A Photochemically Active Particle Derived from Chloroplasts by the Action of the Detergent Triton X-100*

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LEO P. VERNON, ELWOOD R. SHAW, AND BACON KE

From the Charles F. Kettering Research Laboratory, Yellow Springs, Ohio 45387

SUMMARY

Treatment of spinach chloroplasts with 4% Triton X-100 yields fragments which are separable by centrifugation. In the medium used (which is 4% in Triton X-100 and 0.5 M in sucrose) for the initial incubation particles sedimented by centrifugation at 144,000 × g for 1, 3, and 8 hours are relatively inactive in the partial photochemical reactions representative of photosynthesis. These particles contain an increased amount of chlorophyll b when compared with chloroplasts. These particles could represent Pigment System 2 which is involved in oxygen evolution in the intact chloroplast but is inactivated by the detergent treatment.

The supernatant fluid from the centrifugation described above contains particles which are sedimented by 10 hours of centrifugation at 144,000 × g after diluting with an equal volume of water. These particles exhibit a high rate of nicotinamide adenine dinucleotide phosphate photoreduction, with rates as high as 1980 pmoles of NADP photoreduced per hr per mg of chlorophyll observed. This reaction requires the presence of spinach ferredoxin, ferredoxin-NADP reductase, and plastocyanin in addition to an electron donor system of ascorbate-2,6-dichloroindophenol (ascorbate alone is only moderately active) or ascorbate- N,N,N',N'-tetramethyl-p-phenylenediamine. This activity is heat-labile, being destroyed by heating to 40° for 5 min. Other photochemical reactions supported by this particle include methyl red reduction (supported by ascorbate-dichloroindophenol), ferrocyanochrome c photooxidation (aerobic), and ferricytochrome c photoreduction (supported by reduced trimethylbenzoquinone). Methyl red photoreduction activity is also destroyed by heating, while ferricytochrome c photoreduction activity is only partially destroyed by heating to 70°. Methyl red photoreduction and ferrocyanochrome c photooxidation also are stimulated by plastocyanin.

The photoactive particles contain low amount of chlorophyll b, show an unresolved cytochrome spectrum indicating the presence of cytochrome f and b6, exhibit an increased absorbance at 700 mµ, and show a greatly enhanced P700 absorption change and electron spin resonance spectrum upon illumination. The particle is only weakly fluorescent and does not emit any delayed light following illumination. All these properties indicate that this particle retains the photoactive Pigment System 1 which is responsible for NADP photoreduction in photosynthesis.

Detergents react readily with the chloroplast system to give fragments of varying size, or even solubilized chlorophyll (1, 2). (The reader is referred to Reference 2 for a comprehensive review of the particle types obtained from chloroplasts; for our purposes we need only consider those obtained by the action of detergents.) The three detergents most used for fragmenting chloroplasts are digitonin, sodium deoxycholate, and Triton X-100, but sodium deoxycholate (3), Zephiran chloride and saponin (4), Tween 20 (4, 5), Duponol C and Span 80 (6), and several anionic and cationic detergents have also been employed (7). The use of sodium deoxycholate produces two fragments which are photochemically inactive in the Hill reaction or nicotinamide adenine dinucleotide phosphate photoreduction (8, 9). These particles differ in the ratio of chlorophyll a to chlorophyll b and in carotenoid composition; the particle with the higher chlorophyll a to chlorophyll b ratio contains significantly more β-carotene and relatively less of the oxygenated xanthophylls. The quinones are equally distributed between the two particles. Digitonin treatment produces particles of varying size which differ in terms of their chlorophyll composition and photochemical activity. The experiments performed by Boardman and Anderson (10, 11) and extended by Wessels (12) show that the heavier particles are more active in the dichloroindophenol Hill reaction, and less active in NADP photoreduction coupled to ascorbate-DCl, and contain relatively more chlorophyll b. Therefore, the lighter particles appear to be enriched in the structural assembly related to Pigment System 1 (the long wave length pigment system) which is directly involved in NADP photoreduction. The heavy

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particles are more closely related to Pigment System 2, the oxygen evolving system.

