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PHOTOSYSTEM II ACTIVITY OF CHLOROPLAST FRAGMENTS LACKING P700

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SUMMARY

Chloroplast fragments, prepared with the detergent Triton X-100, had a pigment composition characteristic of Photosystem II. The fragments had chlorophyll a/chlorophyll b ratios of 1.7–1.9 and had no detectable P700, either by photochemical or chemical assay. In addition, the fragments were devoid of cytochrome *f* and cytochrome b_6 but cytochrome b_{559} was present. The Triton fragments could not photoreduce $NADP^+$ from ascorbate in the presence of 2,6-dichlorophenolindophenol as the electron carrier. The Photosystem II activity of the fragments included electron-transfer reactions with either water or diphenylcarbazide as electron donor which were sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). In addition the fragments could photoreduce $NADP^+$ with ascorbate as electron donor in the presence of plastocyanin as the electron carrier. This reaction was insensitive to DCMU and proceeded more effectively with 664-nm actinic illumination than with 715-nm actinic illumination. These results are consistent with the concept of two Photosystem II reactions cooperating in noncyclic electron transport.

INTRODUCTION

The generally accepted scheme for noncyclic electron transport from water to $NADP^+$ involves the cooperation of two light reactions, one short-wavelength ($\lambda < 700$ nm) and one long-wavelength ($\lambda > 700$ nm)[1,2]. Recent experiments in our laboratory led to a different concept of noncyclic electron transport, in which photoreduction of $NADP^+$ with water is accomplished solely by Photosystem II, comprising, however, not one but two short-wavelength photoreactions operating in series[3]. The new scheme also includes the long-wavelength light reaction as a third light reaction of Photosystem I, operating in parallel to Photosystem II (ref. 4).

An essential feature of the older, two-light-reaction scheme is that the photoreduction of $NADP^+$ from water requires P700, the "reaction center chlorophyll" of Photosystem I (ref. 5), and cytochrome *f*. By contrast, the three-light-reaction concept considers these chloroplast components dispensable for noncyclic electron transport to $NADP^+$ and includes them only in the long-wavelength Photosystem I.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; DCP, diphenylcarbazide.

Although the initial concept of three light reactions was based mainly on studies with chloroplasts[3,4], subsequent support has been derived from investigations with chloroplast fragments, prepared by a combination of several treatments, deficient in P700 and cytochrome *f* and still capable of reducing $NADP^+$ (ref. 6). This communication reports a direct method for preparing Triton chloroplast fragments wholly devoid of P700 and cytochrome *f* but still capable of photoreducing $NADP^+$ and capable of other Photosystem II activity.

METHODS AND MATERIALS

Triton chloroplast fragments having Photosystem II activity were prepared by modification of previously published procedures[7-9]. Whole spinach chloroplasts were prepared by blending 75 g of deveined spinach leaves for approx. 20 sec at 4° in 150 ml of the following blending solution: 0.4 M sucrose –50 mM Tris–HCl (pH 7.8) - 10 mM NaCl. The slurry was squeezed through filtering silk and the filtrate was centrifuged for 1 min at 2500 × *g*. The pellet, containing whole chloroplasts, was resuspended in the blending solution to a final chlorophyll concentration of 1.7 mg/ml. Triton X-100 (in 0.5 M sucrose–50 mM Tris–HCl, pH 7.8) was added quickly to the chloroplast suspension to give a final Triton concentration of 2.5 % (60 mg chlorophyll per g of detergent) and the suspension incubated 5 min at 4°. The Triton suspension was then centrifuged at 2500 × *g* for 3 min; the supernatant solution from this slow centrifugation was immediately centrifuged at 30 000 × *g* for 10 min. The pellet, which was resuspended in the blending solution, was used as the Photosystem II chloroplast fragment. The chlorophyll a/chlorophyll b ratio of the Triton chloroplast fragments varied from 1.7 to 1.9, compared to a ratio of 2.6–2.9 for the starting material.

Photoreductions of $NADP^+$ and 2,6-dichlorophenolindophenol (DCIP) were measured as previously described[10]. The P700 and cytochrome content of chloroplasts and the chloroplast fragments was determined chemically from the oxidized minus reduced difference spectra measured with the Cary 14R spectrophotometer[11]. The photooxidation of P700 was measured photochemically at 77° K with a dual-wavelength spectrophotometer (D. B. KNAFF, in preparation, 1971).

