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Regulation of ferredoxin-catalyzed photosynthetic phosphorylations

(photophosphorylation/photosynthesis/energy conversion)

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ABSTRACT **Under aerobic conditions that are likely to prevail in chloroplasts *in vivo*, the optimal concentration of ferredoxin for cyclic photophosphorylation was found to be equal to that required for NADP reduction and about one-tenth of that needed for cyclic photophosphorylation under anaerobic conditions. In the presence of ferredoxin and NADP, cyclic photophosphorylation operated concurrently with noncyclic photophosphorylation, producing an ATP: NADPH ratio of about 1.5. The effective operation of ferredoxin-catalyzed cyclic photophosphorylation by itself required a curtailment of the electron flow from water which was accomplished experimentally by the use of either an inhibitor or far-red monochromatic light. An unexpected discovery was that the operation of cyclic photophosphorylation by itself was also regulated by a back reaction of NADPH and ferredoxin with two components of chloroplast membranes, component C550 and cytochrome b_{559}. The significance of these findings to photosynthesis *in vivo* is discussed.**

Solar energy is first converted into biologically useful chemical energy by photosynthetic phosphorylation (photophosphorylation), the process by which the photosynthetic apparatus transforms the electromagnetic energy of sunlight into phosphate bond energy of ATP, the universal energy carrier of living cells. The energy of the photochemically generated ATP and reducing power is conserved through the biosynthesis of organic compounds from CO_2. When these are later degraded by fermentation and respiration, reducing power and ATP are regenerated to drive the multitude of endergonic reactions and activities of living cells.

Chloroplasts, the photosynthetic organelles of green plants, have two types of photophosphorylation, cyclic and noncyclic, names devised to denote the coupling of ATP formation to a light-induced electron flow that is either of a closed (cyclic) type that yields only ATP or of a unidirectional (noncyclic) type that yields not only ATP but also NADPH as reducing power (1). Ferredoxin plays a key role in both. In cyclic photophosphorylation ferredoxin is the endogenous catalyst (2, 3) and in noncyclic photophosphorylation ferredoxin is the electron acceptor (4) that in turn reduces NADP by an enzymatic reaction that is independent of light (5).

In this paper we report the concurrent operation of ferredoxin-dependent cyclic and noncyclic photophosphorylation under conditions that are likely to exist in chloroplasts *in vivo*. These experiments led to the discovery that noncyclic electron flow provides a regulatory mechanism that permits cyclic photophosphorylation to operate either concurrently with the noncyclic type (and thereby to increase the ratio of ATP to NADPH as needed for photosynthetic CO_2 assimilation) or to operate by itself and produce only ATP for those endergonic reactions that do not require photochemically generated reducing power.

Abbreviations: Tricine, *N*-tris(hydroxymethyl)methylglycine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

METHODS

Chloroplasts. Whole chloroplasts were isolated from spinach leaves (var. Resistoflay) essentially as described by Kalberer *et al.* (6) except that the preparative solution contained 0.4 M sucrose, 0.02 M *N*-tris(hydroxymethyl)methylglycine (Tricine)–KOH buffer (pH 8.0), and 0.01 M NaCl. The chloroplasts were osmotically disrupted and washed by resuspension in about 40 ml of a hypotonic solution made up of 0.01 M NaCl in 0.02 M Tricine–KOH buffer (pH 8.0), centrifuged, and then the "broken" chloroplasts were resuspended in the same hypotonic solution.

Illumination. Incident monochromatic illumination was provided by a 250 W tungsten-halogen lamp (General Electric type EHN) and appropriate interference filters (Baird-Atomic Co.).

Analytical Procedures. The ATP formed was measured by the method of Hagihara and Lardy (7). C-550 (8) was estimated at −189° from absorbance changes at 546 nm in light minus dark difference spectra obtained with an Aminco model DW-2 spectrophotometer equipped with a low-temperature attachment and operated in the split-beam mode; chlorophyll, NADPH, and ferricyanide were determined as described earlier (9).

Antimycin A was purchased from the Sigma Chemical Co.; dibromothymoquinone was a gift of Prof. A. Trebst.