Triton X-100 at low concentrations has profound effects upon the photosynthetic electron transfer processes and associated adenine triphosphate formation (13–15). Higher concentrations liberate a chlorophyll a-protein complex which has been extensively studied by Kahn (16, 17); it is inactive in the usual photochemical reactions of chloroplasts. Vernon and Shaw (18) studied the effect of varying concentrations of this detergent upon the photochemical reactions of chloroplasts and concluded that whereas low concentrations merely uncouple phosphorylation, high concentrations drastically alter the structure to allow the simplified electron transfer reactions related to Pigment System 1 to become evident, while inhibiting completely oxygen evolution and reactions related to Pigment System 2. The present paper is an extension of the earlier investigation, and concerns the physical and photochemical properties of a small particle isolated after treatment with Triton X-100. The evidence indicates this particle is the portion of the photosynthetic apparatus involved in Pigment System 1.

**EXPERIMENTAL PROCEDURE**

The procedure used for preparation of spinach chloroplasts and the characteristics of the detergent Triton X-100 have been previously described (18). Chloroplasts from 400 g of spinach were exposed to 4% detergent solution (30 mg of chlorophyll per g of detergent) which was 0.5 M in sucrose and 0.05 M in phosphate buffer, pH 7.7. After 1 hour in an ice bath, the suspension was centrifuged at 10,000 × g for 20 min to remove large particles, which were discarded. The supernatant material from this treatment was centrifuged sequentially for separate periods of 1, 3, and 8 hours at 144,000 × g. The material sedimenting during these periods is referred to as P-1, P-3, and P-8, designating the chloroplast particle in the residue remaining after the supernatant material was removed. After the last centrifugation the supernatant material was diluted with an equal volume of distilled water, and recentrifuged for 10 hours. The material sedimenting from this operation is designated P-D10. The supernatant fluid from this final operation still contained chlorophyll which appeared to be primarily solubilized chlorophyll. In all cases the sedimented material was suspended by homogenizing in a solution which was 0.5 M in sucrose and 0.05 M in phosphate buffer, pH 7.7. The concentrations of chlorophyll a and chlorophyll b were determined by the procedure of Vernon (19).

The apparatus used and the procedures employed for determining NADP photoreduction, methyl red photoreduction coupled to ascorbate-DCI and ascorbate TPD, cytochrome e photoreduction coupled to trimethylhydroquinone, and the photooxidation of ferrocytochrome c were the same as previously described (18). The reactions involving NADP photoreduction required the presence of ferredoxin, ferredoxin-NADP reductase, and plastocyanin, which were prepared as before (18). The light intensity employed for photochemical reactions (after passing through the red Corning filter No. 2403) was 2 × 10⁵ ergs per cm² per sec. A modified Beckman DB recording spectrophotometer was utilized (20). The ESR spectra were determined by Mr. R. W. Treharne on the apparatus used in his investigations (21). The absorbance changes related to P700 were determined with equipment described elsewhere (22).

**RESULTS**

The various fractions obtained following treatment with Triton X-100 were all inactive in a DCI Hill reaction, indicating that the oxygen-evolving apparatus has been destroyed. This was expected, since a previous study had shown that concentrations of this detergent above 0.01% inactivated chloroplasts for oxygen evolution (18). Table I shows the distribution of photochemical activities among the various fractions. For all of the reactions studied, the greatest activity was located in the light P-D10 fraction obtained by centrifugation at 144,000 × g for 10 hours following dilution of the suspending medium with distilled water. All of these reactions are catalyzed by spinach chloroplasts which have been damaged to the extent that they will not evolve oxygen. These reactions are representative of Pigment System 1, the long wave length system. A crude preparation of spinach ferredoxin was utilized for the NADP photoreduction. Additional activity could be observed under anaerobic conditions and in some cases upon addition of plastocyanin. The requirement for plastocyanin and the higher rates observed with purified enzymes are shown below in Table II. Plastocyanin markedly stimulated methyl red photoreduction and was required to show significant rates of cytochrome c photooxidation. These reactions are also discussed below.