Chlorophyll was determined as described by ARNON[12]. Plastocyanin, ferredoxin, and ferredoxin–$NADP^+$ reductase were prepared by standard procedures[13-15] and were gifts of Mr. R. Chain. Triton X-100 was purchased from Sigma Chemical Co. and diphenylcarbazide (DPC), from Eastman Chemical Co.

RESULTS

P700 and cytochrome composition of Triton chloroplast fragments

As already stated, treatment of whole spinach chloroplasts with Triton X-100 produces chloroplast fragments with chlorophyll a/chlorophyll b ratios of 1.7–1.9 characteristic of Photosystem II (ref. 2).

One indication that these fragments are free of Photosystem I contamination is the absence of P700. As shown in Fig. 1, there was essentially no photooxidation of P700 in the fragments. Chemical analyses for P700 also confirmed that the fragments were essentially free of this chlorophyll component; the ferricyanide *minus*

ascorbate difference spectra in the 700-nm region gave no indication of any P700 (Fig. 2).

Of the three known chloroplast cytochromes, cytochrome b_{559} is associated with Photosystem II (refs. 11, 16, 17), whereas cytochrome f and cytochrome b_6 have been found to be associated with Photosystem I (refs. 11, 16, 18). Fig. 3 shows that cytochrome f was absent but that cytochrome b_{559} was present in the Triton fragments. Cytochrome b_{559} showed a slight enrichment in the fragments (1 cytochrome b_{559} per 250 chlorophyll molecules) compared with the starting material

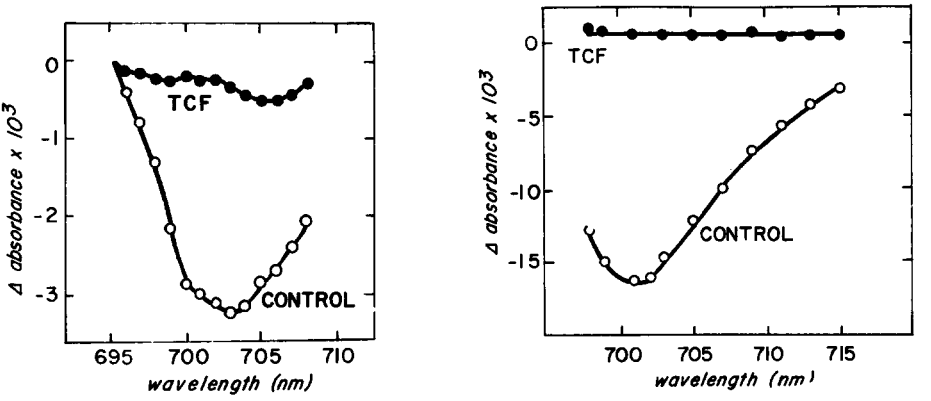

Fig. 1. Photooxidation of P700 at 77° K. The reaction mixture contained (per 1.0 ml) control chloroplasts or Triton chloroplast fragments (TCF) (equivalent to 200 μg chlorophyll), 0.5 ml glycerol, and the following in μmoles: 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer (pH 7.2), 100; sodium ascorbate, 5. Illumination was carried out with 664-nm actinic light which had an intensity of $1.5 \cdot 10^4$ ergs/cm^2 per sec.

Fig. 2. P700 content of Triton chloroplast fragments (TCF). The reaction mixture contained (per 1.0 ml) control chloroplasts or Triton chloroplast fragments (equivalent to 100 μg chlorophyll) and 100 μmoles 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer (pH 7.2). The spectra were obtained by adding 10 μmoles of sodium ascorbate to the reference cuvette and 10 μmoles potassium ferricyanide to the sample cuvette. Spectra were recorded in the Cary 14R spectrophotometer using a 1-cm light path.

TABLE I

PHOTOCHEMICAL ACTIVITIES OF TRITON PHOTOSYSTEM II FRAGMENTS WITH DCIP AS ELECTRON ACCEPTOR

The reaction mixture contained, in a final volume of 1.0 ml, chloroplast fragments (equivalent to 20 μg chlorophyll): 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer (pH 6.7), 50 μmoles; DCIP, 0.25 μmole; and, when present, DPC, 0.5 μmole, and DCMU, 0.001 μmole. All reactions were carried out with 664-nm actinic illumination of intensity $4.0 \cdot 10^4$ ergs/cm^2 per sec.

