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Photoreduction of $NADP^+$ by isolated reaction centers of photosystem II: Requirement for plastocyanin

(photosynthesis/electron transport/pheophytin)

DANIEL I. ARNON* AND JAMES BARBER†

*Department of Plant Biology, University of California, Berkeley, CA 94720; and †Agricultural and Food Research Council Photosynthesis Research Group, Department of Biochemistry, Imperial College, London, United Kingdom

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ABSTRACT **The carrier of photosynthetically generated reducing power is the iron-sulfur protein ferredoxin, which provides directly, or via $NADP^+$, reducing equivalents needed for CO_2 assimilation and other metabolic reactions in the cell. It is now widely held that, in oxygenic photosynthesis, the generation of reduced ferredoxin–$NADP^+$ requires the collaboration in series of two photosystems: photosystem II (PSII), which energizes electrons to an intermediate reducing potential and transfers them to photosystem I (PSI), which in turn is solely competent to energize electrons to the strong reducing potential required for the reduction of ferredoxin–$NADP^+$ (the Z scheme). This investigation tested the premise of an alternative scheme, which envisions that PSII, without the involvement of PSI, is also capable of photoreducing ferredoxin-$NADP^+$. We report here unexpected findings consistent with the alternative scheme. Isolated PSII reaction centers (completely free of PSI components), when supplemented with ferredoxin, ferredoxin–$NADP^+$ oxidoreductase, and a PSII electron donor, 1,5-diphenylcarbazide, gave a significant photoreduction of $NADP^+$. A striking feature of this electron transfer from a PSII donor to the perceived terminal acceptor of PSI was its total dependence on catalytic quantities of plastocyanin, a copper-containing electron-transport protein hitherto known only as an electron donor to PSI.**

One of the notable achievements of modern biochemical research is the elucidation in molecular terms of the mechanisms of photosynthesis, the process that sustains virtually all life on our planet. The core of oxygenic photosynthesis is the conversion of the electromagnetic energy of sunlight into two forms of biologically useful chemical energy—(*i*) phosphate bond energy whose carrier is ATP, the universal energy currency of living cells; and (*ii*) reducing equivalents—i.e., energized electrons whose carrier is the iron-sulfur protein ferredoxin (1–4). ATP and reduced ferredoxin [directly or after it enzymatically reduces $NADP^+$ (5, 6)] provide the assimilatory power used for CO_2 assimilation (7, 8) and for other endergonic cellular reactions, such as reduction of nitrite to ammonia (9), sulfite to sulfide (10), and peptide synthesis (11, 12).

It is now well established that the photosynthetic apparatus is subdivided into two photosystems, I and II (PSI and PSII), each with its own reaction center, light-harvesting pigments, proteins, and other electron carriers. Jointly PSI and PSII account for photosynthetic energy conversion by two processes: cyclic photophosphorylation that generates only ATP and noncyclic photophosphorylation in which ATP formation is accompanied by oxygen evolution and reduction of ferredoxin (1, 2). The terms cyclic and noncyclic photophosphorylation derive, respectively, from the type of electron flow induced by light: a type in which electrons flow in a cyclic path and a type in which electrons flow in a linear, noncyclic path from water to ferredoxin (2, 4). The light-induced electron flow gives rise to electrochemical proton gradients that account for the respective "cyclic" and "noncyclic" ATP formation (13).

The specific roles of PSI and PSII in inducing cyclic and noncyclic electron flow are defined by the currently prevalent doctrine embodied in the Z scheme, so called because of the shape of diagrams by which it is represented. The Z scheme is widely regarded as the conceptual cornerstone of modern research in photosynthesis (14).

There is now agreement on some points bearing on the Z scheme: (*i*) PSII photooxidizes water and thereby initiates the noncyclic electron flow of the water-derived electrons that ultimately reduce ferredoxin, and (*ii*) PSI drives cyclic electron flow whose physiological catalyst undergoing oxidation–reduction is ferredoxin (15, 16). Experimentally, the capacity of PSI to photoreduce ferredoxin is readily demonstrable by inhibiting PSII activity, providing a PSI electron donor and adding $NADP^+$ to trap electrons that normally would cycle. However, there is disagreement about the role of PSII in the reduction of ferredoxin.

