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ACTION SPECTRA OF THE THREE LIGHT REACTIONS IN PHOTOSYNTHESIS

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SUMMARY

Action spectra for the photoreduction of cytochrome b_{559} and C550 and for the photooxidation of cytochromes b_{559} and f in spinach chloroplasts were measured in the region from 640 to 720 nm. The action spectra for the photoreduction of cytochrome b_{559} and C550 are closely similar and show marked decreases in efficiency at wavelengths longer than 680 nm. These findings are consistent with the conclusion that the two components are reduced by the same photoact of Photosystem II.

The action spectrum for the photooxidation of cytochrome b_{559} also showed a decrease in efficiency at wavelengths longer than 680 nm, characteristic of Photosystem II, but the photooxidation of cytochrome f showed an increase in efficiency at long wavelengths, characteristic of Photosystem I.

These action spectra show a pattern consistent with a recently proposed concept of three light reactions in plant photosynthesis.

INTRODUCTION

Recent studies of electron transport in photosynthesis led to the discovery of C550, a chloroplast electron carrier that accepts electrons released by the photooxidation of water and in turn reduces cytochrome b_{559} (refs. 1–3):

$$H_2O \rightarrow chl_{h\nu} \rightarrow C550 \rightarrow cyt.\ b_{559} \rightarrow \cdots$$

Experiments with monochromatic light indicated that the photoreductions of C550 (ref. 1) and cytochrome b_{559} (refs. 3–6) were of greater magnitude in Photosystem II light ($\lambda < 680$ nm) than in Photosystem I light ($\lambda > 680$ nm). This and other evidence[1,7–9] indentified C550 and cytochrome b_{559} as components of Photosystem II.

The identification of the photoreduction of C550 and cytochrome b_{559} with Photosystem II was based on experiments at only two wavelengths of monochromatic light at saturating intensity[1,3]. It seemed desirable, therefore, to extend these measurements over a broader spectral range and to use limiting light intensities, thereby making it possible to obtain an action spectrum in which the effectiveness of each wavelength would be expressed on the basis of absorbed quanta.

METHODS

Broken spinach chloroplasts (P_{1S}) were prepared according to the method of WHATLEY AND ARNON[10], and Tris-treated chloroplasts by a modification[11] of the procedure of YAMASHITA AND BUTLER[12]. Sonicated chloroplasts (sonicated P_{1S1}) were prepared as described previously[3,11]. Chlorophyll was determined as described by ARNON[13].

Absorbance changes were measured with a dual wavelength spectrophotometer (Phoenix Precision Instrument Co.) as described previously[1-3,11,14].

Monochromatic light[15] was introduced through a hole in the side of the spectrophotometer as described previously[1-3,14]. The intensity of the incident monochromatic light was measured with a YSI-Kettering Model 65 radiometer and controlled with neutral density filters. The light absorbed by the samples was measured with an integrating sphere as described previously[15].

The rates reported were measured at light intensities where the reaction rates were linearly proportional to the light intensity. Averages of at least three measurements were used.

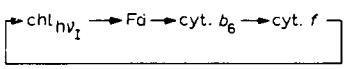
RESULTS AND DISCUSSION

Fig. 1 shows that the photoreductions of C550 and cytochrome b_{559} exhibit identical action spectra in the red region, as would be expected from the postulation that they are reduced by the same photoreaction[2,3,9]. The action spectra show a pronounced decrease in efficiency at wavelengths longer than 680 nm ("red drop"[16]) characteristic of Photosystem II (ref. 15).

Evidence was presented elsewhere[2,3] that the photoreaction ($chl_{h\nu IIb}$) that extracts electrons from water and reduces C550 and cytochrome b_{559} is linked through the copper protein plastocyanin (PC)[2,3,11] to a second short-wavelength photoreaction[2,11,14] ($chl_{h\nu IIa}$) that is responsible for the photoreduction of ferredoxin (Fd)/NADP as shown in Scheme 1:

$$H_2O \rightarrow chl_{h\nu IIb} \rightarrow C550 \rightarrow \text{cyt. } b_{559} \rightarrow PC \rightarrow chl_{h\nu IIa} \rightarrow Fd \rightarrow NADP \qquad (1)$$

A corollary of including two light reactions in Photosystem II is that it locates the other two cytochromes of chloroplasts, *f* and b_6 (refs. 7, 8), in a parallel photosystem, Photosystem I, which is driven by a third light reaction[2,9] (Scheme 2):



(2)

Photosystem I is concerned solely with cyclic electron transport and phosphorylation (and their variants) and functions most efficiently in long-wavelength light ($\lambda > 680$ nm)[15]. Thus, the three light reactions of plant photosynthesis consist of the two short-wavelength light reactions of Photosystem II acting in series and, parallel to Photosystem II, the single long-wavelength light reaction of Photosystem I.