**TABLE I**

<table>
<thead>
<tr>
<th>Particle</th>
<th>Photochemical activities</th>
<th>NADP reduction</th>
<th>Methyl red reduction</th>
<th>Cytochrome c oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>umoles/hr/mg of chlorophyll</td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>P-1</td>
<td>7</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>P-3</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>P-8</td>
<td>193</td>
<td>71</td>
<td>25</td>
<td>55</td>
</tr>
<tr>
<td>P-D10</td>
<td>320</td>
<td>92</td>
<td>30</td>
<td>60</td>
</tr>
</tbody>
</table>

**NADP Photoreduction—**NADP photoreduction activity with P-D10 depends upon the presence of an appropriate electron donor, and the fastest rates were observed when the DCI ascorbate couple was used (Fig. 1). Ascorbate alone was active, but the addition of TPD or DCI increased the activity. The chloroplast particle derived by sonic disruption (23) is less dependent upon the presence of DCI in the reaction mixture. The reason for this difference is not immediately apparent but is most likely due to different reaction conditions. Katoh and San Pietro employed higher concentrations of plastocyanin along with purified ferredoxin and ferredoxin-NADP reductase (23).

The photochemically active P-D10 particle requires three enzymes to accomplish NADP photoreduction: ferredoxin, ferredoxin-NADP reductase, and plastocyanin, as shown in Table II. There appears to be some ferredoxin-NADP reductase and plastocyanin on the particle, since addition of purified ferredoxin alone

The terms P-D10, P-1, P-3, and P-8 are defined in "Experimental Procedure," Paragraph 1.
TABLE II
Enzymatic requirements for NADP photoreduction by spinach chloroplast fragment

In Experiment A the reaction mixture contained 0.04 M phosphate buffer (pH 7.0), 0.4 M sucrose, 0.5 mM NADP, 5 mM ascorbate, 67 μM DCI, P D10 fraction equivalent to 28 μg of chlorophyll and, where indicated, saturating amounts of crude spinach ferredoxin (24), 3.0 units of purified spinach ferredoxin having a specific activity of 22 (25), 1.0 μM plastocyanin, and 85 μg of a purified ferredoxin:NADP reductase. In Experiment B the experimental conditions are those given by Katoh and San Pietro (23). These experiments were performed at pH 6.0 with higher concentrations of ferredoxin and ferredoxin-NADP reductase.

<table>
<thead>
<tr>
<th>Additions made to P-D10 particle</th>
<th>NADF reduced (μmoles/hr/mg chlorophyll)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment A</td>
<td></td>
</tr>
<tr>
<td>1. Crude ferredoxin</td>
<td>250</td>
</tr>
<tr>
<td>2. Purified ferredoxin</td>
<td>10</td>
</tr>
<tr>
<td>3. Ferredoxin-NADP reductase</td>
<td>0</td>
</tr>
<tr>
<td>4. Plastocyanin</td>
<td>0</td>
</tr>
<tr>
<td>2 + 3</td>
<td>30</td>
</tr>
<tr>
<td>2 + 4</td>
<td>82</td>
</tr>
<tr>
<td>3 + 4</td>
<td>0</td>
</tr>
<tr>
<td>2 + 3 + 4</td>
<td>238</td>
</tr>
<tr>
<td>Experiment B</td>
<td></td>
</tr>
<tr>
<td>1. Purified ferredoxin, ferredoxin-NADP reductase</td>
<td>1025</td>
</tr>
<tr>
<td>+ 4.7 μM plastocyanin</td>
<td></td>
</tr>
<tr>
<td>+ 9.0 μM plastocyanin</td>
<td>1660</td>
</tr>
<tr>
<td>+13.7 μM plastocyanin</td>
<td>1980</td>
</tr>
<tr>
<td>+13.7 μM plastocyanin minus DCI</td>
<td>675</td>
</tr>
</tbody>
</table>

causes slight activity. It is clear, however, from the reactivation when all three purified enzymes are present, that all three are required in the reaction. This resembles the pattern observed for intact chloroplasts, and indicates that there is considerable organization and structural integrity remaining in this particle even after treatment with the detergent. This is borne out by the data presented below on heat inactivation of the particle.