Anaerobic Versus Aerobic Conditions. Unless otherwise indicated, the capped cuvettes filled with reaction mixture had little or no free gas phase. Aerobic conditions mean here that the reaction mixture was equilibrated with air and contained about 0.25 μmol of dissolved oxygen per ml. Anaerobic conditions were provided by equilibrating the reaction mixture with pure nitrogen gas to displace the dissolved air with dissolved nitrogen.

RESULTS AND DISCUSSION

Rates of Ferredoxin-dependent Electron Transport and Phosphorylation. Since this study was concerned with cyclic and noncyclic photophosphorylation as they might operate *in vivo*, it was useful to establish that the chloroplast preparations used had high rates of photochemical activity. Most of the experiments were performed under conditions not suitable for calculating activity rates (light below saturation and high concentrations of chlorophyll). Separate determinations made in saturating white light and with low chlorophyll concentrations (75 μg/ml) on a representative chloroplast preparation gave rates (in μmol/mg of chlorophyll per hr) of 235 for $NADP^+$ reduction by water and correspondingly high rates of ATP formation. These rates are compatible with high rates of photosynthesis *in vivo* (cf. 10).

Cyclic Photophosphorylation under Aerobic Conditions. Cyclic photophosphorylation *in vivo* (11) takes place in the chloroplast lamellar system which liberates oxygen

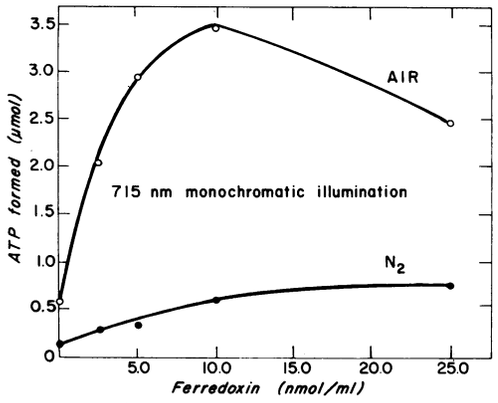

FIG. 1. Effect of ferredoxin on cyclic photophosphorylation under aerobic and anaerobic conditions. The reaction mixture which filled the capped glass cuvettes (optical path, 2 mm) included (per 1 ml) broken chloroplasts containing 332 μg of chlorophyll and the following (μmol): Tricine buffer (pH 8.2), 100; $MgCl_2$, 5.0; ADP, 5.0; $K_2H^{32}PO_4$, 5.0; and spinach ferredoxin as indicated. The 715-nm illumination (for 40 min) had an intensity of 2×10^4 ergs (= 2 mJ)/cm^2 per sec. Incident light intensity was measured by a Yellow Springs Instrument Co. model 65 radiometer or by a quartz-window Eppley surface-type linear thermopile, both of which were calibrated against a National Bureau of Standards radiation standard.

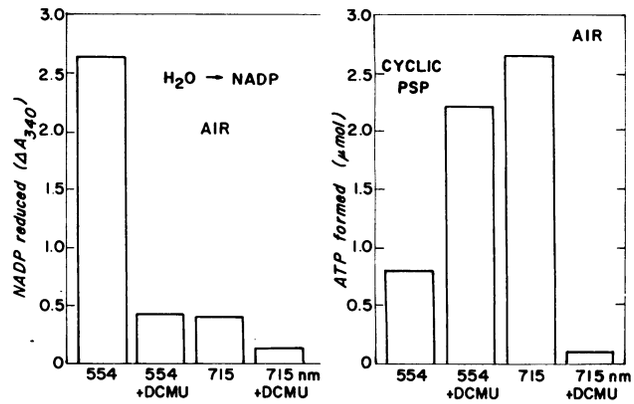

FIG. 2. Comparison of noncyclic electron transport (left) with ferredoxin-catalyzed cyclic photophosphorylation (right). Experimental conditions as in legend to Fig. 1 except that 0.01 μmol of ferredoxin was used throughout and, where indicated, 2 μmol of $NADP^+$ and 0.0012 μmol of DCMU were added. The chloroplast fragments and DCMU were preincubated together for 2 min in the dark. Incident light intensity for each wavelength was 2×10^4 ergs/cm^2 per sec. The photoreduction of NADP was measured at 340 nm in a Beckman-Gilford spectrophotometer (23).

and is in contact with the stroma fluid that contains the soluble enzymes for CO_2 assimilation (12). It seems reasonable to conclude therefore that cyclic photophosphorylation normally occurs in an environment containing dissolved oxygen. Experimentally, however, cyclic photophosphorylation by chloroplasts has usually been studied under anaerobic conditions, for reasons that are partly historical and partly of current validity.

