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ROLE OF CYCLIC PHOTOPHOSPHORYLATION IN PHOTOSYNTHETIC CARBON DIOXIDE ASSIMILATION BY ISOLATED CHLOROPLASTS

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SUMMARY

The role of cyclic photophosphorylation in photosynthetic CO_2 assimilation was investigated in isolated, intact chloroplasts capable of high rates of CO_2 fixation. ATP produced by endogenous cyclic photophosphorylation was found to play an important role in shortening the lag period in CO_2 assimilation and in the formation of sugar phosphates. Inhibition of either cyclic or noncyclic photophosphorylation severely lowered total CO_2 fixation but gave contrasting patterns of products formed: increased sugar phosphates and decreased phosphoglycerate when noncyclic photophosphorylation was inhibited and decreased sugar phosphates and increased phosphoglycerate when cyclic photophosphorylation was inhibited. Antimycin A, which inhibits ferredoxin-catalyzed cyclic photophosphorylation in broken chloroplasts, was also found to inhibit endogenous cyclic photophosphorylation in intact chloroplasts with a resultant decrease in total CO_2 assimilation and the characteristic shift toward increased phosphoglycerate and decreased sugar phosphate formation. The addition of ATP to chloroplasts inhibited by antimycin A quadrupled the rate of CO_2 fixation and restored the products to their original pattern. These results support the view that the ATP produced by cyclic photophosphorylation is essential in sustaining a high rate of CO_2 assimilation and maintaining a high ATP:NADPH ratio that favors the conversion of phosphoglycerate to carbohydrate.

INTRODUCTION

Interest in the origin of ATP in photosynthesis was aroused when the intermediates of CO_2 assimilation were found to be phosphorylated[1-4]—an indication that they were probably formed by reactions involving ATP. The phosphorylated compounds were isolated from whole cells[1-4]; the requisite ATP was thought to be produced by the oxidation of photochemically generated reductants with molecular O_2 (ref. 4). A contemporary cellular model for such a process appeared to be a collaboration between chloroplasts and mitochondria[5].

A different concept of the origin of ATP in photosynthesis began to emerge after isolated chloroplasts were found[6] to assimilate CO_2 and to form phosphorylated intermediates[7] similar to those observed in whole cells. The ATP needed for CO_2

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2 ethanesulfonic acid.

assimilation by chloroplasts appeared to come from a new cyclic photophosphorylation process localized in chloroplasts and independent of mitochondria, O_2, or any external electron donor or acceptor[6,8–10]. The source of photosynthetic ATP was reassessed when a second (noncyclic) type of photophosphorylation was discovered, in which ATP formation was stoichiometrically coupled with the photoreduction of NADP (ref. 11). Since noncyclic photophosphorylation supplied both ATP and the requisite reductant, NADPH, and was, like CO_2 assimilation itself, accompanied by O_2 evolution, it seemed clearly to be the main source of assimilatory power in photosynthesis[11,12]. Cyclic photophosphorylation was now viewed as a reaction[11] that only supplemented the ATP requirement for CO_2 assimilation and that, in the main, supplied ATP for other ATP-dependent processes (*e.g.* protein synthesis). Alternatively, cyclic photophosphorylation came to be regarded as an artifact, peculiar to isolated chloroplasts[13].

Later work disposed of the possibility that cyclic photophosphorylation was an experimental artifact. In intact cells, cyclic photophosphorylation was found to supply ATP for photokinesis[14,15], the incorporation of ^{32}P into cellular constituents[16,17] and for anaerobic photoassimilation of glucose[18–20] or acetate[21,22]. Cyclic photophosphorylation was observed in leaves[23] and, more recently, as an endogenous process in isolated, intact chloroplasts[24,25]. Evidence has also been obtained for a role of cyclic photophosphorylation in protein synthesis[26] and ion uptake[27–29].

Although the physiological nature of cyclic photophosphorylation is no longer in doubt, there is still a divergence of views concerning its involvement in CO_2 assimilation. A need for cyclic photophosphorylation stems from the requirement of 3 moles of ATP and 2 of NADPH for the assimilation of 1 mole of CO_2 to the level of carbohydrate[30] — a requirement that cannot be met by noncyclic photophosphorylation alone which produces ATP and NADPH in a ratio of 1 to 1 (ref. 11, 31–34).

