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SEPARATION OF THE LIGHT AND DARK REACTIONS IN ELECTRON TRANSFER DURING PHOTOSYNTHESIS

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Ever since the discovery of oxygen production by green plants, almost two hundred years ago,¹ a continuing objective of research in photosynthesis has been the chemical isolation of the first stable molecular species that is formed by trapping and converting radiant energy into chemical energy. Based on chemical isolation alone, the first physiological, stable products formed at the expense of radiant energy were identified as starch about a hundred years ago,² as phosphoglyceric acid twelve years ago,³ as TPNH_2 † eleven years ago,⁴⁻⁶ and as ATP eight years ago.⁷ In 1957 ATP formation was found to be coupled to TPN reduction⁸ and these two compounds, ATP and TPNH_2 , jointly called assimilatory power, were put forward as the first stable chemical products of photosynthesis that are subsequently used to drive the dark reactions of CO_2 assimilation.⁹ By fractionating isolated chloroplasts, the photochemical formation of ATP and TPNH_2 was separated, in time and space, from the subsequent dark reactions of carbon assimilation in chloroplasts.⁹

This article describes further progress in the separation of the light and dark reactions of photosynthesis. The earliest, chemically isolated, reductant formed at the expense of radiant energy trapped during photosynthesis has now been identified as reduced ferredoxin. These findings stem from the recent subdivision¹⁰ of the TPN reducing system in chloroplasts into two components: (a) a flavo-protein enzyme (the TPN reductase proper, now prepared in crystalline form¹¹); and (b) a nonheme iron-protein electron carrier, one of a family of ferredoxins, members of which have been shown in the last year to be widely distributed in hydrogenase-containing nonphotosynthetic bacteria^{12, 13} and also in green plants

and photosynthetic bacteria.¹⁰ (Ferredoxin of chloroplasts is the same substance as the photosynthetic pyridine nucleotide reductase (PPNR) of San Pietro, the haem-reducing factor of Davenport, Hill, and Whatley and the TPN-reducing factor of Arnon, Whatley, and Allen. This change in nomenclature, discussed in more detail elsewhere,¹⁰ is based on the recently discovered chemical and functional similarities of ferredoxins from bacteria and chloroplasts.¹⁰)

The flavoprotein TPN reductase of chloroplasts was shown to catalyze, with the aid of ferredoxin, the reduction of TPN by one of two electron donor systems: by chloroplast fragments (grana) in the light (the physiological system), or by hydrogen gas plus hydrogenase in the dark (an experimental system). Spinach ferredoxin was found¹⁰ to have a redox potential, $E_0' = -432$ mV (pH 7.55), close to that of hydrogen electrode, and is thus capable of transferring to appropriate enzyme systems the most reducing electrons in cellular metabolism: those that come from hydrogen gas or from chlorophyll "activated" by light.

The photoreduction of TPN by illuminated chloroplasts, which is reported here, has been physically separated into two steps: (a) a light-induced reduction of ferredoxin by the chloroplast pigment system, followed by (b) the dark reduction of TPN by reduced ferredoxin—a step that is catalyzed by the TPN reductase present in the chloroplasts.

Methods.—The reactions were carried out in Thunberg-type cuvettes which contained in the optical compartment the chloroplast preparation plus the ferredoxin, and had TPN in the sidearm. The progressive photoreduction of ferredoxin by illuminated chloroplasts was followed by measuring spectral changes with a Cary recording spectrophotometer. After the ferredoxin was reduced by the illuminated chloroplasts, TPN was tipped from the sidearm in the dark and the resultant reoxidation of ferredoxin was measured by the reappearance of its oxidized spectrum. Ferredoxin was prepared as previously described.¹⁰

Results.—Figure 1 shows a progressive photoreduction of "substrate" amounts of ferredoxin by the illuminated chloroplast preparation, in the absence of TPN. The ferredoxin reduced by light was stable and, under the strictly anaerobic conditions of the experiments, was not spontaneously reoxidized in a subsequent dark period. However, the reduced ferredoxin became quickly and fully reoxidized in the dark when an excess of TPN was added (Fig. 2).