An earlier two-light-reactions scheme[17] envisages that the photoreduction of ferredoxin/NADP by water requires the collaboration of a single short-wavelength photoreaction ($chl_{h\nu II}$) with a long-wavelength photoreaction ($chl_{h\nu I}$). The two

photoreactions were thought to be connected by a dark electron transport chain that includes cytochrome b_{559}, cytochrome *f*, and plastocyanin (Scheme 3):

$$H_2O \rightarrow chl_{h\nu}II \rightarrow cyt.\ b_{559} \rightarrow cyt.\ f \rightarrow PC \rightarrow chl_{h\nu}I \rightarrow Fd \rightarrow NADP \quad (3)$$

Both Schemes 2 and 3 predict that cytochrome *f* is photooxidized by a long-wavelength photoreaction and therefore would be oxidized most efficiently at wavelengths longer than 680 nm ("red rise"[16]). However, the two concepts differ in their predictions about the wavelength dependence for the photooxidation of cytochrome b_{599}. The three-light-reactions concept predicts that cytochrome b_{559} would be photo-

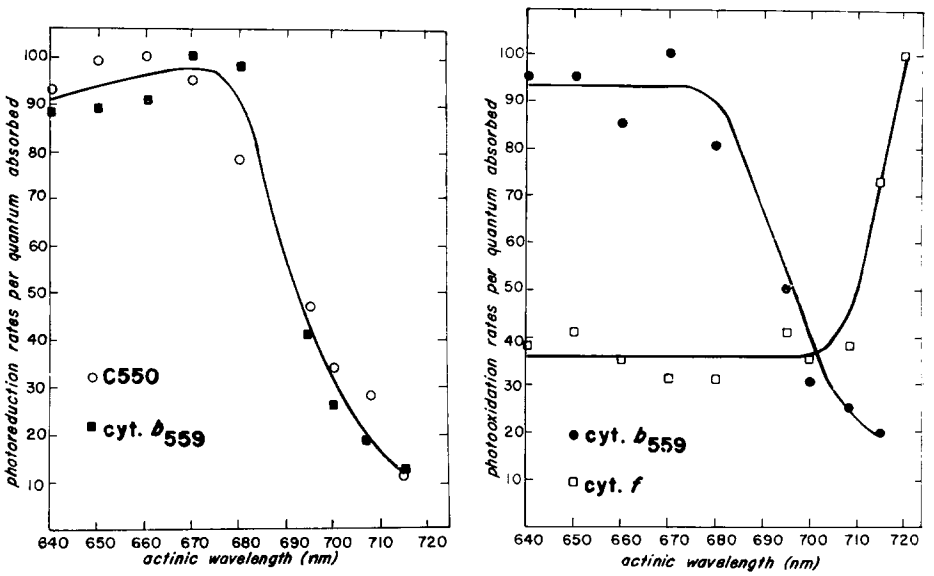


Fig. 1. Action spectra for the photoreduction of C550 (550–540 nm) and cytochrome b_{559} (560–570 nm). The photoreduction rates are given as relative rates in arbitrary units. The reaction mixture for the C550 photoreduction measurements contained, per 1.0 ml, P_{18} chloroplasts (equivalent to 37.5 μg chlorophyll) and the following in μmoles: $MgCl_2$, 2; K_2HPO_4, 5; potassium ferricyanide, 5; and Tricine [*N*-Tris(hydroxymethyl)methylglycine] buffer (pH 8.2), 33.3. The reaction mixture for the cytochrome b_{559} photoreduction measurements contained, per 1.0 ml, sonicated P_{18I} chloroplasts (equivalent to 37.5 μg chlorophyll) and the following in μmoles: $MgCl_2$, 2; K_2HPO_4, 5; potassium ferricyanide, 0.025; and MES [2-(*N*-morpholino)ethanesulfonic acid] buffer (pH 6.2), 33.3. Sonicated chloroplasts, depleted of plastocyanin, were used to prevent any competing photooxidation of cytochrome b_{559} (refs. 2, 3, 11). Gas phase, air. The incident light intensities used for C550 photoreduction ranged from 2.8 to $4.5 \cdot 10^3$ ergs/cm^2 per sec in the 640 to 695 nm region and from 1.2 to $1.6 \cdot 10^4$ ergs/cm^2 per sec in the 700 to 715 nm region. The incident light intensities used for cytochrome b_{559} photoreduction ranged from 0.64 to $1.4 \cdot 10^3$ ergs/cm^2 per sec in the 640 to 695 nm region and from 7.0 to $9.2 \cdot 10^3$ ergs/cm^2 per sec in the 700 to 715 nm region.