Most investigators accept the basic premises of the Z scheme: (*i*) that PSII can energize the water-derived electrons only to an intermediate reducing potential at pH 7 ($E_{m,7}$, *ca.* 0 V) that is insufficient for the reduction of ferredoxin ($E_{m,7} = -420$ mV) and $NADP^+$ ($E_{m,7} = -320$ mV), and (*ii*) that only PSI is competent to energize electrons to a strong reducing potential adequate for the reduction of ferredoxin and the more electronegative bound iron-sulfur centers in PSI ($E_m = -540$ to -580 mV), whose reduction is a precondition for the reduction of ferredoxin by PSI (17). Accordingly, the key tenet of the Z scheme is that a collaboration of PSII and PSI, linked by a chain of electron carriers ending with plastocyanin (a copper-containing electron-carrier protein) is required for the complete noncyclic electron transport from water to ferredoxin (17, 18).

This article reports findings that seem at variance with this key tenet of the Z scheme. We found that isolated PSII reaction centers, completely devoid of PSI components, photoreduced ferredoxin–$NADP^+$ and that this photoreduction was totally dependent on added plastocyanin, hitherto thought to donate electrons exclusively to PSI reaction centers (18). However, these findings are relevant to an alternative concept of photosynthetic electron transport (19–21) and its recent refinements (22–24) proposed by one of us (D.I.A.), which envisions that PSII (renamed the oxygenic photosystem) drives the complete noncyclic electron transport from water to ferredoxin without the collaboration of PSI (renamed the anoxygenic photosystem), whose physiological role centers on cyclic electron flow and the resultant cyclic phosphorylation. The alternative scheme regards the two photosystems as being basically autonomous and as

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Abbreviations: PSI and PSII, photosystems I and II.

operating not collaboratively in series but synchronously in parallel.

METHODS

$NADP^+$ reduction was followed by absorbance changes at 340 nm photoinduced by isolated PSII reaction centers and measured with a dual-wavelength spectrophotometer (Aminco DW-2 or Perkin–Elmer 557). Actinic light was supplied by side illumination from a tungsten lamp. The photomultiplier was protected from red actinic light by a narrow band 340-nm interference filter (Baird).

PSII reaction centers were isolated from peas as described by Chapman *et al.* (25), except that the second chromatographic step involved the use of 2 mM dodecylmaltoside instead of Triton X-100, a procedure that greatly enhances the stability of the complex (26). The isolated complex was characterized by its absorbance spectrum (red peak at 675 nm) at room temperature (Fig. 1) and its chlorophyll-to-cytochrome *b*-559 ratio; the preparation was stored at −196°C.

Protein analyses of the isolated PSII reaction centers, confirming the absence of PSI components (Fig. 2), were carried out by SDS/PAGE as described (28). Profiles of separated proteins were electrophoretically transferred from the polyacrylamide gels onto nitrocellulose and detected (29) by using rabbit primary antibodies to a maize PSI-200 preparation (30), which was a gift from G. Giacometti of the University of Padova.

RESULTS

Background of Investigation. Until recently the doctrine that PSII lacks competence to photoreduce ferredoxin was supported by seemingly unassailable thermodynamic considerations that guaranteed its validity irrespective of other supporting evidence: PSII was known to generate the strong oxidant that oxidizes water but to generate only a weak reductant incapable of reducing ferredoxin (14).

This thermodynamic barrier was removed when pheophytin (E_m *ca.* −610 mV) was found to be the primary electron acceptor in PSII (31–33). Reduced pheophytin is thermodynamically competent to reduce ferredoxin, and such a role for pheophytin was proposed in the alternative scheme (19, 20) and rationalized as advantageous from the standpoint of energy conservation (24). On the other hand, Klimov *et al.* (31–33) assigned to pheophytin a role that did not conflict with the essentiality of PSI for ferredoxin reduction, as represented by the Z scheme. More recently, when a *Chlamydomonas* mutant lacking PSI was found capable of photoreducing $NADP^+$ (and hence ferredoxin) solely by PSII, such activity by PSII was explained as a special case possible only at high light intensities (34).