Soon after photophosphorylation was discovered (13), cofactors like menadione were found that could support it at good rates and for extended periods of time under anaerobic conditions (14–16). This ATP formation in the absence of oxygen played an important part in overcoming the early resistance (see ref. 17) to the concept that light-induced ATP synthesis by chloroplasts was totally independent of oxidative phosphorylation by mitochondria (18). Although this matter is no longer in dispute, anaerobic conditions have continued to be widely used in studies of cyclic photophosphorylation in chloroplasts.

A more recent reason came from the finding that ferredoxin, the low-potential (19) iron–sulfur protein native to chloroplasts, is the endogenous cofactor for cyclic photophosphorylation (2, 3). Reduced ferredoxin can be reoxidized by oxygen (19, 4) and may thus catalyze a pseudocyclic photophosphorylation which, like the true cyclic type, yields only ATP but in reality is a variant of noncyclic photophosphorylation that depends on continuous production and consumption of oxygen (20, 10).

Putting historical and experimental reasons aside, the compelling consideration remains that, *in vivo*, cyclic photophosphorylation in chloroplasts most likely proceeds in the presence of oxygen. Indeed, early evidence indicated that ferredoxin was a more effective catalyst of cyclic photophosphorylation in air than under anaerobic conditions (21, 22). We undertook, therefore, to characterize more fully cyclic photophosphorylation under aerobic conditions.

Fig. 1 shows that ferredoxin was a far more effective catalyst of cyclic photophosphorylation when the reaction mixture contained dissolved air rather than dissolved N_2. The optimum ferredoxin concentration in air was about 10 μM, about an order of magnitude less than used earlier under argon (10) and about the same as that needed to catalyze noncyclic photophosphorylation. Much higher concentrations were less effective, probably because they facilitated a reoxidation of reduced ferredoxin by oxygen rather than by the cyclic electron transport chain of chloroplasts. Similar results were obtained in parallel experiments with green monochromatic illumination (554 nm). Here again, low concentrations of ferredoxin were found to catalyze cyclic photophosphorylation more effectively under aerobic than under anaerobic conditions but—and this was crucial—only when the light-induced electron flow from water was severely inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

In sum, ferredoxin-catalyzed cyclic photophosphorylation functioned best when light-induced electron flow from water (and hence noncyclic photophosphorylation and its pseudocyclic variant) was materially restricted either by the use of 715-nm illumination (22) or by combining 554-nm illumination with the addition of DCMU (Fig. 2). The extent to which these conditions restricted electron flow from water is reflected in the inhibition of the photoreduction of $NADP^+$ by water (Fig. 2).

Another observation was that under the aerobic conditions of these experiments a light-induced electron flow from water, though severely restricted, could not be dispensed with entirely. When the "trickle" electron flow from water was totally suppressed, as by adding DCMU to chloroplasts illuminated with 715-nm light, cyclic photophosphorylation stopped (Fig. 2). This observation is contrary to the generally held view that DCMU does not inhibit cyclic photophosphorylation (24) but is in agreement with the findings of Kaiser and Urbach (25) that in far-red light endogenous cyclic photophosphorylation in isolated chloroplasts was inhibited by low concentrations of DCMU.

In the presence of oxygen, the trickle of electrons from water appeared to maintain the proper oxidation-reduction balance or "poising" that is required for cyclic photophosphorylation. The need for poising of ferredoxin-catalyzed cyclic photophosphorylation in chloroplasts had already be-