The 1:1 ratio of ATP:NAPDH in noncyclic photophosphorylation was reexamined and reconfirmed in a recent study[35] but other investigators[36–39] reported ATP:NADPH (or ATP:O_2) ratios greater than 1. Such ratios could account for an excess of ATP over NADPH without an ATP contribution from cyclic photophosphorylation. Furthermore, Kandler and co-workers[40–42] concluded from experiments with algal cells that there was no direct relationship between cyclic photophosphorylation (measured as glucose uptake) and CO_2 assimilation since the two processes differed in light saturation and in the degree of sensitivity to certain inhibitors. Another possibility, *i.e.*, that CO_2 assimilation may depend not on the ATP produced by cyclic photophosphorylation but on a postulated high-energy precursor(s), was suggested by Urbach and Gimmler[17] from experiments with algal cells and by Champigny and Miginiac-Maslow[43,44] from experiments with chloroplasts.

To resolve these conflicting views, evidence has been sought for a direct role of cyclic photophosphorylation in photosynthetic CO_2 assimilation by isolated, intact chloroplasts[45] capable of high rates of CO_2 assimilation (25–50 % of the rates in intact leaves). Earlier experiments with broken chloroplasts have already shown that appreciable sugar phosphates were formed only when both cyclic and noncyclic photophosphorylation operated in a proper balance[46]. The new evidence reported here shows that the extra ATP generated by cyclic photophosphorylation shortens the lag period in CO_2 assimilation by chloroplasts and enhances the formation of sugar phosphates relative to 3-phosphoglyceric acid.

METHODS

Chloroplasts were isolated in sorbitol[45] from freshly harvested spinach leaves (*Spinacea oleracea*, var. Viroflay) grown in a nutrient solution in a greenhouse. As estimated by phase contrast microscopy[47,48], 60 to 90 % of the chloroplasts isolated by this procedure had intact outer membranes.

Experiments with $^{14}CO_2$ were carried out as described previously[45]. $^{12}CO_2$ assimilation by illuminated chloroplasts was measured by the accompanying O_2 evolution[49,50]. Actinic beams of monochromatic light of 664 and 720 nm were isolated as previously described[51]. O_2 evolution was measured separately for each treatment. To insure stability of cloroplasts they were stored in darkness at 0° as a pellet[52] and were resuspended and gassed with Ar (5 min) prior to measuring O_2 evolution.

3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), *o*-phenanthroline, and salicylaldoxime were added in aqueous solution, whereas antimycin A and oligomycin were added in methanol solution in a volume not exceeding 20 μl. Additions of methanol at this level had no measurable effect on either CO_2 assimilation or photophosphorylation; higher levels lowered the rate of CO_2 assimilation but gave no consistent change in products.

To identify the products of $^{14}CO_2$ fixation, 5 to 30 μl of acidified reaction mixture was spotted on thin-layer plates prepared with cellulose powder[53]. The plates were developed in one dimension by electrophoresis and in the second dimension by chromatography[53] and then exposed to X-ray film to locate the individual ^{14}C-labeled products. The ^{14}C-labeled compounds formed by chloroplasts (except for sedoheptulose phosphates) were identified by co-chromatography with the authentic compounds; coincidence of the radioactive spot with the spot of the unlabeled authentic compound established the identity of the radioactive compound. Phosphate compounds were located by Hanes–Isherwood spray[54]; amino acids by ninhydrin spray (1 mg ninhydrin per ml ethanol); and organic acids by an acid indicator (2',7-dichlorofluorescein) added to the chromatography solvent[55]. Sedoheptulose phosphates were identified as the free sugars after removal of the phosphate group by enzymic hydrolysis whit wheat germ acid phosphatase.

Radioactivity in products was counted in a scintillation counter. The ^{14}C-labeled products were removed from the plates with a razor blade (the accompanying cellulose powder had no effect on counting efficiency) and placed in a vial containing 0.5 ml water and 10 ml of the scintillation mixture[56] [150 g naphthalene, 10.5 g 2,5-diphenyloxazole (PPO), 450 mg 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene (dimethyl-POPOP), dioxane to give a vol. of 1500 ml, and water to a final vol. of 1800 ml].

RESULTS AND DISCUSSION

Effect of antimycin A on endogenous cyclic photophosphorylation

Before investigating the effect of cyclic photophosphorylation on CO_2 assimilation it was deemed desirable to establish that this process was actually occurring endogenously in the intact chloroplast preparations used in the CO_2 assimilation experiments. Evidence for endogenous cyclic photophosphorylation in our chloroplast preparations was obtained with the use of antimycin A; an inhibitor which was found by Tagawa *et al.*[57] to inhibit ferredoxin-catalyzed cyclic photophosphorylation in