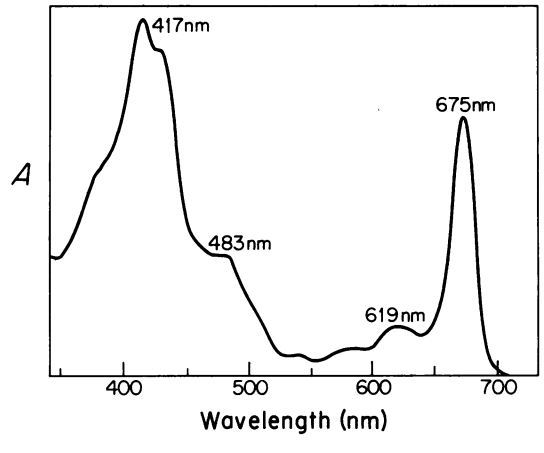

FIG. 1. Room temperature absorption spectrum of isolated PSII reaction centers suspended in 50 mM Tris buffer (pH 7.2) containing 2 mM dodecylmaltoside.

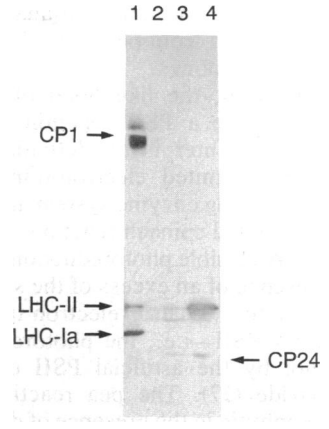

FIG. 2. Immunoblots with antibody raised to PSI-200 preparation as described in ref. 30. This antibody reacts not only with the P700-containing complex of the PSI reaction center (CP1) but also with the chlorophyll a/b light-harvesting complexes of PSI (LHC-Ia) and PSII (LHC-II). CP24, 24-kDa chlorophyll protein complex. Lanes: 1, stromal membranes; 2 and 3, isolated PS reaction centers; 4, grana membranes prepared as in ref. 27 and used for isolation for PSII reaction centers shown in lane 2.

When tested (24), the proposed effect of high light intensity (34) was not supported by experiments with inside-out vesicles enriched in PSII and made from spinach chloroplasts by the two-phase aqueous polymer partition method (21). This PSII preparation photoreduced ferredoxin–$NADP^+$ at various light intensities ranging from limiting to saturating, under experimental conditions that indicated no involvement of PSI (24). A striking finding in this and earlier work (21) was the dependence of the oxygenic photoreduction of ferredoxin–$NADP^+$ by PSII on added plastocyanin, the copper-containing electron-carrier protein of chloroplasts that is lost during the preparation of inside-out vesicles. As stated, up to that time plastocyanin was known only as an electron donor to PSI (18).

In sum, inside-out vesicles enriched in PSII and a *Chlamydomonas* mutant lacking PSI provided direct evidence that PSII can drive light-induced electron transport from water to ferredoxin without the collaboration of PSI. Moreover, experiments with inside-out vesicles established that this activity of PSII was dependent on plastocyanin. Both findings were incompatible with key premises of the Z scheme.

PSII Reaction Centers. The *Chlamydomonas* mutant lacking PSI constituted, in effect, a PSII specimen free from preparative artifacts and PSI contamination. The inside-out vesicles enriched in PSII did have a small PSI component, but, as was shown by specific experiments, PSI participation in the plastocyanin-dependent electron transport from water to $NADP^+$ was negligible (see figure 3 in ref. 21 and figure 1 in ref. 24). Nevertheless, the alternative concept that PSII can directly reduce ferredoxin in a plastocyanin-dependent reaction elicited some skepticism that could be dispelled if these earlier findings were validated with a PSII preparation completely free of any PSI contamination. Ideally, such a preparation would be the PSII reaction center.

A photosynthetic reaction center is a membrane-bound protein–pigment complex that catalyzes the primary photochemical events. The recently isolated PSII reaction center from spinach chloroplasts (35) was found to contain only the D1,D2 polypeptides and the apoproteins of cytochrome *b*-559 and to bind chlorophyll *a*, pheophytin, and the heme of cytochrome *b*-559 in an approximate ratio of 5:2:1; the

complex also contained β-carotene. An analogous PSII reaction center was isolated from peas (36). Neither preparation contained bound quinones.

On first consideration, the likelihood of validating the earlier findings by using a PSII reaction center seemed remote. The reaction center is by definition a truncated system with severely limited electron-transport capacity lacking the water-oxidizing enzyme system and various electron carriers. The isolated spinach reaction center exhibited upon illumination a reversible photoreduction of pheophytin, but only in the presence of an excess of the strong reductant dithionite; it was inactive (35) in an electron-transfer reaction that is diagnostic of PSII—i.e., the photoreduction of 2,6-dichloroindophenol by the artificial PSII electron donor, 1,5-diphenylcarbazide (37). The pea reaction center also photoreduced pheophytin in the presence of dithionite, but in addition it was able to catalyze the photoreduction by 1,5-diphenylcarbazide of an artificial PSII electron acceptor, silicomolybdate (36).

