A CONCEPT OF THREE LIGHT REACTIONS IN PHOTOSYNTHESIS BY GREEN PLANTS

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Communicated August 18, 1969

Abstract.—It has generally been accepted that plant photosynthesis involves two light reactions, one that proceeds best in short-wavelength light and is identified with oxygen evolution (System II) and another that proceeds best in long-wavelength light and is identified with a cyclic electron flow (System I). This paper presents a concept of three light reactions in photosynthesis, based on new evidence that System II comprises two rather than one short-wavelength light reaction. These appear to operate in series and to be connected by an electron transport chain peculiar to System II. Parallel to System II is the long-wavelength light reaction of System I.

There is now wide agreement that photosynthesis in green plants involves two light reactions, one that proceeds best in short wavelength ($\lambda < 685$ mμ) light (known as Photosystem II or simply System II) and another—known as System I—which proceeds best in long wavelength ($\lambda > 685$ mμ) light. System I is identified with a light-induced cyclic electron transport among chloroplast constituents (cytochromes → chlorophyll → ferredoxin → cytochromes) that produces ATP without any net change in the redox state of any electron donor or acceptor (cyclic photophosphorylation). System II is identified with a light-induced electron transport from water to a terminal electron acceptor with a concomitant evolution of oxygen and production of ATP (noncyclic photophosphorylation) (references cited in reviews[1, 2]).

We have recently reported[3, 4] two new light reactions characteristic of System II which, because of their insensitivity to temperature (they occur even at −189°C), appear to lie close to the primary photochemical events in that system. The first of these light reactions is the photooxidation of cytochrome b_{559} (ref. 3) and the second is a spectral change, which now appears to be a reduction, of a new photoreactive chloroplast component which we have provisionally named C550.[4] This paper presents evidence in support of a hypothesis which holds that System II includes one light reaction (IIb) in which C550 is reduced and water is oxidized (liberating oxygen) and a second light reaction (IIa) in which cytochrome b_{559} is oxidized and ferredoxin is reduced. Our results suggest that light reactions IIb and IIa operate in series and are linked by an electron transport chain which includes (but is not limited to) C550, cytochrome b_{559}, and plastocyanin. This formulation leads to a concept of three light reactions in photosynthesis, comprising the two short-wavelength light reactions of System II and, parallel to it, the one long-wavelength light reaction of System I.

Methods.—"Broken" spinach chloroplasts were prepared according to the method of Whatley and Arnon[5] and Tris-treated chloroplasts by a modification of the procedure of Yamashita and Butler.[6] Sonicated chloroplasts were prepared by sonicating a chloroplast suspension (0.5 mg chlorophyll/ml) with a Branson sonifier for 3 min at power setting 3.

The sonicate was centrifuged for 30 min at 40,000 rpm in a Spinco model L centrifuge and the pellet resuspended in 0.2% NaCl. Chlorophyll was determined as described by Arnon.[7]

Plastocyanin was isolated from spinach leaves and purified according to a modification of the procedure of Katoh *et al.*[8] It had an A_{278}/A_{597} ratio of 1.4.

Absorbance changes were measured with a dual wavelength spectrophotometer (Phoenix Precision Instrument Co.) as described previously.[3, 4] Oxidation-reduction potentials were measured using a Radiometer model 26 pH meter with a PK 149 platinum electrode.

Monochromatic illumination was introduced through a hole in the side of the spectrophotometer.[3, 4, 9, 10] Incident illumination intensity was measured with a YSI-Kettering model 65 radiometer. In quantum requirement determinations, the absorbed light was measured in an integrating sphere as described previously.[10]

Results and Discussion.—Intermediate position of cytochrome b_{559} between two photoacts of System II: As recently reported,[3] the photooxidation of cytochrome b_{559} was measured spectrophotometrically when its reduction was blocked, either by performing the experiments at $-189°C$ or by treating the chloroplasts with Tris, a treatment[6, 11] that inactivates the electron flow from water but does not interfere otherwise with System II activity. That the photooxidation of cytochrome b_{559} is indeed part of System II was indicated by the much greater effectiveness of short-wavelength (664 mμ) than long-wavelength (715 mμ) light in that reaction.[3] We now find that, on the basis of absorbed quanta, the photooxidation of cytochrome b_{559} in Tris-treated chloroplasts proceeds at least three times more effectively in System II light (664 mμ) than in System I light (715 mμ). These findings do not agree with those of Cramer and Butler,[12] and Levine and Gorman[28] who reported that the photooxidation of cytochrome b_{559} was a System I reaction.