chloroplasts at concentrations at which noncyclic photophosphorylation or cyclic photophosphorylation with other catalysts remained unaffected. The sensitivity of ferredoxin-catalyzed cyclic photophosphorylation in chloroplasts to antimycin A was confirmed by Izawa *et al.*[58] and Drechsler *et al.*[59] but these authors also reported an inhibition of noncyclic photophosphorylation under special conditions, *e.g.* low light intensity[58]. The significance of antimycin A as an inhibitor is two-fold: (i) in isolated chloroplasts it inhibits at selected low concentrations only the ferredoxin-catalyzed cyclic photophosphorylation[60,61] (a conclusion also reached recently on the basis of independent evidence by Böhme *et al.*[62]) and (ii) in whole cells it selectively inhibits processes requiring only ATP, *i.e.* processes that can be supported solely by cyclic photophosphorylation[14,16,19,20]. For these and other reasons discussed elsewhere[61] ferredoxin is considered to be the endogenous catalyst of cyclic photophosphorylation in chloroplasts.

Fig. 1 shows that 50% inhibition of the endogenous cyclic photophosphorylation in intact chloroplasts was produced by $1.2 \cdot 10^{-5}$ M antimycin A. This concentration of antimycin A was about 100 times greater than that used to give 50% inhibition of cyclic photophosphorylation in disrupted chloroplasts (see Fig. 6 in ref. 61). The low rate of endogenous ATP formation in the absence of an inhibitor (compare ref. 24) is attributed to a slow turnover of ADP and a permeability barrier to adenylates in intact chloroplasts. Much higher rates of cyclic photophosphorylation would be expected to occur when ADP turnover is stimulated by ATP utilization in

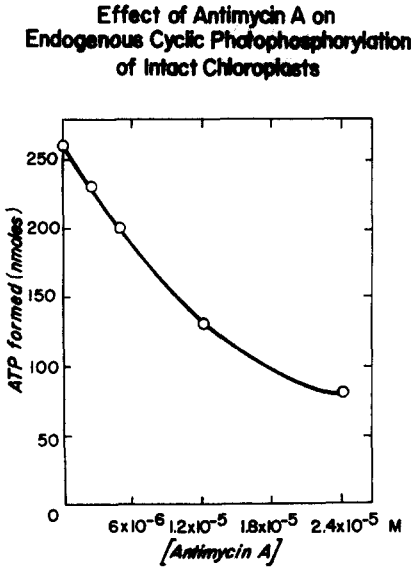

Fig. 1. Effect of antimycin A on endogenous cyclic photophosphorylation of intact chloroplasts. The reaction mixture (final vol. 1 ml) contained chloroplasts equivalent to 0.3 mg chlorophyll; 0.05 mg yeast hexokinase (Type III, Sigma Chemical Co.); 0.02 M glucose; 0.3 M sucrose; 0.05 M HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.6; $1.7 \cdot 10^{-4}$ M isoascorbic acid; $5 \cdot 10^{-5}$ M *o*-phenanthroline; 0.0025 M $^{32}P_i$; 0.001 M ADP; 0.003 M $MgCl_2$; and antimycin A as indicated. The reaction was carried out for 30 min in Warburg vessels equilibrated with argon; temperature, 20°; light intensity, 30000 lux. The reaction was stopped and the newly formed ATP (trapped as glucose 6-phosphate) was isolated and counted as described previously[57].

the course of CO_2 assimilation. Likewise, osmotically disrupted spinach chloroplasts with lower permeability barriers and an unimpaired capacity for ATP accumulation in the external medium gave rates of ferredoxin-catalyzed cyclic photophosphorylation of about 180 μmoles ATP per mg chlorophyll per h (ref. 60).

Effect of ATP and cyclic photophosphorylation on the lag period in CO_2 assimilation by chloroplasts

When light-dependent CO_2 assimilation by isolated chloroplasts was discovered, a lag period was found to precede the onset of linear CO_2 fixation[6,7]. The lag period could be shortened either by preillumination[63,52] or by the addition of certain intermediates of the reductive pentose phosphate cycle[63–66] (*e.g.* ribose 5-phosphate or fructose 1,6-diphosphate).

The effectiveness of phosphorylated intermediates on shortening the lag period suggested that the lag period might be related to a need for ATP. This was found to be the case. Fig. 2 shows that the lag period preceeding a linear rate of CO_2 assimilation was markedly shortened by the addition of ATP. ATP could not be replaced by AMP.