Despite the limited electron-transport capacity of the PSII reaction centers, we considered it worthwhile to test the remote possibility that under favorable experimental conditions, especially in the presence of plastocyanin (21, 24), the reaction centers might conform to the postulates of the alternative scheme of photosynthetic electron transport and catalyze a photoinduced electron transport from a PSII donor (other than water) to the primary PSII acceptor, pheophytin, then to ferredoxin, and, enzymatically, to $NADP^+$. This tenuous expectation was experimentally substantiated by using 1,5-diphenylcarbazide as electron donor.

Activity of Reaction Centers. The electron transport activity of the PSII reaction center measured here was the reduction of $NADP^+$ as evidenced by increase in absorbance at 340 nm, characteristic of NADPH. In previous experiments photoreduction of $NADP^+$ by PSII preparations (inside-out vesicles) was measured under aerobic conditions (21, 24). Similar measurements with PSII reaction centers showed an increase in absorbance at 340 nm that was totally dependent on the presence of the electron donor, 1,5-diphenylcarbazide, and was somewhat stimulated by plastocyanin and ferredoxin (data not shown). However, the light-dependent absorption

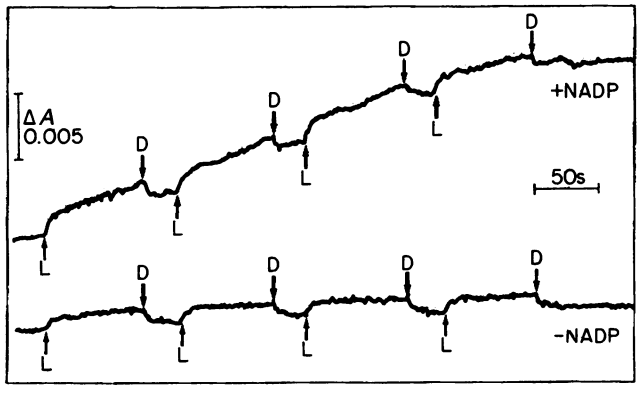

FIG. 3. Absorbance increase at 340 nm, in the presence and absence of $NADP^+$, photoinduced under anaerobic conditions by isolated PSII reaction centers. The complete reaction mixture contained PSII reaction centers at a concentration of 10 μg of chlorophyll per ml, 50 mM Tricine buffer (pH 8), 5 mM $MgCl_2$, 10 μM spinach ferredoxin, 2 mM $NADP^+$, 3 μM plastocyanin, 0.5 mM 2,5-diphenylcarbazide, and a saturating amount of ferredoxin–$NADP^+$ oxidoreductase. The reaction mixture was prepared under N_2 and maintained anaerobic by an oxygen trap consisting of 10 mM glucose, 13 units of glucose oxidase per ml, and 1200 units of catalase per ml. The actinic light intensity supplied by the side illumination was about 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the surface of a glass 1-ml cuvette and was transmitted by a 3-mm 222-58 Corning glass filter. L, light on; D, light off.

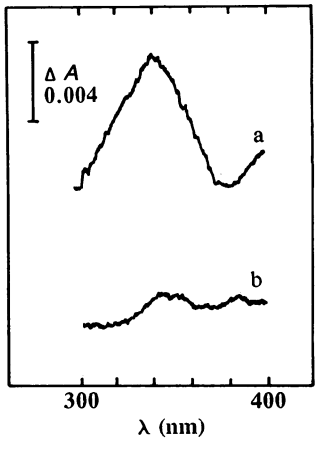

FIG. 4. Spectra of absorbance changes from 300 to 400 nm of PSII reaction centers prior to (trace b) and immediately after (trace a) illumination. Experimental conditions were as in Fig. 3.

increase at 340 nm could not be fully attributed to the reduction of $NADP^+$. The same light-induced absorbance increase at 340 nm, although at a lower rate, was observed when $NADP^+$ was omitted from the reaction mixture (traces not shown).