Fig. 2. Action spectra for the photooxidation of cytochrome *f* (554–540 nm) and cytochrome b_{559} (561–570 nm). The photooxidation rates are given as relative rates in arbitrary units. The reaction mixture contained, per 1.0 ml, Tris-treated chloroplasts (equivalent to 37.5 μg chlorophyll) and the following in μmoles: $MgCl_2$, 2; K_2HPO_4, 5; sodium ascorbate, 1; NADP, 1; ferredoxin, 0.01; and Tricine buffer (pH 8.2), 33.3. Tris-treated chloroplasts which are unable to photooxidize water[2, 11, 12, 14] were used to prevent possible competing photoreductions. The incident light intensities used for cytochrome b_{559} photooxidation ranged from 1.1 to $1.4 \cdot 10^4$ ergs/cm^2 per sec in the 640 to 695 nm region and from 2.2 to $2.8 \cdot 10^4$ ergs/cm^2 per sec in the 700 to 715 nm region. The incident light intensities used for cytochrome *f* photooxidation ranged from 1.2 to $2.2 \cdot 10^3$ ergs/cm^2 per sec in the 640 to 695 nm region and from 3.4 to $4.7 \cdot 10^3$ ergs/cm^2 per sec in the 700 to 720 nm region. Other experimental conditions were as for Fig. 1.

oxidized by the short-wavelength photoreaction ($chl_{h\nu}IIa$ in Scheme 1) and hence show a "red drop," whereas the two-light-reactions concept predicts that cytochrome b_{559} would be photooxidized by the long-wavelength photoreaction ($chl_{h\nu}I$ in Scheme 3) and therefore show a "red rise."

The action spectra (Fig. 2) show a red rise for cytochrome *f* photooxidation and a red drop for cytochrome b_{559} photooxidation. These results are in agreement with Scheme 1 but are incompatible with Scheme 3.

The observation that the photooxidation of cytochrome *f* is a Photosystem I reaction is in agreement with the findings of several laboratories[1,4-6,11,18,19]. The observation that the photooxidation of cytochrome b_{559} is a Photosystem II reaction is in agreement with our earlier results at high light intensities[3,11,14] but at variance with reports of other workers[4-6] who have attributed this reaction to Photosystem I.

In sum, the action spectra of the photooxidation of cytochrome b_{559} and cytochrome *f* show a pattern that is consistent with the concept of three light reactions in plant photosynthesis.

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REFERENCES

1 D. B. KNAFF AND D. I. ARNON, *Proc. Natl. Acad. Sci. U.S.*, 63 (1969) 963.
2 D. B. KNAFF AND D. I. ARNON, *Proc. Natl. Acad. Sci. U.S.*, 64 (1969) 715.
3 D. B. KNAFF AND D. I. ARNON, *Biochim. Biophys. Acta*, 226 (1971) 400.
4 W. A. CRAMER AND W. L. BUTLER, *Biochim. Biophys. Acta*, 143 (1967) 332.
5 G. BEN-HAYYIM AND M. AVRON, *European J. Biochem.*, 14 (1970) 205.
6 R. P. LEVINE AND D. S. GORMAN, *Plant Physiol.*, 41 (1966) 1293.
7 N. K. BOARDMAN AND J. M. ANDERSON, *Biochim. Biophys. Acta*, 143 (1967) 187.
8 D. I. ARNON, H. Y. TSUJIMOTO, B. D. MCSWAIN AND R. K. CHAIN, in K. SHIBATA, A. TAKAMIYA, A. T. JAGENDORF AND R. C. FULLER, *Comparative Biochemistry and Biophysics of Photosynthesis*, University of Tokyo Press, Tokyo ,and University Park Press, State College, Pa., 1968, p. 113.
9 D. I. ARNON, R. K. CHAIN, B. D. MCSWAIN, H. Y. TSUJIMOTO AND D. B. KNAFF, *Proc. Natl. Acad. Sci. U.S.*, 67 (1970) 1404.
10 F. R. WHATLEY AND D. I. ARNON, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 6, Academic Press, New York, 1963, p. 308.
11 D. B. KNAFF AND D. I. ARNON, *Biochim. Biophys. Acta*, 223 (1970) 201.
12 T. YAMASHITA AND W. L. BUTLER, *Plant Physiol.*, 43 (1968) 1978.
13 D. I. ARNON, *Plant Physiol.*, 24 (1949) 1.
14 D. B. KNAFF AND D. I. ARNON, *Proc. Natl. Acad. Sci. U.S.*, 63 (1969) 956.
15 B. D. MCSWAIN AND D. I. ARNON, *Proc. Natl. Acad. Sci. U.S.*, 61 (1968) 989.
16 D. I. ARNON, H. Y. TSUJIMOTO AND B. D. MCSWAIN, *Nature*, 214 (1967) 562.
17 N. K. BOARDMAN, *Ann. Rev. Plant Physiol.*, 21 (1970) 115.
18 L. N. M. DUYSENS, J. AMESZ AND B. M. KAMP, *Nature*, 190 (1961) 510.
19 M. AVRON AND B. CHANCE, *Brookhaven Symp. Biol.*, 19 (1966) 149.