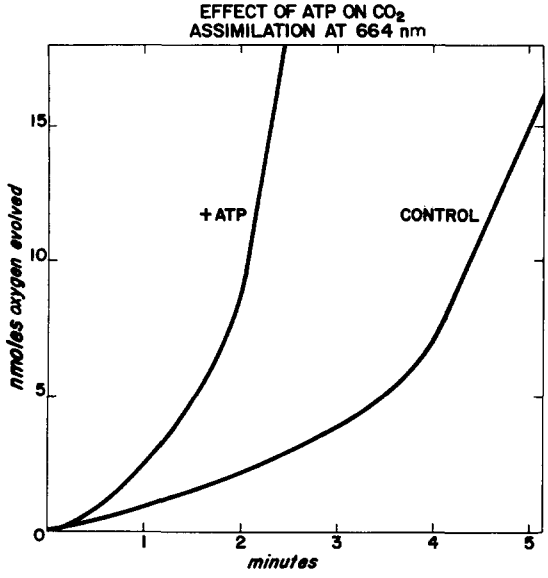

Fig. 2. Effect of ATP on the lag period in CO_2 assimilation by chloroplasts illuminated with monochromatic light at 664 nm. The reaction mixture (vol. 2.5 ml) contained chloroplasts equivalent to 0.13 mg chlorophyll per ml; 0.3 M sorbitol; 0.05 M HEPES buffer, pH 7.6; $1.7 \cdot 10^{-4}$ M isoascorbic acid; 0.004 M sodium pyrophosphate, pH 7.6; 0.0067 M $KHCO_3$. ATP, 0.002 M, was added where indicated. The reaction was carried out in an oxygen electrode[49,50] and was followed by measuring O_2 evolution. Temperature, 25°; gas phase, Ar; light intensity, $2.5 \cdot 10^4$ ergs/cm^2 per sec.

Since ATP alone was effective in shortening the lag period of photosynthesis, it seemed likely that a similar effect could be produced by cyclic photophosphorylation in intact chloroplasts. This possibility was tested by overcoming the lag period with preillumination at a long wavelength of monochromatic light that can effectively

support cyclic but not noncyclic photophosphorylation[60]. The selected wavelength, 720 nm, which, unlike 664 nm, could not by itself support appreciable CO_2 assimilation (Fig. 3) was found to be as effective as 664-nm light in overcoming the lag period in CO_2 assimilation (Fig. 4). The effectiveness of either 720-nm light or of

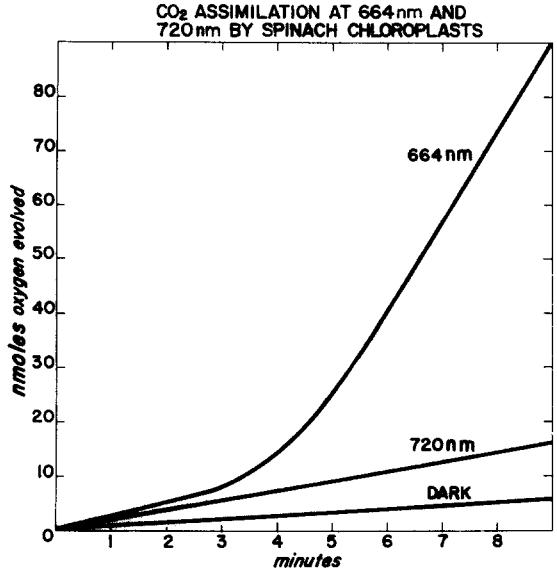

Fig. 3. CO_2 assimilation by intact spinach chloroplasts in red (664 nm) and far-red (720 nm) monochromatic light. Light intensity for each wavelength was $2.5 \cdot 10^4$ ergs/cm^2 per sec. Other experimental conditions were as described for the control treatment in Fig. 2.

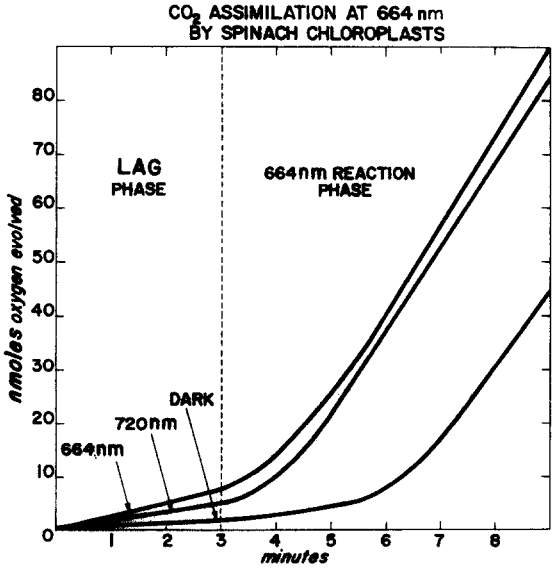

Fig. 4. Equivalence of red and far-red monochromatic light during the lag period of CO_2 assimilation by chloroplasts. For the first 3 min (lag period) chloroplasts were kept in the dark or illuminated by 664- or 720-nm light. After 3 min chloroplasts from each of the three previous treatments were illuminated with 664-nm light. Experimental conditions were as given for Fig. 3.