The photooxidation of cytochrome b_{559} was of unusual interest because the redox potential of this chloroplast component is only 0.33 v (see below). It has been commonly assumed that, since System II photooxidizes water, it functions to oxidize chloroplast constituents with a redox potential close to that of water ($E'_0 = 0.82$ v). The half-volt difference between these potentials made it highly improbable that the same primary photochemical reaction oxidized both cytochrome b_{559} and water. A more likely possibility would be that System II has a second light reaction (IIb) that generates a stronger oxidant capable of extracting electrons from water and liberating oxygen. The acceptor of the electrons from water in light reaction IIb would then provide the reducing power needed to reduce cytochrome b_{559} after it is oxidized by light reaction IIa.

We had previously explained the lack of any detectable cytochrome b_{559} photooxidation in untreated chloroplasts as being due to an equally rapid photoreduction caused by electrons from water. If this explanation is correct, measurable photooxidation of cytochrome b_{559} in Tris-treated chloroplasts should also be counterbalanced by its reduction when an artificial electron donor[6] that can replace water in Photosystem II is added to the reaction mixture.

As shown in Fig. 1, the predicted result of diminished photooxidation of cytochrome b_{559} was obtained by adding *p*-phenylenediamine[6] as the substitute electron donor. Figure 1 also shows that the further addition of ferredoxin and NADP, the physiological acceptors of electrons in noncyclic electron transport,[13]

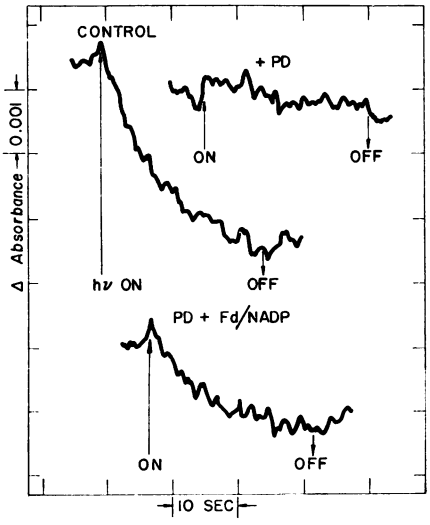

FIG. 1.—Photooxidation of cytochrome b_{559} in Tris-treated chloroplasts (561 mμ minus 570 mμ). The reaction mixture contained (per 1.0 ml) Tris-treated spinach chloroplasts (equivalent to 75 μg chlorophyll) and the following in μmoles: Tricine [*N*-tris(hydroxymethyl)methylglycine] buffer (pH 8.2), 33.3; K_2HPO_4, 5; $MgCl_2$, 2; ascorbate, 1; and, where indicated, *p*-phenylenediamine (PD), 0.033; ferredoxin, 0.01; and NADP, 1. Gas phase, nitrogen. The 664 mμ actinic light had an intensity of approximately 1.5×10^4 ergs/cm²/sec.