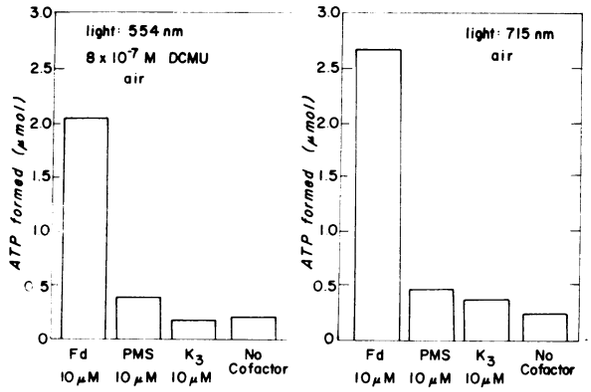

FIG. 3. Comparison of ferredoxin with other cofactors of cyclic photophosphorylation. Experimental conditions as in legend to Fig. 1 except that ferredoxin (Fd), phenazine methosulfate (PMS), and menadione (K_3) were added as indicated. The incident light intensities for both wavelengths were adjusted to give equal absorbed illumination of 3×10^3 ergs/cm^2 per sec.

come apparent in earlier experiments under conditions different from those used here (21, 26). The general importance of poising for cyclic photophosphorylation in both chloroplasts and bacterial chromatophores has been stressed by Avron and Neumann (24).

Comparison of Ferredoxin with Other Catalysts of Cyclic Photophosphorylation. It was previously reported (3) that under anaerobic conditions and in limiting light ferredoxin was a more effective catalyst of cyclic photophosphorylation than either menadione or the widely used phenazine methosulfate (27). A new comparison of these three catalysts, at equal concentrations (10 μM) and in limiting light, was now made under aerobic conditions. As shown in Fig. 3, with either 715- or 554-nm illumination (in the presence of DCMU) ferredoxin was a decisively more effective catalyst of cyclic photophosphorylation than either menadione or phenazine methosulfate. The quantum efficiency of this and other ferredoxin-catalyzed photophosphorylations will be reported in another paper.

Effect of Dibromothymoquinone. Trebst *et al.* (28) have introduced a new inhibitor, dibromothymoquinone, which acts as an antagonist of the plastoquinone component of chloroplast membranes and inhibits chloroplast reactions in which plastoquinone is involved. Hauska *et al.* (29) recently found that ferredoxin-catalyzed cyclic photophosphorylation (under argon) was inhibited by dibromothymoquinone

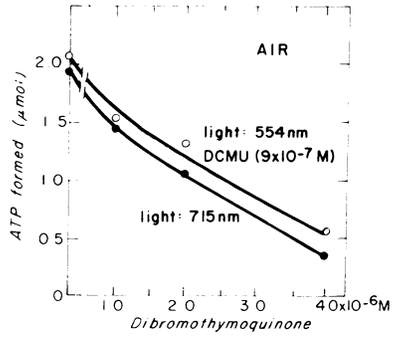

FIG. 4. Effect of dibromothymoquinone on ferredoxin-catalyzed cyclic photophosphorylation. Experimental conditions as in Fig. 1 except that 0.01 μmol of ferredoxin was used throughout and the quinone and DCMU were added as indicated. Chloroplast fragments were preincubated with dibromothymoquinone as described for DCMU in Fig. 2. Illumination was as in Fig. 2.

and concluded that ferredoxin is the probable cofactor of cyclic photophosphorylation *in vivo* in which plastoquinone is the native energy-conserving site. We set out to determine, therefore, the effect of dibromothymoquinone on ferredoxin-catalyzed cyclic photophosphorylation under aerobic conditions. Under these, dibromothymoquinone also strongly inhibited ferredoxin-catalyzed cyclic photophosphorylation under either 715- or 554-nm illumination (with DCMU present) (Fig. 4)—evidence that here too plastoquinone was essential.

Concurrent Cyclic and Noncyclic Photophosphorylation. In the experiments discussed so far, the poising of cyclic photophosphorylation under aerobic conditions was maintained by decreasing the electron flow from water by means foreign to photosynthesis in nature: far-red monochromatic illumination or shorter wavelength illumination combined with an addition of DCMU. Seeking a physiological regulation mechanism, we formulated a hypothesis that poising of cyclic photophosphorylation *in vivo* is accomplished by its concurrent operation with noncyclic photophosphorylation. During CO_2 assimilation, the electrons from water would be used (via ferredoxin and $NADP^+$) for the reduction of phosphoglycerate. Thus, an overreduction of the chloroplast milieu would be avoided and cyclic photophosphorylation could operate concurrently with the noncyclic type. Moreover, cyclic and noncyclic photophosphorylation operating concurrently would yield more than one ATP molecule for each pair of electrons transferred from water to $NADP^+$, i.e., the ATP/e_2 ratio would be greater than one. Such a result would help to resolve a current controversy over the stoichiometry of noncyclic photophosphorylation and the role of cyclic photophosphorylation in CO_2 assimilation. The basis of the controversy is as follows.

