxaloacetate, oxidation of $(OH)_2$acetone-P should not occur and indeed no $(OH)_2$ acetone-P-dependent CO_2 fixation in the dark could be measured. Thus, in the absence of oxaloacetate, ATP for $(OH)_2$acetone-P-dependent CO_2 fixation can be formed only in the light by different types of endogenous photophosphorylation.

As no 3-P-glycerate reduction occurs in presence of high concentrations of $(OH)_2$acetone-P, ATP should be necessary only for the formation of ribulose diphosphate. Thus, the ATP/CO_2 ratio in our system should not be 3, as expected for the normal Calvin cycle, but about 1. This means that the rates of $(OH)_2$acetone-P-dependent CO_2 fixation reflect directly the rates of different reactions of endogenous photophosphorylation which can take place independently of a non-cyclic electron flow. In air, where a cyclic and/or a pseudo-cyclic phosphorylation proceed, a rate of $(OH)_2$acetone-P-dependent CO_2 fixation of 40–70 μmol · mg chlorophyll^{-1} · h^{-1} indicates that 40–70 μmol ATP · mg chlorophyll^{-1} · h^{-1} are formed by both types of photophosphorylation. Normal CO_2 fixation, which usually occurs with the same rate (compare Fig. 4), requires then 120–210 μmol ATP · mg chlorophyll^{-1} · h^{-1}. Therefore, we can conclude that the capacity of endogenous cyclic and/or pseudo-cyclic photophosphorylation is at least 1/3 of the total phosphorylation capacity of the chloroplast. This value is probably too low, since the capacity of the Calvin cycle might limit the rate of $(OH)_2$acetone-P-dependent CO_2 fixation rather than the capacity of cyclic and pseudo-cyclic photophosphorylation.

From a number of investigations we know that besides NADPH and ATP some other conditions seem to be necessary for an optimal fixation of CO_2, e.g. an efflux of Mg^{2+} from the thylakoids into the stroma [28, 29], an alkaline pH of the stroma which might be produced by an influx of protons from the stroma into the thylakoids [30] and light-activation of enzymes by reduced ferredoxin [31, 32]. As CO_2 can be fixed with reasonable rates without $NADP^+$ reduction, it might be concluded that all the conditions mentioned above are independent of $NADP^+$-reduction and can also be produced by cyclic and pseudo-cyclic electron transport. At present we cannot decide whether cyclic or pseudo-cyclic photophosphorylation is predominant under aerobic conditions. Cyclic photophosphorylation, however, can be measured easily either under anaerobic conditions where no acceptor (O_2) is present for pseudo-cyclic electron transport, or in far-red light, where only Photosystem I is excited. As yet rates of only 5–10 μmol · mg chlorophyll^{-1} · h^{-1} for CO_2 fixation in far-red light have been found presumably because of an insufficient light intensity. The observation that the rates of CO_2 fixation in presence of $(OH)_2$acetone-P are equally low under anaerobic conditions in saturating white light does not necessitate a low

capacity for endogenous cyclic photophosphorylation because these rates are greatly increased by DCMU up to the rate obtained in air without DCMU (60 μmol · mg chlorophyll^{-1} · h^{-1}). We again conclude therefore that the capacity of endogenous cyclic photophosphorylation under anaerobic conditions is also at least 1/3 of the total phosphorylation capacity of the chloroplast. The stimulation of $(OH)_2$acetone-P-dependent CO_2 fixation by DCMU might be due to an "overreduction" of the cyclic electron transport system in strong white light in N_2. This overreduction might be prevented under anaerobic conditions by DCMU in suitable concentrations and in air by O_2, which could draw off electrons from the system by acting as electron acceptor. Because DCMU shows no stimulatory effect on $(OH)_2$acetone-P-dependent CO_2 fixation in air, we conclude that no overreduction takes place under these conditions. Evidence for an overreduction only under anaerobic conditions was obtained also by other authors, e.g. in measuring ferredoxin-catalyzed cyclic photophosphorylation of isolated broken chloroplasts [33] and of a cell-free system of the blue-green alga *Anacystis nidulans* [34] as well as in experiments on chloroplast shrinkage in intact leaves [8]. Furthermore, our results show that DCMU in concentrations higher than $5 \cdot 10^{-7}$ M inhibits DHAP-dependent CO_2 fixation in N_2 nearly as strongly as normal CO_2 fixation. This confirms our earlier finding [6] that endogenous cyclic photophosphorylation of isolated intact chloroplasts is rather sensitive to DCMU. As $(OH)_2$acetone-P-dependent CO_2 fixation is stimulated under anaerobic conditions by DCMU in low concentrations and inhibited by higher concentrations, one can conclude that the cyclic electron transport system needs a properly poised redox state to occur with optimal efficiency. In vivo such a poising might be caused by different factors e.g. light intensity and availability of NADP and O_2.

The participation of plastoquinone in endogenous cyclic photophosphorylation

In contrast to DCMU, dibromothymoquinone never stimulated $(OH)_2$acetone-P-dependent CO_2 fixation under anaerobic conditions, i.e. the inhibitor apparently could not prevent the overreduction described above. Since dibromothymoquinone interrupts electron flow between the two photosystems like DCMU, this result can be explained only, if dibromothymoquinone inhibits also cyclic electron transport. Further evidence for the inhibition of endogenous cyclic electron transport by dibromothymoquinone comes from the finding that DCMU-stimulated CO_2 fixation in the presence of $(OH)_2$acetone-P under anaerobic conditions is blocked by this inhibitor similar to normal CO_2 fixation. In agreement with earlier results obtained in vivo [11] we therefore suppose that plastoquinone is an essential component also of the endogenous cyclic electron transport chain of isolated intact chloroplasts.

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