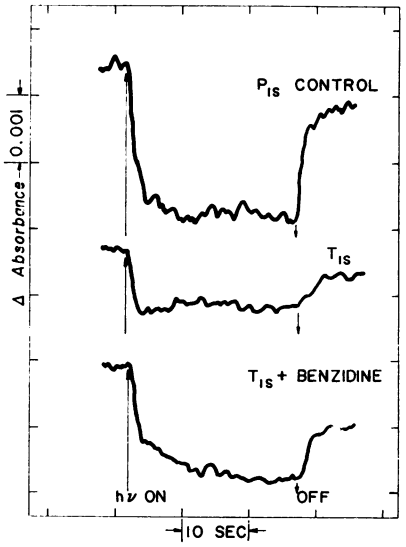

FIG. 2.—Effect of Tris-treatment on photoreduction of C550 (550 mμ minus 540 mμ). The reaction mixture contained (per 1.0 ml) spinach chloroplasts (P_{1S}) or Tris-treated spinach chloroplasts (T_{1S}) (equivalent to 75 μg chlorophyll) and the following in μmoles: Tricine buffer (pH 8.2), 33.3; K_2HPO_4, 5; $MgCl_2$, 2; potassium ferricyanide, 0.5; and, where indicated, benzidine, 0.067. Gas phase, nitrogen. Illumination as in Fig. 1.

restored some of the cytochrome b_{559} photooxidation. Similar results were obtained using benzidine and semicarbazide[14] as electron donors.

The results with ferredoxin and NADP suggest that light reaction IIa, which is responsible for cytochrome b_{559} oxidation, is also responsible for the photoreduction of ferredoxin. It seems reasonable to conclude that the presence of ferredoxin-NADP enhanced the oxidation of cytochrome b_{559} by providing an acceptor system for electrons removed from that cytochrome by light reaction IIa. We thus picture noncyclic electron transport as proceeding according to the following sequence:

$$H_2O \rightarrow \text{IIb} \rightarrow \text{cyt } b_{559} \rightarrow \text{IIa} \rightarrow \text{Fd} \rightarrow \text{NADP} \qquad (1)$$

Role of C550 in the electron transport chain of System II: Since reduction of cytochrome b_{559} does not occur at −189°C,[3] its postulated reduction by light reaction IIb must involve at least one intermediate thermochemical electron transfer step between light reaction IIb and cytochrome b_{559}. It now appears that the electron carrier which mediates this step may be C550—a provisional name given to a new chloroplast component[4] which, upon illumination by short-wavelength (System II) light, shows a decrease of absorbance with a maximum

at 550 mμ. Figure 2 shows that in Tris-treated chloroplasts, the magnitude of the light-induced C550 change was considerably decreased. Since Tris treatment decreases the flow of electrons from water into System II, the effect on C550 suggests that the light-induced decrease in absorbance is a photoreduction.

The correctness of this interpretation was confirmed by adding benzidine,[14] an artificial donor that restored electron flow to Tris-treated chloroplasts and thereby increased the magnitude of the absorbance change in C550 (Fig. 2).

Figure 3 shows the spectra of light-induced C550 changes in Tris-treated chloroplasts in the presence and absence of benzidine. The maxima at 550 mμ indicate that the changes are indeed due to C550 and that there is no interference from cytochrome *f* (cf. ref. 4). Similar results were obtained with semicarbazide[14] as the electron donor.

Once it was established with Tris-treated chloroplasts that the light-induced decrease in absorbance in C550 is a photoreduction, it became clear that, in untreated chloroplasts, C550 is photoreduced by electrons from water. Since it was established earlier that the photoreduction of C550 in untreated chloroplasts is a temperature-independent reaction,[4] it seems reasonable to conclude that C550 is the primary acceptor of electrons from water via light reaction IIb (eq. 2).

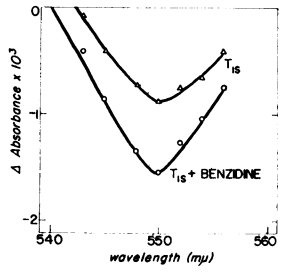

FIG. 3.—Photoreduction of C550 in Tris-treated chloroplasts (540 mμ reference). Experimental conditions were as in Fig. 2.

$$H_2O \rightarrow \text{IIb} \rightarrow \text{C550} \rightarrow \text{cyt } b_{559} \rightarrow \text{IIa} \rightarrow \text{Fd} \quad (2)$$