When noncyclic photophosphorylation was discovered, a P/e_2 ratio (or, with reference to the oxygen produced, a P/O ratio) equal to one was obtained with either NADP or its nonphysiological substitute, ferricyanide, as the terminal electron acceptor (30). A P/e_2 ratio equal to one for noncyclic photophosphorylation was confirmed by a later study in this laboratory (9) and by many other investigators (31–34) but higher P/e_2 (or P/O) ratios were also reported (35, 36) and led, on the basis of certain assumptions, to proposals that noncyclic photophosphorylation produces 2 ATPs per pair of electrons transferred (35–40). Only a negligible amount of the ATP was thought to come from a possibly concurrent cyclic or pseudocyclic photophosphorylation (37, 39).

The P/e_2 ratio of noncyclic photophosphorylation is of considerable importance for the assessment of the role of cyclic photophosphorylation in photosynthetic CO_2 assimilation, which requires 3 molecules of ATP and 2 molecules of NADPH for each molecule of CO_2 (41). A P/e_2 ratio of noncyclic photophosphorylation greater than one could account for all the ATP needs of CO_2 assimilation. Conversely, with a P/e_2 ratio of one, a contribution of ATP from cyclic photophosphorylation would be required for CO_2 assimilation, as some results do indeed indicate (42).

We undertook, therefore, to reinvestigate P/e_2 ratios of noncyclic photophosphorylation on the premise that under our present experimental conditions the ratios might depend on a concurrent operation of cyclic photophosphorylation. As shown in Fig. 5, with ferricyanide as the terminal electron acceptor (and in the absence of ferredoxin), the P/e_2 ratio was again found to be one; but with the physiological acceptor $NADP^+$ (and ferredoxin present) the P/e_2 ratio was about 1.5. Next, we used antimycin A, an inhibitor of ferre-

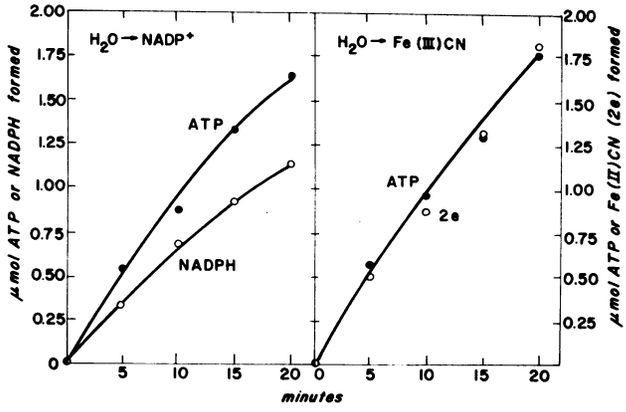

FIG. 5. Stoichiometry of noncyclic photophosphorylation with $NADP^+$ or ferricyanide as the terminal electron acceptor. The reaction mixture contained (per 1 ml) broken chloroplasts (332 µg of chlorophyll) and the following (µmol): Tricine–KOH buffer (pH 8.2), 200; $MgCl_2$, 5.0; ADP, 7.5; $K_2H^{32}PO_4$, 7.5; and $K_3(FeCN)_6$, 5.0, or $NADP^+$, 2.5, plus ferredoxin, 0.01. The 554-nm light gave an incident intensity of 8×10^3 ergs/cm^2 per sec. Cuvettes were full but open to air.

doxin-catalyzed cyclic photophosphorylation (2, 10, 43, 44) to test whether the excess ATP was of cyclic origin. Fig. 6 shows that this proved to be the case. Antimycin A, within a concentration range that did not inhibit noncyclic photophosphorylation, inhibited the extra ATP formed by cyclic photophosphorylation, thereby restoring to noncyclic photophosphorylation its P/e_2 ratio of one.