The spectral changes in ferredoxin at 340, 420, and 463 $m\mu$ resulting from photoreduction and dark reoxidation by TPN are summarized in Table 1. When the photoreduced ferredoxin was reoxidized by TPN (Table 1, line 3), the increase in optical density at 340 $m\mu$ included reduced TPN which absorbs strongly at this wavelength. From this increment it was possible to compute the amount of TPN reduced and to compare it with the amount of ferredoxin reoxidized. The amount of ferredoxin was computed from the changes in optical density at 420 and 463 $m\mu$ (Table 1).

The computations show that 1 mole of TPN reoxidized 2 moles of ferredoxin according to equation (1).

Discussion and Conclusions.—The experimental approach used here in isolating and separating the light and dark steps in TPN reduction was similar to that used

FIG. 1.—Reduction of ferredoxin by light. Spectral changes in oxidized ferredoxin on illumination. Spectral changes were recorded at 2 min intervals as a difference between a sample cuvette containing 1.75 mg (0.125 μ moles) ferredoxin and a reference cuvette without ferredoxin. Both cuvettes contained, in a final volume of 3 ml washed broken chloroplasts (P_{142}), heated at 55°C for 5 min, containing 5 μ g chlorophyll and the following (μ moles): tris buffer pH 7.3, 100; sodium ascorbate, 10; and 2,6-dichlorophenolindophenol, 0.2. The reaction was carried out in Thunberg-type cuvettes in an atmosphere of argon at 20°C. Illumination was 10,000 lux.

FIG. 2.—Reoxidation of ferredoxin by TPN in the dark. The initial spectrum for photoreduced ferredoxin is that shown at 6' illumination in Figure 1. The spectrum of ferredoxin reoxidized by TPN in the dark was recorded as the difference between the sample cuvette containing 0.2 μ moles TPN (in 0.05 ml) tipped from the side arm, and the reference cuvette containing the same components except that TPN was replaced by 0.05 ml of water. Spectral change was recorded about 1 min after tipping.

TABLE 1

STOICHIOMETRY OF PHOTOREDUCTION OF SPINACH FERREDOXIN (Fd) AND ITS SUBSEQUENT REOXIDATION BY TRIPHOSPHOPYRIDINE NUCLEOTIDE (TPN) IN THE DARK

(Experimental conditions were as described in Figs. 1 and 2 except that the amount of chlorophyll was changed to 10 μ g and that of TPN to 2 μ moles)

	Wavelength (m μ) $\rightarrow$	340	Optical density 420	463
1. Oxidized ferredoxin		0.547	0.434	0.383
2. Photoreduced ferredoxin		0.461	0.257	0.196
3. Ferredoxin reoxidized by TPN in the dark		0.644	0.440	0.337
4. μ moles Fd photoreduced*		...	0.102	0.110
5. μ moles Fd reoxidized by TPN in the dark*		...	0.106	0.108
6. μ moles TPN reduced†		0.047		

* The provisional millimolar extinction coefficients for oxidized and reduced spinach ferredoxin (assuming a molecular weight of 14,000) are based on measurements of our purest preparation. Complete reduction was obtained with dithionite. The following values were obtained:

	Oxidized Fd	Reduced Fd	$\Delta\epsilon(\text{ox-red})$
$\epsilon_{340}\text{cm}^{-1}, \text{mM}^{-1}$	10.32	5.16	5.16
$\epsilon_{420}\text{cm}^{-1}, \text{mM}^{-1}$	9.30	4.25	5.05

† The millimolar extinction coefficient used for TPNH_2 is $\epsilon_{340} = 6.22 \text{ cm}^{-1}, \text{mM}^{-1}$.

earlier in the separation of the photochemical ATP and TPNH_2 formation from the dark CO_2 assimilation reactions in chloroplasts.⁹ The photochemical reaction, which is "catalytic" under physiological conditions, has been isolated and transformed into a "substrate" reaction by supplying substrate amounts of the catalyst, and by preventing the product of the photochemical reaction (reduced ferredoxin) from reacting immediately with its normal physiological acceptor, TPN.