Role of plastocyanin in the electron transport chain of System II: Since plastocyanin, a copper protein first isolated from *Chlorella* by Katoh[15] and from spinach by Katoh *et al.*,[8] has been implicated in noncyclic electron transport from water to NADP,[16–19] we investigated its effect on the photooxidation of cytochrome b_{559}. Figure 4 shows that sonication (a treatment that removes plastocyanin[16, 17, 19, 20]) of the Tris-treated chloroplasts eliminates the photooxidation of cytochrome b_{559}. The addition of plastocyanin to the sonicated chloroplasts restores the photooxidation of cytochrome b_{559}. The increased absorbance in the upper trace of Fig. 4 was found to be caused by the photoreduction of cytochrome b_6, whose peak absorption in the α band is at 563 mμ.[21–23]

The absorbance changes (Fig. 4) induced by system II light (664 mμ) in sonicated chloroplasts, supplemented with plastocyanin, gave a spectrum characteristic of cytochrome b_{559} (ref. 3). Illumination of the sonicated chloroplasts supplemented with plastocyanin by System I light (715 mμ) gave no photooxidation of cytochrome b_{559}—an observation consistent with a System II reaction.

The plastocyanin requirement for the oxidation of cytochrome b_{559} was observed in sonicated chloroplasts under conditions when the oxidation could in turn be stimulated by the addition of ferredoxin-NADP, i.e. when the oxidation was a part of the usual noncyclic electron flow in chloroplasts. However, no

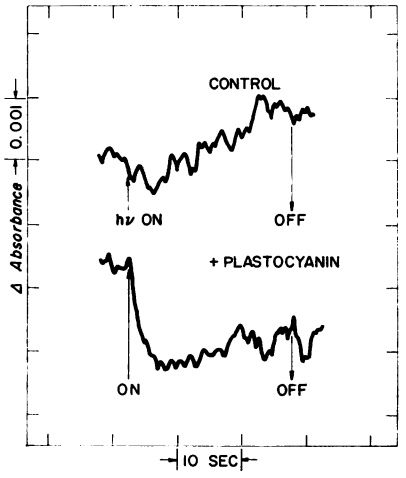

FIG. 4.—Effect of plastocyanin on photooxidation of cytochrome b_{559} (561 mμ minus 570 mμ) in chloroplasts depleted of plastocyanin. The reaction mixture contained (per 1.0 ml) sonicated, Tris-treated spinach chloroplasts (equivalent to 75 μg chlorophyll) and the following in μmoles: MES [2(N-morpholino)-ethane sulfonic acid] buffer (pH 6.2), 33.3; K_2HPO_4, 5; $MgCl_2$, 2; ascorbate, 1; ferredoxin, 0.01; NADP, 1; and, where indicated, plastocyanin, 0.015. Gas phase, nitrogen. Illumination as in Fig. 1.

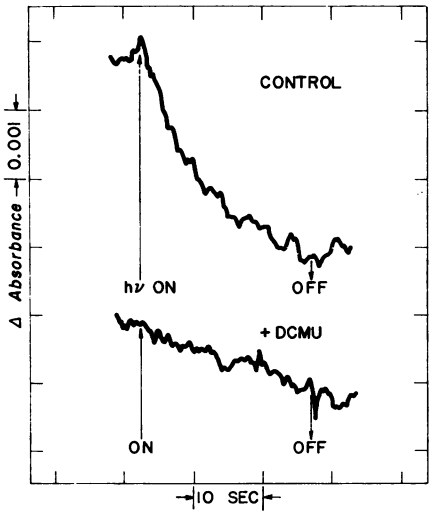

FIG. 5.—Effect of DCMU on cytochrome b_{559} photooxidation in Tris-treated chloroplasts (561 mμ minus 570 mμ). Experimental conditions were as in Fig. 1, with 0.001 μm (per 1.0 ml) DCMU added where indicated.

plastocyanin requirement was observed under each of two experimental conditions that no longer gave a stimulation of cytochrome b_{559} oxidation by ferredoxin-NADP: at −189° or at alkaline pH's. It is possible that under these conditions the plastocyanin site was by-passed.