We conclude, therefore, that, in the presence of $NADP^+$, noncyclic photophosphorylation poises the chloroplast system to permit the concurrent operation of ferredoxin-catalyzed cyclic photophosphorylation. The same concentration of ferredoxin serves both types of photophosphorylation. The concurrent operation of cyclic and noncyclic photophosphorylation would prevail during CO_2 assimilation and provide the needed extra ATP ($P/e_2 > 1$).

The rates of concurrent noncyclic and cyclic photophosphorylation fluctuated during the year and gave variable P/e_2 ratios with different chloroplast preparations. Despite this variability, the capacity of chloroplasts to carry on concurrently ferredoxin-catalyzed cyclic and noncyclic photophosphorylation was never in question.

Allen (45) has recently proposed that the extra ATP needed for CO_2 assimilation comes from ferredoxin-catalyzed

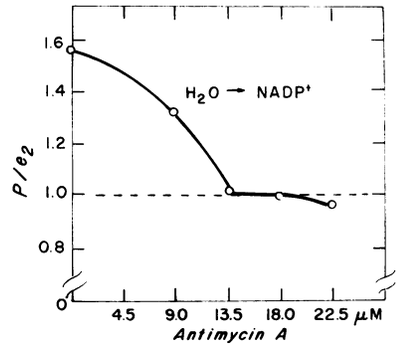

FIG. 6. Effect of antimycin A on concurrent cyclic and noncyclic photophosphorylation. The reaction mixtures were as in Fig. 1 except that 0.01 µmol of ferredoxin and 2.5 µmol of $NADP^+$ were used throughout. The chloroplasts and the antimycin A were incubated together as described for DCMU in Fig. 2. Reaction time was 15 min. Other conditions were as in Fig. 5.

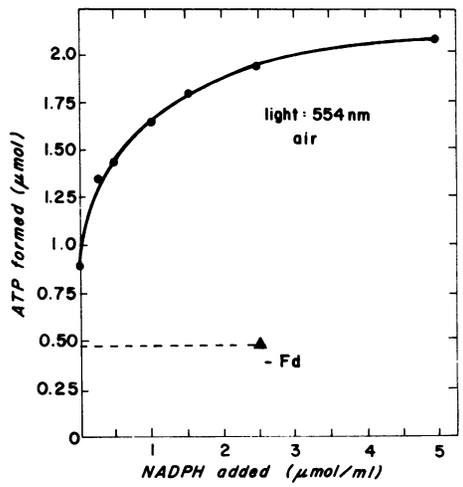

FIG. 7. Effect of NADPH on ferredoxin-catalyzed cyclic photophosphorylation. Reaction mixture was as in the $NADP^+$ treatment in Fig. 5 except that $NADP^+$ was omitted and NADPH was added as indicated. The 554-nm illumination was as in Fig. 2. The reaction was carried out in cuvettes filled with reaction mixture and sealed with serum caps. Reaction time, 30 min.

pseudocyclic photophosphorylation. We consider this an unlikely possibility. Much higher concentrations of ferredoxin are required for pseudocyclic photophosphorylation (10, 45) than for cyclic photophosphorylation under aerobic conditions.

Regulation of Cyclic Photophosphorylation. The ATP photochemically generated by cyclic photophosphorylation may be used not only for CO_2 assimilation but also for other ATP-requiring cellular activities (11). One example of such "nonphotosynthetic" use of ATP derived from cyclic photophosphorylation is amino acid incorporation by isolated chloroplasts (46).

As pointed out earlier (30), cyclic photophosphorylation may continue to produce ATP even when CO_2 assimilation

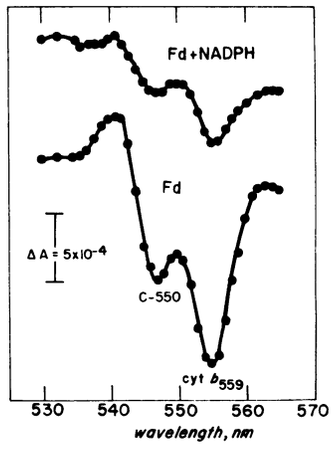

FIG. 8. Effect of preincubation with and without NADPH on subsequent photoreduction of C-550 and photooxidation of cytochrome b_{559} at low temperature. The reaction mixture contained (per 1 ml) chloroplasts (0.25 mg of chlorophyll) and the following (µmol): Tricine-KOH buffer (pH 8.2), 100; $MgCl_2$, 5.0; ferredoxin, 0.01; and, where indicated, NADPH, 2.5. After the reaction was incubated for 17 min in the dark, glycerol was added (50% final concentration); the mixture was frozen, and was then illuminated at −189°. Each point on the graph was obtained after correction for the base-line changes. The cuvette (2 mm light path) was illuminated by a 150 W Sylvania FCS projection lamp whose light was filtered through Corning 2-58 and 1-69 filters.