The observed light-induced absorbance increase at 340 nm under aerobic conditions in the presence or absence of $NADP^+$ appeared to reflect a redox change because it depended on the presence of 1,5-diphenylcarbazide as the electron donor. However, the nature of this absorbance change needs further study. It should be noted that chlorophyll *a* (and by extension chlorophyll derivatives like pheophytin) exhibits appreciable absorption at 340 nm (38).

All subsequent measurements of $NADP^+$ photoreduction were made under anaerobic conditions maintained by an N_2 atmosphere and an oxygen trap.

Fig. 3 shows that under anaerobic conditions in the presence of $NADP^+$, ferredoxin, ferredoxin–$NADP^+$ oxidore-

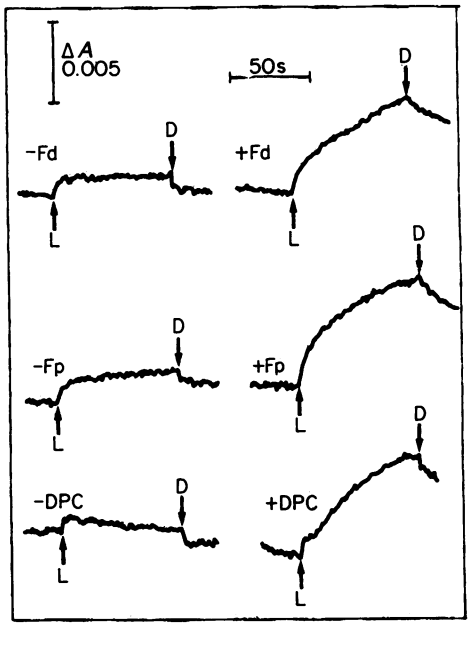

FIG. 5. Requirements of ferredoxin (Fd), ferredoxin–$NADP^+$ oxidoreductase (Fp), and 2,5-diphenylcarbazide (DPC) for $NADP^+$ photoreduction by isolated PSII reaction centers. Experimental conditions were as in Fig. 3. Each factor was initially omitted from the reaction mixture and added, where indicated, in the course of the experiment.

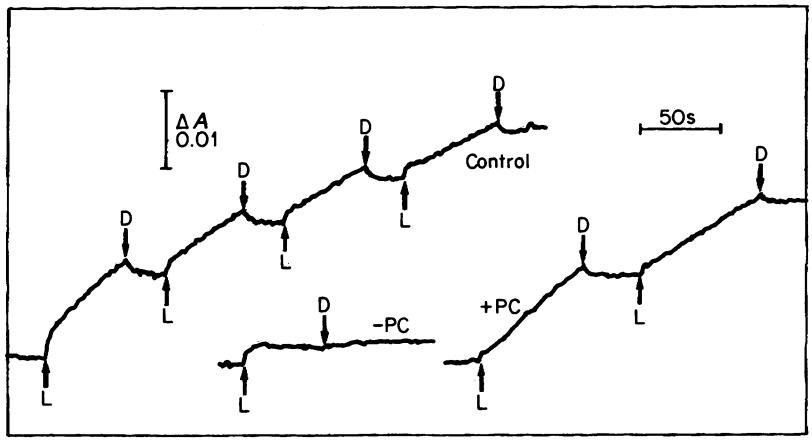

FIG. 6. Plastocyanin (PC) requirement for $NADP^+$ photoreduction by isolated PSII reaction centers. Traces: upper, complete reaction mixture; lower, PC added, where indicated, in the course of the experiment. Experimental conditions were as in Fig. 3.

ductase, plastocyanin, and 1,5-diphenylcarbazide, there was upon illumination a steady increase in absorbance at 340 nm. No such absorbance change was observed when $NADP^+$ was omitted from the reaction mixture (Fig. 3). In the presence of $NADP^+$, the spectrum of the light-induced absorbance change showed a peak at 340 nm (Fig. 4). Therefore, we attribute the steady increase in absorbance at 340 nm seen under anaerobic conditions to photoreduction of $NADP^+$.

A small and rapid reversible absorbance change was also observed at 340 nm, but this signal was not dependent on the presence of $NADP^+$. Its origin is unknown.

If the observed absorbance change at 340 nm reflected $NADP^+$ reduction, it should be dependent on 1,5-diphenylcarbazide as electron donor, on ferredoxin as electron carrier (3, 4), and on ferredoxin–$NADP^+$ oxidoreductase (5, 6). As shown in Fig. 5, this expectation was realized. Omission of the donor, ferredoxin, or the reductase severely inhibited the absorbance rise at 340 nm, which was restored upon the addition of the missing component.