If plastocyanin is the natural oxidant of cytochrome b_{559} in System II, it should have a more positive redox potential than that of the cytochrome. The reported redox potential values for plastocyanin range from 0.37 to 0.39 v[8, 15, 24] whereas those reported for cytochrome b_{559} are 0.32 v[25] and 0.37 v.[26] Measurements in our laboratory gave for plastocyanin[27] a redox potential value of 0.40 v and for cytochrome b_{559}, 0.33 v (both at pH 8.2). These redox potential values are in agreement with the relative positions assigned to these chloroplast constituents in the electron transport chain below.

$$H_2O \rightarrow \text{IIb} \rightarrow \text{C550} \rightarrow \text{cyt } b_{559} \rightarrow \text{PC} \rightarrow \text{IIa} \rightarrow \text{Fd} \qquad (3)$$

Effect of inhibitors: With the inclusion of two light reactions in System II, it became desirable to identify the sites of action of such well-known inhibitors of that system as 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) and *o*-phenanthroline.[13] Figure 5 shows that 1×10^{-6} *M* DCMU eliminates the photooxidation of cytochrome b_{559}. Similar results were obtained with *o*-phenanthroline. It thus appears that DCMU and *o*-phenanthroline impede electron

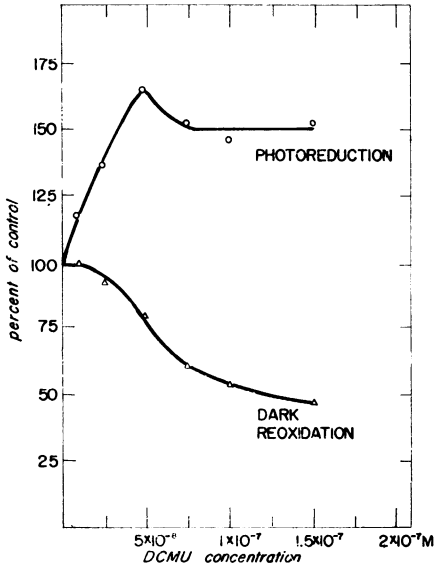

FIG. 6.—Effect of DCMU concentration on the rates of photoreduction and dark reoxidation of C550 (550 mμ minus 540 mμ). The reaction mixture contained (per 1.0 ml) P_{1s} spinach chloroplasts (equivalent to 75 μg chlorophyll) and the following in μmoles: Tricine buffer (pH 8.2), 33.3; and potassium ferricyanide, 5. DCMU added as indicated. Gas phase, air. The 664 mμ actinic light had an intensity of approximately 2.5×10^4 ergs/cm²/sec.

transport at a point between cytochrome b_{559} and light reaction IIa (See eq. 3). For reasons which we cannot explain, these findings are not in agreement with those of Cramer and Butler[12] and Levine and Gorman,[28] who reported that DCMU (1×10^{-5} *M*) blocks not the oxidation, but the reduction of cytochrome b_{559} by System II light.

As for the effect of DCMU and *o*-phenanthroline on C550, we reported earlier[4] that they do not inhibit its decrease in absorbance (photoreduction) but inhibit the dark reversal (oxidation). Figure 6 shows that DCMU increases the initial rate of photoreduction—probably by preventing electron outflow—and decreases the apparent first-order rate constant for the dark reoxidation of C550. Similar results were obtained with *o*-phenanthroline. These results are consistent with the idea that the two inhibitors block electron transport between cytochrome b_{559} and light reaction IIa.