Reaction	*Q**
$H_2O \rightarrow$ DCIP	20
$H_2O \xrightarrow{+\text{DCMU}}$ DCIP	0
DPC $\rightarrow$ DCIP	90
DPC $\xrightarrow{+\text{DCMU}}$ DCIP	15

* μmoles DCIP reduced per mg chlorophyll per h.

(1 cytochrome b_{559} per 400 chlorophyll molecules). The absence of cytochrome *f* in these fragments was also confirmed by examining the ascorbate *minus* ferricyanide difference spectrum of fragments which had been extracted with 80 % acetone[11,16].

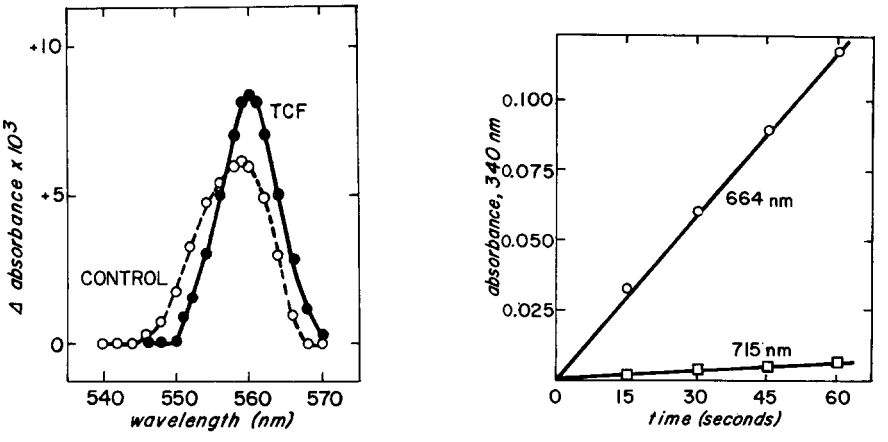

Fig. 3. Reduced-*minus*-oxidized difference spectra in the cytochrome region of Triton chloroplast fragments (TCF). The reaction mixture contained (per 1.0 ml) control chloroplasts or Triton chloroplast fragments (equivalent to 100 μg chlorophyll) and 100 μmoles 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer (pH 7.2). The spectra were obtained by adding 10 μmoles sodium ascorbate to the sample cuvette and 10 μmoles potassium ferricyanide to the reference cuvette. Light path, 1 cm. Gas phase, air.

Fig. 4. Effect of monochromatic illumination on $NADP^+$ photoreduction by Triton chloroplast fragments. The reaction mixture contained (per 1.0 ml) Triton chlorophyll fragments (equivalent to 50 μg chlorophyll) and the following in μmoles: 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer (pH 6.7), 50; ferredoxin, 0.01; $NADP^+$, 2; sodium ascorbate, 20; DCMU, 0.001; plastocyanin, 0.01; and ferredoxin–$NADP^+$ reductase equivalent to an absorbance of 0.008 at 456 nm. Both the 664- and 715-nm monochromatic light beams had an intensity of approx. $4 \cdot 10^4$ ergs/cm^2 per sec. Reactions were carried out in a Gilford spectrophotometer at 25° using a light path of 0.2 cm.

TABLE II

PHOTOCHEMICAL ACTIVITIES OF TRITON PHOTOSYSTEM II FRAGMENTS WITH $NADP^+$ AS ELECTRON ACCEPTOR

The reaction mixture contained, in a final volume of 1.0 ml, chloroplast fragments (equivalent to 50 μg chlorophyll): 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer (pH 6.7), 50 μmoles; ferredoxin, 0.01 μmole; $NADP^+$, 2 μmoles; ferredoxin–$NADP^+$ reductase, equivalent to an absorbance of 0.008 at 456 nm; ascorbate, 20 μmoles; DCMU, 0.001 μmole; and, where present, DCIP, 0.1 μmole and plastocyanin, 0.01 μmole. Illumination was carried out as described in Table I.

Reaction	*Q**
Ascorbate $\xrightarrow{+\text{DCMU}}$ $NADP^+$	0
Ascorbate + DCIP $\xrightarrow{+\text{DCMU}}$ $NADP^+$	0
Ascorbate + plastocyanin $\xrightarrow{+\text{DCMU}}$ $NADP^+$	110
Ascorbate + plastocyanin + DCIP $\xrightarrow{+\text{DCMU}}$ $NADP^+$	105

* μmoles $NADP^+$ reduced per mg chlorophyll per h.