Isolated PSII reaction centers catalyze, upon illumination, a separation of charges—i.e., an electron transfer from P680, the special form of chlorophyll acting as the primary electron donor, to pheophytin (Pheo) acting as the primary electron acceptor, giving rise to the $P680^+ \cdot Pheo^-$ state (35, 36). It also has been shown (25, 36) that in the presence of an artificial PSII electron donor such as 1,5-diphenylcarbazide and an artificial secondary PSII electron acceptor (silicomolybdate), PSII reaction centers can sustain a light-induced electron flow from donor to acceptor.

The novel finding here, shown in Figs. 3 and 4, was that isolated PSII reaction centers catalyzed electron flow from 1,5-diphenylcarbazide to what is commonly regarded as the physiological PSI acceptor—i.e., ferredoxin–$NADP^+$. This seemingly improbable electron transport from a PSII reaction center to the perceived acceptor of PSI was made possible, as shown below, by the inclusion of another component in the reaction mixture.

Plastocyanin Requirement. Perhaps the most surprising finding in this investigation was that, in catalyzing electron transport from a PSII donor to ferredoxin–$NADP^+$, the PSII reaction centers were totally dependent on the presence of plastocyanin. No photoreduction of $NADP^+$ occurred in the absence of plastocyanin and, with a reaction mixture lacking plastocyanin, photoreduction of $NADP^+$ was restored upon addition of plastocyanin (Fig. 6). This finding, which decisively validated and extended earlier observations of a plastocyanin requirement for $NADP^+$ reduction by PSII made with preparations that still contained a small PSI component (21, 24), cannot be accommodated by the Z scheme.

The photoreduction of $NADP^+$ by the PSII reaction centers in the complete system and under anaerobic conditions was unequivocal, but the rates in these initial experiments were low, reminiscent of the low rates of photophosphorylation and CO_2 assimilation by isolated chloroplasts when these phenomena were first discovered (1, 2, 7), before experimental conditions were optimized.

DISCUSSION

The findings reported in this paper point to the need to reassess two widely accepted premises in current photosynthesis research—i.e., (*i*) that only PSI is competent to photoreduce ferredoxin–$NADP^+$ and the corollary that PSII lacks such competence, and (*ii*) that plastocyanin functions only as an electron donor to PSI and cannot function as a donor to PSII.

The ability of PSII to photoreduce ferredoxin–$NADP^+$ without the collaboration of PSI has been observed previously in a *Chlamydomonas* mutant lacking PSI (34) and in inside-out vesicles enriched in PSII (21, 24). These observations now have been unexpectedly validated in a simpler, fully defined experimental system that contained only the essential characteristic component of PSII—i.e., its reaction center—and excluded any PSI contamination, however small.

Our results also validated and extended to a reconstituted system totally free of PSI the requirement of plastocyanin for ferredoxin–$NADP^+$ reduction by PSII, as initially observed with inside-out vesicles that still contained a small PSI component (21, 24). The results reported here open a new and conceptually intriguing area of inquiry about the mode of action of plastocyanin as an electron carrier capable of interacting not only with the reaction center of PSI but also with the reaction center of PSII.

Modern research provides several instances of novel findings that were out of harmony with dominant contemporary concepts but have, despite strong resistance, prevailed in time and led to advances in the knowledge of photosynthesis (2, 4, 7, 39). It now seems possible that the plastocyanin-dependent photoreduction of ferredoxin–$NADP^+$ by isolated PSII reaction centers may be added to this category and lead to a reassessment of currently prevalent concepts.