is curtailed or stopped altogether because of the well-known midday closure of stomata in leaves (47, 48). A curtailment of CO_2 assimilation would result in an accumulation of NADPH; hence the possibility arose that NADPH might have an effect on the operation of cyclic photophosphorylation.

Fig. 7 shows that under aerobic conditions the addition of NADPH greatly stimulated ferredoxin-catalyzed cyclic photophosphorylation (sensitive to antimycin A) in 554-nm light. Unexpectedly, no DCMU was required for poising (compare Fig. 2). It appeared that NADPH, like DCMU, diminished the electron flow from water (Photosystem II activity). Such a novel role of NADPH was tested further by measuring its effect on the photoreduction of C-550 (8), a primary indicator of Photosystem II activity (49). Fig. 8 (upper curve) shows that preincubation of chloroplasts with NADPH (in the presence of ferredoxin) in the dark diminished the subsequent photoreduction of C-550 (and photooxidation of cytochrome b_{559}). NADPH without ferredoxin was ineffective.

It appears, therefore, that when CO_2 assimilation is curtailed and NADPH accumulates, the back reaction of NADPH and ferredoxin with C-550 and possibly cytochrome b_{559} provides a regulatory mechanism which by diminishing the activity of Photosystem II maintains proper poising for the operation of cyclic photophosphorylation as a source of ATP for diverse cellular needs.