added ATP suggests that the lag period in CO_2 assimilation results from a need for extra ATP which is contributed by cyclic photophosphorylation. Since the lag in CO_2 assimilation can also be shortened by adding phosphorylated intermediates of the carbon cycle[63–66], it would appear that one role of cyclic photophosphorylation is to supply the ATP needed to form these intermediates at the beginning of photosynthetic activity that follows a period of darkness.

Products of photosynthetic CO_2 assimilation

Aside from initiation of photosynthetic activity, cyclic photophosphorylation was also found to influence the pattern of the products of $^{14}CO_2$ assimilation. Fig. 5 shows a radioautograph of the soluble products formed from $^{14}CO_2$ by the illuminated intact chloroplasts used in our experiments. The thin-layer electrophoresis-chromatography technique gave an especially sharp separation of the individual products which were similar to those found originally with saline chloroplasts[7] and later with various other chloroplast preparations[46, 48, 52, 64, 67]. At no time did we observe sucrose, which has been reported[68] to be a seasonal product in spinach chloroplasts.

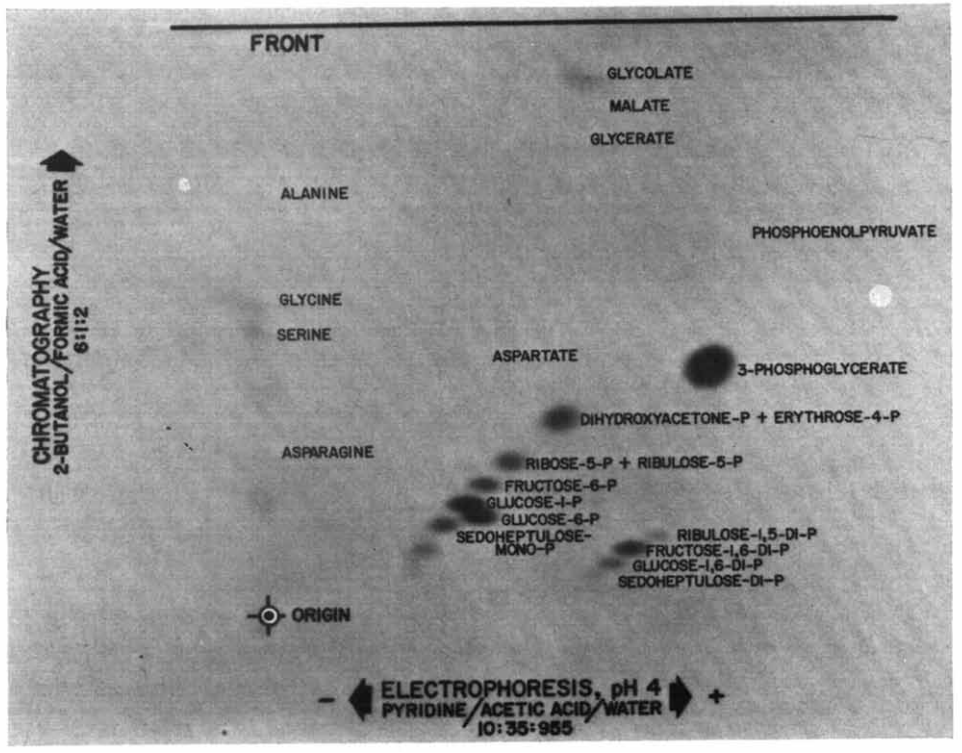

Fig. 5. Radioautograph of $^{14}CO_2$ assimilation by isolated chloroplasts. Products were separated by thin-layer electrophoresis–chromatography technique (see Methods). The reaction was carried out in Warburg vessels, the main compartment of which contained chloroplasts equivalent to 0.2 mg chlorophyll and 1.4 ml of the reaction mixture described for Fig. 2 except that 0.1 ml $KH^{14}CO_3$ (100000 counts/min per μmole) (added from side-arm) replaced $KHCO_3$. Temperature, 20°; gas phase, air. Vessels were preilluminated (yellow light, 10000 lux) for 3 min prior to adding the [^{14}C]bicarbonate to start the reaction. The reaction was stopped after 12 min by adding 0.1 ml of 1 M HCl.