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1. Arnon, D. I., Allen, M. B. & Whatley, F. R. (1954) *Nature (London)* **174,** 394–396.
2. Arnon, D. I. (1984) *Trends Biochem. Sci.* **9,** 1–5.
3. Tagawa, K. & Arnon, D. I. (1962) *Nature (London)* **195,** 537–543.
4. Arnon, D. I. (1988) *Trends Biochem. Sci.* **13,** 30–33.
5. Shin, M., Tagawa, K. & Arnon, D. I. (1963) *Biochem. Z.* **338,** 84–86.
6. Shin, M. & Arnon, D. I. (1965) *J. Biol. Chem.* **240,** 1405–1411.
7. Arnon, D. I. (1987) *Trends Biochem. Res.* **12,** 39–42.
8. Schurmann, P., Buchanan, B. B. & Arnon, D. I. (1971) *Biochim. Biophys. Acta* **267,** 111–124.
9. Ramirez, J. M., Del Campo, F. F., Paneque, A. & Losada, M. (1966) *Biochim. Biophys. Acta* **118,** 58–71.
10. Schmidt, A. & Trebst, A. (1969) *Biochim. Biophys. Acta* **180,** 529–535.
11. Ramirez, J., Del Campo, F. F. & Arnon, D. I. (1968) *Proc. Natl. Acad. Sci. USA* **59,** 606–612.
12. Ellis, R. J. (1977) *Biochim. Biophys. Acta* **463,** 185–215.
13. Mitchell, P. (1979) *Science* **206,** 1148–1159.
14. Stryer, L. (1988) *Biochemistry* (Freeman, New York), 3rd Ed.
15. Tagawa, K., Tsujimoto, H. Y. & Arnon, D. I. (1963) *Proc. Natl. Acad. Sci. USA* **49,** 567–575.
16. Trebst, A. (1974) *Annu. Rev. Plant Physiol.* **25,** 423–458.
17. Malkin, R. (1987) in *The Light Reactions of Photosynthesis,* ed. Barber, J. (Elsevier, Amsterdam), pp. 495–526.
18. Haehnel, W. (1984) *Annu. Rev. Plant Physiol.* **35,** 659–693.
19. Arnon, D. I., Tsujimoto, H. & Tang, G. M.-S. (1981) in *Photosynthesis II: Electron Transport and Photophosphorylation,* Proceedings of the 5th International Photosynthesis Congress, ed. Akoyunoglou, G. (Balaban International Science Services, Philadelphia), pp. 7–18.
20. Arnon, D. I., Tsujimoto, H. & Tang, G. M.-S. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2942–2946.
21. Albertsson, P.-A., Hsu, B.-D., Tang, G. M.-S. & Arnon, D. I. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3971–3975.
22. Arnon, D. I. & Tang, G. M.-S. (1986) *Biochim. Biophys. Acta* **849,** 347–354.
23. Arnon, D. I. & Tang, G. M.-S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9524–9528.
24. Arnon, D. I. & Tang, G. M.-S. (1989) *FEBS Lett.* **253,** 253–256.
25. Chapman, D. J., Gounaris, K. & Barber, J. (1988) *Biochim. Biophys. Acta* **933,** 423–431.
26. Crystall, B., Booth, P. J., Klug, D. R., Barber, J. & Porter, G. (1989) *FEBS Lett.* **249,** 75–78.
27. Berthold, D. A., Babcock, G. T. & Yocum, C. F. (1981) *FEBS Lett.* **134,** 231–234.
28. Marder, M. B., Mattoo, A. K. & Edelman, M. (1986) *Methods Enzymol.* **118,** 384–396.
29. Shipton, C. A., Marder, J. B. & Barber, J. (1990) *Z. Naturforsch. Teil C* **45,** 37–43.
30. Bassi, R. & Simpson, D. J. (1987) *Eur. J. Biochem.* **163,** 221–230.
31. Klimov, V. V., Klevanik, A. V., Shuvalov, V. A. & Krasnovsky, A. A. (1977) *FEBS Lett.* **82,** 183–186.
32. Klimov, V. V. & Krasnovsky, A. A. (1981) *Photosynthetica* **15,** 592–609.
33. Klimov, V. V., Allakhverdiev, S. I., Demeter, S. & Krasnovsky, A. A. (1979) *Dokl. Akad. Nauk SSSR* **249,** 227–230.
34. Klimov, V. V., Allakhverdiev, S. I. & Ladygin, V. G. (1986) *Photosynth. Res.* **10,** 355–361.
35. Nanba, O. & Satoh, K. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 109–112.
36. Barber, J., Chapman, D. J. & Telfer, A. (1987) *FEBS Lett.* **220,** 67–73.
37. Vernon, L. P. & Shaw, E. R. (1969) *Plant Physiol.* **44,** 1645–1649.
38. Rabinowitch, E. I. (1951) *Photosynthesis and Related Processes* (Interscience, New York), Vol. 2, Part 1, p. 610.
39. Buchanan, B. B. & Arnon, D. I. (1990) *Photosynth. Res.* **24,** 47–53.