In addition, cytochrome b_6 was absent. These findings are similar to those recently reported by other workers for their heavy Triton fragments[8,9].

Thus, the Triton fragments were found to lack two components, P700 and cytochrome *f*, considered essential in the two-light-reaction scheme for electron transport from water to $NADP^+$.

Photochemical activity of Triton chloroplast fragments

The photochemical activity of the Triton fragments was measured using DCIP and $NADP^+$ as electron acceptors. The results with DCIP are shown in Table I. The fragments were able to photoreduce DCIP with water as the electron donor or with the artificial electron donor, DPC. In each case, the reaction was sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). The light-induced electron transport from DPC to DCIP observed here has been shown by VERNON AND SHAW[19] to be a probe for Photosystem II activity in photosynthetic systems where oxygen-evolving capacity has been lost or damaged.

The photoreduction of $NADP^+$ by the Triton fragments was measured with either water or ascorbate as the electron donor. With ascorbate, various carriers were tested for their ability to mediate electron transfer, and the results of these photochemical assays are shown in Table II. The fragments could not photoreduce $NADP^+$ with DCIP as the electron carrier; other carriers, such as diaminodurene, *p*-phenylenediamine, and tetramethyl-*p*-phenylenediamine (each at a concentration of 0.1 mM) were also inactive in supporting $NADP^+$ photoreduction.

The Triton fragments were, however, able to photoreduce $NADP^+$ when ascorbate–plastocyanin acted as the electron donor couple. This reaction was found to be insensitive to DCMU. These characteristics would indicate that this reaction is associated with Photosystem I but the finding that the Triton fragments lack P700 indicated that the photoreduction of $NADP^+$ from ascorbate–plastocyanin might proceed *via* Photosystem II instead of Photosystem I. Support for this idea was obtained by studying the effect of monochromatic illumination on the ascorbate–plastocyanin → $NADP^+$ reaction (Fig. 4) because $NADP^+$ photoreduction was found to be more effective with Photosystem II light (664 nm) than with Photosystem I light (715 nm).

Although the Triton fragments were capable of photoreducing $NADP^+$ from ascorbate–plastocyanin, no photoreduction of $NADP^+$ occurred with water as the electron donor. It has previously been shown that detergent treatment removes plastocyanin from chloroplasts[11] and that the subsequent addition of plastocyanin restores $NADP^+$ photoreduction from water. However, in the case of the Triton fragments, no $NADP^+$ photoreduction with water as donor could be detected even when plastocyanin was added to the reaction mixture.

CONCLUDING REMARKS

The new concept of noncyclic electron transfer in chloroplasts by our laboratory includes the cooperation of two Photosystem II reactions in the photoreduction of $NADP^+$ from water[3]:

$$H_2O \rightarrow h\nu_{IIb} \rightarrow C550 \rightarrow \text{cyt } b_{559} \rightarrow \text{plastocyanin} \rightarrow h\nu_{IIa} \rightarrow \text{ferredoxin} \rightarrow NADP^+$$

According to this scheme, photoreaction IIb oxidizes water and reduces C550, while photoreaction IIa oxidizes plastocyanin and reduces ferredoxin. These two light reactions are joined by a dark electron-transfer chain which includes cytochrome b_{559}; cytochrome *f* and P700 are not considered to be carriers in the noncyclic electron-transfer chain.

The Triton fragments have photochemical activity associated with photoreaction IIb since they are able to carry out DCMU-sensitive electron-transfer reactions. DCMU has previously been shown to inhibit Photosystem II reactions at a site between C550 and cytochrome b_{559} (ref. 3). The demonstration of DCMU-sensitive reactions in these fragments is in agreement with the previously reported properties of the Triton fragments prepared by VERNON and co-workers[8,19].

The Triton fragments had no Photosystem I activity as measured by the characteristic and widely used test for such activity, *i.e.* the photoreduction of $NADP^+$ by the ascorbate–DCIP couple[7,18,20–24]. Although the Triton fragments were unable to use the ascorbate–DCIP couple as an electron donor, they were capable of photoreducing $NADP^+$ with an ascorbate–plastocyanin donor system, and this reaction was not stimulated by the addition of DCIP. The inability of DCIP to influence electron transfer from ascorbate to $NADP^+$, either in the presence or in the absence of plastocyanin, distinguishes the Triton fragments from other subchloroplast preparations[7,18,24].