In the absence of TPN, reduced ferredoxin is rapidly oxidized by oxygen. It has also been our experience that the accumulation of photochemically reduced ferredoxin proceeds best with a chloroplast preparation that was heated at 55°C for 5 min and thus had lost its capacity for photoproduction of oxygen¹⁴ and for re-oxidizing ferredoxin anaerobically, but still retained its ability to use ascorbate as the electron donor for the photoreduction of ferredoxin.

Bleaching by light of photosynthetic pyridine nucleotide reductase (i.e., ferredoxin in our terminology), under apparently aerobic conditions, was observed by Chance and San Pietro (cf. ref. 15) and by Horio and Yamashita.¹⁶ However, the light-induced spectral changes shown by Horio and Yamashita (see curve "L"—"D" in Fig. 2 of ref. 16) do not correspond to the spectrum of reduced ferredoxin (Figs. 1 and 2). Moreover, they report¹⁶ that "the bleached PPNR is not restored to its original color by addition of TPN in darkness immediately after illumination"—a result that is contrary to ours (Fig. 2).

Equation (1) suggests that the photoreduction of ferredoxin occurs by single electron transfer which follows photon capture in the primary photochemical act. Ferredoxin thus replaces TPN as the first isolated electron acceptor in photosynthesis. The transfer of electrons from ferredoxin to TPN is a dark reaction catalyzed by the chloroplast flavoprotein TPN reductase.

The place assigned to ferredoxin in the noncyclic electron transport system of chloroplasts is shown in Figure 3. According to this scheme the photoreduction of ferredoxin would be a prerequisite for noncyclic photophosphorylation but only when it is coupled with TPN reduction. In this sequence of reactions ferredoxin is an electron carrier between the chlorophyll pigment system and TPN. Under non-physiological conditions, when TPN is replaced by ferricyanide, noncyclic photophosphorylation occurs (in the cytochrome-chlorophyll region^{17, 18}) without ferredoxin since the photoreduction of ferricyanide is independent of ferredoxin.

As for cyclic photophosphorylation, we have previously reported¹⁰ that ferredoxin is not required for the non-physiological type that is catalyzed by phenazine methosulfate. But, as will

FIG. 3.—Diagrammatic representation of non-cyclic electron flow in chloroplasts as a composite of two light reactions and associated dark reactions. The light reactions are indicated by open arrows, the dark reactions by black arrows. OH^- is the electron donor and TPN is the electron acceptor. "Fd" stands for ferredoxin; "Q" tentatively represents plastoquinone. Standard redox potentials of some of the intermediates are indicated on the adjoining redox scale.

be reported elsewhere, ferredoxin is required for physiological cyclic photophosphorylation where it is also a component of the electron transport chain. The involvement of ferredoxin (under the name PPNR) in photosynthetic phosphorylation has also been reported by Black *et al.*¹⁹ and Horio and Yamashita.¹⁶

To recapitulate, reduced ferredoxin emerges as the most electro-negative, chemically identified substance that has been isolated following the photochemical events of photosynthesis. It is the physiological electron carrier in the reduction of TPN and in photosynthetic phosphorylation, cyclic and noncyclic.

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† Oxidized and reduced triphosphopyridine nucleotide are abbreviated, respectively, TPN and TPNH₂; adenosine triphosphate is abbreviated ATP.

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INTERACTION OF GENOTYPES DETERMINING VIABILITY IN *DROSOPHILA BUSCKII**

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It is well known that the relative fitnesses of genotypes in a population are influenced by the physical factors of the environment. There is an increasing body of experimental evidence to show that the biotic components of the environment, especially the density and genotypic composition of the population, also may have effects on relative fitness. The literature on the effect of larval density on viability