The distribution of radioactivity among the soluble products formed after 12-min illumination (Table I) indicated that about 90 % of it was accounted for by 3-phosphoglyceric acid and sugar phosphates (dihydroxyacetone phosphate, sugar mono- and diphosphates). Accordingly, in later experiments the small amount of radioactivity in other compounds was disregarded and total $^{14}CO_2$ fixation was expressed as the sum of 3-phosphoglyceric acid and sugar phosphates formed.

TABLE I

DISTRIBUTION OF ^{14}C IN PHOTOSYNTHETIC PRODUCTS FORMED BY CHLOROPLASTS

Experimental conditions were as described for Fig. 5.

Products	% *of total activity*	*Products*	% *of total activity*
3-phosphoglyceric acid	36.6	Unknown compound	0.2
Dihydroxyacetone phosphate	10.1	Fructose di-phosphate	4.3
Glucose 6-phosphate	9.9	Glucose di-phosphate	1.6
Glucose 1-phosphate	8.4	Ribulose di-phosphate	0.7
Fructose 6-phosphate	3.2	Sedoheptulose di-phosphate	0.7
Ribose 5-phosphate + ribulose 5-phosphate	3.8	Diphosphate, unidentified	0.5
		Serine + glycine	2.1
Sedoheptulose monophosphate	2.3	Alanine	0.7
Monophosphates, unidentified	2.3	Aspartate	0.4
Glycolate	0.9	Asparagine	0.5
Malate	0.7	Compounds 1 and 2	1.7
Phosphoenolpyruvate	0.2	Insoluble products	8.1

Effect of ATP on CO_2 assimilation

Since cyclic photophosphorylation would affect the pattern of CO_2 assimilation by increasing the supply of ATP, the effect of extra ATP was first investigated by adding ATP to the reaction mixture prior to turning on the light. As shown in Table II, the addition of ATP markedly altered the pattern of photosynthetic products formed by chloroplasts: the relative proportion of sugar phosphates was increased, whereas that of 3-phosphoglyceric acid was halved. The overall rate of photosynthesis, measured by total $^{14}CO_2$ fixation, was not affected by added ATP but in certain other experiments a similar change in the pattern of products was accompanied by an increase (up to 80 %) in the total CO_2 assimilation.

TABLE II

EFFECT OF ADDED ATP ON $^{14}CO_2$ ASSIMILATION BY SPINACH CHLOROPLASTS

ATP, 0.002 M, added where indicated. Other experimental conditions were as given for Fig. 5. Rates of fixation in the control and +ATP treatments were, respectively, 26.6 and 27.0 μmoles CO_2 fixed per mg chlorophyll per h.

Products formed	% *of total* $^{14}CO_2$ *fixed*	
	Control	*+ATP*
3-phosphoglyceric acid	44	24
Sugar phosphates	56	76

These results suggested that cyclic photophosphorylation, by increasing the supply of ATP, shifts the pattern of CO_2 assimilation toward sugar phosphates. Conversely, a decrease in the supply of ATP would be expected to produce an increase in 3-phosphoglyceric acid and a decrease in sugar phosphates. This conclusion was tested by inhibiting cyclic photophosphorylation.

Effect of inhibitors of cyclic photophosphorylation on CO_2 assimilation

Two inhibitors of ferredoxin-catalyzed cyclic photophosphorylation[61], oligomycin and antimycin A, were used and each inhibited total CO_2 assimilation and influenced the pattern of products of the residual CO_2 fixation in a manner opposite to that of added ATP, as shown in Table II. At $1.2 \cdot 10^{-5}$ M antimycin A, the percentage of $^{14}CO_2$ fixed as 3-phosphoglyceric acid was doubled and that of sugar phosphates decreased by about 30 % (Table III). Similar results were obtained with oligomycin (Table IV).

Table V shows that both the shift in products due to antimycin A and the parallel decrease of the rate of CO_2 assimilation were markedly reversed by the addition of ATP. ATP, added in the presence of antimycin A, quadrupled the rate of CO_2 assimilation and restored the pattern of products to that observed in the absence of antimycin A. AMP was totally ineffective in replacing ATP.

TABLE III

EFFECT OF ANTIMYCIN A ON $^{14}CO_2$ ASSIMILATION BY CHLOROPLASTS

Experimental conditions were as given for Fig. 5. The control treatment gave a rate of 26.6 μmoles CO_2 fixed per mg chlorophyll per h.