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1. Arnon, D. I. (1961) in *Light and Life*, eds. McElroy, W. D. & Glass, B. (The Johns Hopkins Press, Baltimore, Md.), pp. 489–566.
2. Tagawa, K., Tsujimoto, H. Y. & Arnon, D. I. (1963) *Proc. Nat. Acad. Sci. USA* **49**, 567–572.
3. Arnon, D. I. (1969) *Naturwissenschaften* **56**, 295–305.
4. Arnon, D. I., Tsujimoto, H. Y. & McSwain, B. D. (1964) *Proc. Nat. Acad. Sci. USA* **51**, 1274–1282.
5. Shin, M. & Arnon, D. I. (1965) *J. Biol. Chem.* **240**, 1405–1411.
6. Kalberer, P. P., Buchanan, B. B. & Arnon, D. I. (1967) *Proc. Nat. Acad. Sci. USA* **57**, 1542–1549.
7. Hagihara, B. & Lardy, H. A. (1969) *J. Biol. Chem.* **235**, 889–894.
8. Knaff, D. B. & Arnon, D. I. (1969) *Proc. Nat. Acad. Sci. USA* **63**, 963–969.
9. Del Campo, F. F., Ramirez, J. M. & Arnon, D. I. (1968) *J. Biol. Chem.* **243**, 2805–2809.
10. Arnon, D. I., Tsujimoto, H. Y. & McSwain, B. D. (1967) *Nature* **214**, 562–566.
11. Simonis, W. & Urbach, W. (1973) *Annu. Rev. Plant Physiol.* **24**, 89–114.
12. Whatley, F. R., Allen, M. B., Rosenberg, L. L., Capindale, J. B. & Arnon, D. I. (1956) *Biochim. Biophys. Acta* **20**, 462–468.
13. Arnon, D. I., Allen, M. B. & Whatley, F. R. (1954) *Nature* **174**, 394–396.
14. Arnon, D. I., Whatley, F. R. & Allen, M. B. (1954) *J. Am. Chem. Soc.* **76**, 6324–6329.
15. Arnon, D. I., Whatley, F. R. & Allen, M. B. (1955) *Biochim. Biophys. Acta* **16**, 607–608.
16. Allen, M. B., Whatley, F. R. & Arnon, D. I. (1958) *Biochim. Biophys. Acta* **27**, 16–23.
17. Arnon, D. I. (1956) *Annu. Rev. Plant Physiol.* **7**, 325–354.
18. Arnon, D. I. (1955) *Science* **122**, 9–16.
19. Tagawa, K. & Arnon, D. I. (1962) *Nature* **195**, 537–543.
20. Arnon, D. I., Losada, M., Whatley, F. R., Tsujimoto, H. Y., Hall, D. O. & Horton, A. A. (1961) *Proc. Nat. Acad. Sci. USA* **47**, 1314–1334.
21. Tagawa, K., Tsujimoto, H. Y. & Arnon, D. I. (1963) *Nature* **199**, 1247–1252.
22. Tagawa, K., Tsujimoto, H. Y. & Arnon, D. I. (1963) *Proc. Nat. Acad. Sci. USA* **50**, 544–549.
23. McSwain, B. D. & Arnon, D. I. (1968) *Proc. Nat. Acad. Sci. USA* **61**, 989–996.
24. Avron, M. & Neumann, J. (1968) *Annu. Rev. Plant Physiol.* **19**, 137–166.
25. Kaiser, W. & Urbach, W. (1973) *Ber. Dtsch. Bot. Ges.* **86**, 213–226.
26. Grant, B. R. & Whatley, F. R. (1967) in *Biochemistry of Chloroplasts*, ed. Goodwin, T. W. (Academic Press, New York), Vol. 2, pp. 505–521.
27. Jagendorf, A. T. & Avron, M. (1958) *J. Biol. Chem.* **231**, 277–290.
28. Trebst, A., Harth, E. & Draber, W. (1970) *Z. Naturforsch. Teil B* **25**, 1157–1159.
29. Hauska, G., Reimer, S. & Trebst, A. (1974) *Biochim. Biophys. Acta* **357**, 1–12.
30. Arnon, D. I., Whatley, F. R. & Allen, M. B. (1958) *Science* **127**, 1026–1034.
31. Jagendorf, A. T. (1958) *Brookhaven Symp. Biol.* **11**, 236–258.
32. Avron, M. & Jagendorf, A. T. (1959) *J. Biol. Chem.* **234**, 1315–1320.
33. Stiller, M. & Vennesland, B. (1962) *Biochim. Biophys. Acta* **60**, 562–579.
34. Turner, J. F., Black, C. C. & Gibbs, M. (1962) *J. Biol. Chem.* **237**, 577–579.
35. Winget, G. D., Izawa, S. & Good, N. E. (1965) *Biochem. Biophys. Res. Commun.* **21**, 438–443.
36. Hall, D. O., Reeves, S. G. & Baltscheffsky, H. (1971) *Biochem. Biophys. Res. Commun.* **43**, 359–366.
37. Izawa, S. & Good, N. E. (1968) *Biochim. Biophys. Acta* **162**, 380–391.
38. Saha, S. & Good, N. E. (1970) *J. Biol. Chem.* **245**, 5017–5021.
39. Reeves, S. G. & Hall, D. O. (1973) *Biochim. Biophys. Acta* **314**, 66–78.
40. West, K. R. & Wiskich, J. T. (1973) *Biochim. Biophys. Acta* **292**, 197–205.
41. Bassham, J. M. & Calvin, M. (1957) *The Path of Carbon in Photosynthesis* (Prentice-Hall, Englewood Cliffs, N.J.).
42. Schürmann, P., Buchanan, B. B. & Arnon, D. I. (1972) *Biochim. Biophys. Acta* **267**, 111–124.
43. Urbach, W. & Simonis, W. (1964) *Biochem. Biophys. Res. Commun.* **17**, 39–45.
44. Tanner, W., Loos, E. & Kindler, O. (1965) in *Currents in Photosynthesis* eds. Thomas, J. B. & Goedheer, J. C. (Donker, Rotterdam), pp. 243–251.
45. Allen, J. F. (1975) *Nature* **256**, 599–600.
46. Ramirez, J. M., Del Campo, F. F. & Arnon, D. I. (1968) *Proc. Nat. Acad. Sci. USA* **59**, 606–612.
47. Stålfelt, M. G. (1955) *Physiol. Plant.* **8**, 572–593.
48. Heath, O. V. S. & Orchard, B. (1957) *Nature* **180**, 180–181.
49. Butler, W. L. (1973) *Acc. Chem. Res.* **6**, 177–184.