Recent experiments with Photosystem I chloroplast fragments have shown that plastocyanin (reduced by ascorbate) can donate electrons directly to P700 and hence serve as the electron donor for $NADP^+$ photoreduction[26]. The experiments with the Triton fragments have shown that the ascorbate-plastocyanin couple can also serve as the electron donor for $NADP^+$ photoreduction in a chloroplast preparation lacking detectable P700. The evidence presented in this study is therefore in agreement with the new scheme for noncyclic electron transport in chloroplasts in which the photoreduction of $NADP^+$ by ascorbate–plastocyanin would proceed *via* photoreaction IIa in the absence of P700.

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REFERENCES

1 N. K. BOARDMAN, *Adv. Enzymol.*, 30 (1968) 1.
2 N. K. BOARDMAN, *Annu. Rev. Plant Physiol.*, 21 (1970) 115.
3 D. B. KNAFF AND D. I. ARNON, *Biochim. Biophys. Acta*, 226 (1971) 400.
4 D. B. KNAFF AND D. I. ARNON, *Proc. Natl. Acad. Sci. U.S.*, 64 (1969) 715.
5 B. KOK AND G. HOCH, in W. D. MC ELROY AND B. GLASS, *Light and Life*, Johns Hopkins Press, Baltimore, 1961, p. 397.
6 D. I. ARNON, R. K. CHAIN, B. D. MC SWAIN, H. Y. TSUJIMOTO, AND D. B. KNAFF, *Proc. Natl. Acad. Sci. U.S.*, 67 (1970) 1404.
7 L. P. VERNON, E. R. SHAW AND B. KE, *J. Biol. Chem.*, 241 (1966) 4101.
8 L. P. VERNON, B. KE, H. H. MOLLENHAUER, AND E. R. SHAW, in H. METZNER, *Progress in Photosynthesis Research*, Vol. 1, Laupp, Tübingen, 1969, p. 137.
9 G. HIND AND H. Y. NAKATANI, *Biochim. Biophys. Acta*, 216 (1970) 223.

10 B. D. Mc Swain and D. I. Arnon, *Proc. Natl. Acad. Sci. U.S.*, 61 (1968) 989.
11 D. I. Arnon, H. Y. Tsujimoto, B. D. Mc Swain, 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.
12 D. I. Arnon, *Plant Physiol.*, 24 (1949) 1.
13 S. Katoh, I. Shiratori and A. Takamiya, *J. Biochem.* Tokyo, 51 (1962) 32.
14 M. Shin, K. Tagawa and D. I. Arnon, *Biochem. Z.*, 338 (1963) 84.
15 M. Losada and D. I. Arnon, in H. F. Linskens, B. D. Sanwal, and M. V. Tracey, *Modern Methods of Plant Analysis*, Vol. 6, Springer, Berlin, 1964, p. 569.
16 N. K. Boardman and J. Anderson, *Biochim. Biophys. Acta*, 143 (1967) 187.
17 D. B. Knaff and D. I. Arnon, *Proc. Natl. Acad. Sci. U.S.*, 63 (1969) 956.
18 P. V. Sane, D. J. Goodchild and R. B. Park, *Biochim. Biophys. Acta*, 216 (1970) 162.
19 L. P. Vernon and E. R. Shaw, *Biochem. Biophys. Res. Commun.*, 36 (1969) 878.
20 L. P. Vernon and W. S. Zaugg, *J. Biol. Chem.*, 235 (1960) 2728.
21 A. T. Jagendorf and M. Margulies, *Arch. Biochem. Biophys.*, 90 (1960) 184.
22 D. S. Gorman and R. P. Levine, *Proc. Natl. Acad. Sci. U.S.*, 54 (1965) 1665.
23 M. Avron and A. Shneyour, *Biochim. Biophys. Acta*, 226 (1971) 498.
24 J. M. Anderson and N. K. Boardman, *Biochim. Biophys. Acta*, 112 (1966) 403.
25 H. Y. Tsujimoto, B. D. Mc Swain, R. K. Chain and D. I. Arnon, in H. Metzner, *Progress in Photosynthesis Research*, Vol. 3, Laupp, Tübingen, 1969, p. 1241.
26 D. C. Fork and N. Murata, *Photochem. Photobiol.*, 13 (1971) 33.