[*Antimycin A*] (*M*)	*Inhibition of total $^{14}CO_2$ fixation* (%)	*$^{14}CO_2$ fixed as*	
		3-phospho-glyceric acid (% *of total*)	*Sugar phosphates*
0	0	21	79
$6 \cdot 10^{-6}$	14	45	55
$1.2 \cdot 10^{-5}$	27	46	54
$2.4 \cdot 10^{-5}$	70	39	61

TABLE IV

EFFECT OF OLIGOMYCIN ON $^{14}CO_2$ ASSIMILATION BY CHLOROPLASTS

Experimental conditions were as given for Fig. 5. The control treatment gave a rate of 42.0 μmoles CO_2 fixed per mg chlorophyll per h.

[*Oligomycin*] (*M*)	*Inhibition of total $^{14}CO_2$ fixation* (%)	*$^{14}CO_2$ fixed as*	
		3-phospho-glyceric acid (% *of total*)	*Sugar phosphates*
0	0	24	76
$4.2 \cdot 10^{-5}$	9	27	73
$8.4 \cdot 10^{-5}$	29	33	67
$8.4 \cdot 10^{-4}$	73	37	63

TABLE V

REVERSAL BY ATP OF THE EFFECT OF ANTIMYCIN A ON $^{14}CO_2$ ASSIMILATION BY CHLOROPLASTS

Experimental conditions were as given for Fig. 5. The control treatment gave a rate of 30.8 μmoles CO_2 fixed per mg chlorophyll per h.

Additions to control	*Relative rate of CO_2 assimilation*	*$^{14}CO_2$ fixed as*	
		3-phospho-glyceric acid (% of total)	*Sugar phosphates*
None	100	36	64
ATP (0.003 M)	131	22	78
Antimycin A ($4.8 \cdot 10^{-6}$ M)	11	49	51
Antimycin A, ATP	47	36	64

Tables II to V show that inhibition by antimycin A and the relative percentages of $^{14}CO_2$ fixed as 3-phosphoglyceric acid and sugar phosphates varied from experiment to experiment, reflecting probably the different physiological status of chloroplasts isolated on different days from a different sample of leaves. What remained constant in every experiment was the characteristic shift in products: more sugar phosphates and less 3-phosphoglyceric acid in treatments in which ATP was added (or cyclic photophosphorylation allowed to proceed) and less sugar phosphates and more 3-phosphoglyceric acid in treatments in which cylcic photophosphorylation was inhibited and ATP was not added.

The sensitivity of CO_2 assimilation by intact chloroplasts to antimycin A and oligomycin and the observed shift in the pattern of products (reversible by added ATP) supports the view that the ATP formed by cyclic photophosphorylation has an essential function in photosynthetic carbon assimilation which, under physiological conditions, is directed toward the synthesis of sugar phosphates from which mono-, di- and polysaccharides are formed as the final products of photosynthesis. The inhibitory effects of antimycin A and oligomycin on CO_2 assimilation by intact chloroplasts are also consistent with the role assigned to ferredoxin as the physiological catalyst of cyclic photophosphorylation[61,69].

That antimycin A causes a shift among the products of CO_2 assimilation by isolated chloroplasts toward increased 3-phosphoglyceric acid formation was previously reported by Champigny and Gibbs[70] who observed an increase of up to 67 % in the relative amount of 3-phosphoglyceric acid formed in the presence of $5 \cdot 10^{-6}$ M antimycin A. However, contrary to the inhibition by antimycin A of total CO_2 assimilation shown in Table III and the 32 % inhibition of CO_2 fixation by 10^{-5} M antimycin A observed earlier by Bamberger *et al.*[71], Champigny and Gibbs[70] found that antimycin A greatly stimulated the rate of CO_2 assimilation.

It is possible that the increase in the rate of CO_2 assimilation observed in the presence of antimycin A was due to the high light intensity (20000 lux) used in those experiments[70]. To test this possibility we prepared chloroplasts in sorbitol and salt as described by Champigny and Gibbs[70] and found that $5 \cdot 10^{-6}$ M antimycin A consistently inhibited CO_2 assimilation at 10000 lux but increased it (up to 20 %) at 30000 lux. To obtain an inhibition of CO_2 assimilation at 30000 lux, higher concentrations of antimycin A were required.

Kandler and Tanner[40] and Tanner *et al.*[41] have used inhibition by salicylaldoxime as evidence that cyclic photophosphorylation is not involved in CO_2 assimilation. Under their conditions, the photoassimilation of glucose by algal cells was more sensitive to salicylaldoxime than was CO_2 assimilation. In the present study salicylaldoxime inhibited CO_2 assimilation by intact spinach chloroplasts in a pattern similar to that of antimycin A: an inhibition of total CO_2 fixation, accompanied by an increase in 3-phosphoglyceric acid and a decrease in sugar phosphates (Table VI).