Concluding Remarks.—Two alternative hypotheses explain the light-induced transport of electrons from water to ferredoxin, which provides (via NADP) the reducing power needed for CO_2 assimilation. A currently popular hypothesis (which this laboratory adopted in 1961[29] and abandoned in 1965[30]) holds that the photoreduction of ferredoxin by water requires the collaboration in series of System II and System I. An alternative hypothesis put forward by this laboratory[1, 13, 30] envisages that the photoreduction of ferredoxin by water involves only System II and limits the function of System I to cyclic electron transport and its experimental variant: the reduction of the ferredoxin-NADP couple by an artificial electron donor such as reduced dichlorophenol indophenol. More recently, Arnold and Azzi[31] have also concluded, on the basis of quite different considerations, that the photoreduction of ferredoxin by water is accomplished solely by System II and that System I is limited to cyclic electron flow (and phosphorylation). Their scheme envisages that System II (contain-

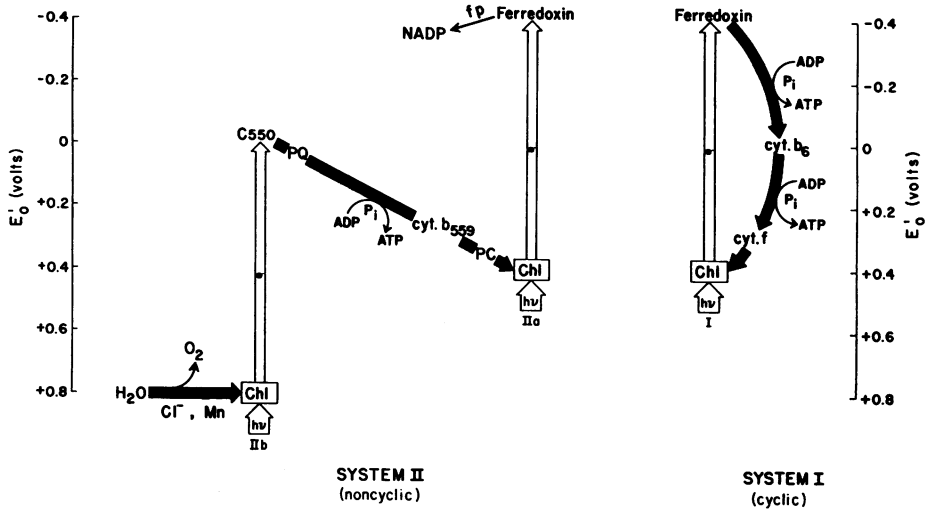

FIG. 7—Scheme for three light reactions in plant photosynthesis. System II consists of two "short wavelength" light reactions (IIb and IIa) operating in series and linked by a "dark" electron transport chain associated with noncyclic phosphorylation. Parallel to System II is System I, consisting of a "long wavelength" light reaction linked to another dark electron transport chain associated with cyclic phosphorylation. Discussed elsewhere[1, 2] are the roles of Cl^-, manganese, plastoquinone (PQ), ferredoxin-NADP reductase (fp) in System II; the roles of cytochromes b_6 and f in System I and the experimental modification of System I to give ferredoxin (and NADP) reduction by such artificial electron donors as reduced dichlorophenol indophenol dye.

ing chlorophyll *b* and *a*) absorbs two quanta of light for each electron transferred from water to ferredoxin. Their scheme provides for no chemical reactions or intermediates between the two quantum absorption acts but only for an electronic conductor system between the site of oxidation and the site of reduction.[31]

Our present concept of electron transport in System II envisages two short-wavelength light reactions (IIb and IIa) operating in series and joined by an electron transport chain that includes (but is not limited to) C550, cytochrome b_{559}, and plastocyanin and is coupled to noncyclic photophosphorylation. According to this concept, System II (comprising two light reactions) and System I (comprising one light reaction) operate in parallel (Fig. 7). The proposed concept is consistent with the following considerations: (i) The electron flow from water to ferredoxin involves only System II.[1, 31] (ii) The transfer of one electron from water to ferredoxin requires no less than two quanta.[10, 31–34] (iii) There is no enhancement[10] in the light-dependent reduction of NADP by water. (iv) Plastocyanin is a component of System II and is required for NADP reduction by electrons from water.[16–19]

Note added in proof: Our recent experiments with techniques described herein show that the photooxidation of cytochrome *f* in System I, unlike that of cytochrome b_{559} in System II, does not require plastocyanin.

We thank William Ufert for excellent technical assistance and Richard K. Chain for the preparation of plastocyanin.

* Aided by a grant from the National Institute of General Medical Sciences.

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