TABLE VI

EFFECT OF SALICYLALDOXIME ON $^{14}CO_2$ ASSIMILATION BY CHLOROPLASTS

Experimental conditions were as given for Fig. 5. The control treatment gave a rate of 28 μmoles CO_2 fixed per mg chlorophyll per h.

[Salicylaldoxime] (*M*)	*Inhibition of total $^{14}CO_2$ fixation* (%)	$^{14}CO_2$ *fixed as*	
		3-phospho-glyceric acid (% *of total*)	*Sugar phosphates*
0	0	20	80
$5 \cdot 10^{-4}$	22	52	48
$1 \cdot 10^{-3}$	44	64	36
$2.5 \cdot 10^{-3}$	88	60	40

TABLE VII

EFFECT OF DCMU ON $^{14}CO_2$ ASSIMILATION BY CHLOROPLASTS

Experimental conditions were as given for Fig. 5. The control treatment gave a rate of 45.0 μmoles CO_2 fixed per mg chlorophyll per h.

[DCMU] (*M*)	*Inhibition of total $^{14}CO_2$ fixation* (%)	$^{14}CO_2$ *fixed as*	
		3-phospho-glyceric acid (% *of total*)	*Sugar phosphates*
0	0	31	69
$2.5 \cdot 10^{-7}$	32	14	86
$5 \cdot 10^{-7}$	64	13	87
$7.5 \cdot 10^{-7}$	76	16	84

Effect of inhibitors of noncyclic photophosphorylation on CO_2 assimilation by chloroplasts

The characteristic pattern of inhibition exhibited by inhibitors of cyclic photophosphorylation was compared with the effects of inhibitors of noncyclic photophosphorylation. As already noted by other investigators[19,26,71], DCMU strongly inhibited CO_2 assimilation, *i.e.*, 64% inhibition at $5 \cdot 10^{-7}$ M (Table VII). Of special interest was that DCMU effected a shift in the products of CO_2 assimilation by chloroplasts in a pattern opposite to that shown by the inhibitors of cyclic photophosphorylation: the percent of $^{14}CO_2$ fixed as 3-phosphoglyceric acid decreased and that fixed as sugar phosphates increased. *o*-Phenanthroline, another inhibitor of noncyclic

photophosphorylation, behaved like DCMU (Table VIII): $1 \cdot 10^{-5}$ M *o*-phenanthroline inhibited CO_2 assimilation 53 % and caused a shift in products toward less 3-phosphoglyceric acid and more sugar phosphates.

TABLE VIII

EFFECT OF *o*-PHENANTHROLINE ON $^{14}CO_2$ ASSIMILATION BY CHLOROPLASTS

Experimental conditions were as given for Fig. 5. The control treatment gave a rate of 34.8 μmoles CO_2 fixed per mg chlorophyll per h.

[o-Phenanthroline] (M)	*Inhibition of total $^{14}CO_2$ fixation (%)*	*$^{14}CO_2$ fixed as*	
		3-phospho-glyceric acid (% of total)	*Sugar phosphates*
0	0	23	77
$5 \cdot 10^{-6}$	26	19	81
$1 \cdot 10^{-5}$	53	17	83
$5 \cdot 10^{-5}$	81	13	87

CONCLUDING REMARKS

The results of this investigation support the view that cyclic photophosphorylation is needed for CO_2 assimilation by chloroplasts. CO_2 assimilation was strongly inhibited by inhibitors of ferredoxin-catalyzed cyclic photophosphorylation such as antimycin A at concentrations similar to those that inhibited endogenous cyclic photophosphorylation in intact chloroplasts.

The finding, that inhibitors of either cyclic or noncyclic photophosphorylation severely inhibit total CO_2 assimilation but affect the pattern of photosynthetic products differently, supports the view that this pattern is governed by the ratio of ATP to NADPH. The percent of $^{14}CO_2$ fixed as 3-phosphoglyceric acid increased and that fixed as sugar phosphates decreased when cyclic photophosphorylation was inhibited, *i.e.* when a lower ratio of ATP to NADPH would be expected. Conversely, the percentage of $^{14}CO_2$ fixed as sugar phosphates increased when noncyclic photophosphorylation was inhibited, *i.e.* when a decrease in NADPH (produced only by noncyclic photophosphorylation) would result in a higher ratio of ATP to NADPH. Such a differential effect of the two types of inhibitors suggests that, under physiological conditions, the ATP produced by cyclic photophosphorylation supplements the ATP produced by noncyclic photophosphorylation and thereby promotes conversion of 3-phosphoglyceric acid to sugar phosphates.

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