The rates observed for NADP photoreduction by the P-D10 particle under the conditions given for Experiment A of Table II were consistently lower than those observed by Katoh and San Pietro for the same reaction with chloroplast particles obtained by sonic treatment (23). The maximal rate observed for a number of preparations under the conditions of Experiment A was 475 μmoles per hour per mg of chlorophyll. The data of Experiment B in Table II (which were made available by Dr. S. Katoh) show that much higher rates were obtained for the P-D10 particle under the experimental conditions of Katoh and San Pietro (23), which include a pH of 6.0 and higher levels of plastocyanin. At a concentration of 13.7 μM plastocyanin, the reaction was not saturated, and the observed rate of 1850 μmoles per hour per mg of chlorophyll is not the maximal rate possible. In such a complex system involving the chloroplast fragment, three enzymes, ascorbate, DCI, and light intensity as variables which would affect the rate of the reaction, it is not possible to predict what might be the maximal rate of the photoreaction. The data do show, however, that the particle is capable of catalyzing this reaction at a rate that is higher than the photoreactions usually observed for the intact chloroplast.

**Fig. 1.** Photoreduction of NADP by spinach chloroplast particle (P-D10) in the presence of ascorbate-DCI (ASC, DPIP) or ascorbate-TPD (ASC, TMPD) as electron donor systems. The experimental conditions are described in “Experimental Procedure.” The reaction mixture of 3.0 ml contained 5.3 mM ascorbate, 67 μM DCI or TPD, 0.05 M phosphate buffer (pH 7.0), 0.5 mM NADP, chloroplast particles (P-D10) equivalent to 22 μg of chlorophyll, and a saturating amount of crude spinach ferredoxin (0.3 ml of a preparation which contained 14 mg of protein per ml). The reactions were performed under aerobic conditions.

**Fig. 2.** Stimulation of methyl red photoreduction by chloroplast particle (P-D10) by plastocyanin, under anaerobic conditions. The reaction mixture contained 0.1 mM methyl red, 5.3 mM ascorbate (ASC), 0.05 M phosphate buffer, pH 7.0, chloroplast fragments (P-D10) equivalent to 33 μg of chlorophyll, and, where indicated, 0.13 mM TPD (TMPD) 0.2 mM DCI (DPIP), and 1.0 μM plastocyanin (PC).
cyanin either to the ascorbate-DC1 or ascorbate-TPD systems (26). Since the rate of methyl red photoreduction by isolated, solubilized chlorophyll a is about 4 times that of chlorophyll in the chloroplast fragment, the earlier data reflect primarily the reaction catalyzed by the solubilized chlorophyll. In the present case, there is not an absolute requirement for plastocyanin, but it does increase the rate observed with ascorbate-DC1 or ascorbate-TPD.

**Cytochrome c Photooxidation**—Chloroplasts treated with detergents gain the ability to photooxidize ferrocytochrome c (5, 18, 27). The P-D10 fraction also has this activity if plastocyanin is present. Fig. 3 shows the marked stimulation observed in the presence of the copper protein. The plastocyanin requirement for cytochrome c photooxidation was shown earlier by Kok and Ruranski for Tween 20-treated chloroplasts (5) and by Vernon and Shaw (18) for chloroplasts treated with Triton X-100.

**Cytochrome c Photoreduction**—Vernon and Shaw have reported on a reaction catalyzed by both chloroplasts and solubilized chlorophyll a, the photoreduction of ferricytochrome c in the presence of trimethylhydroquinone (28). Fig. 4 presents data on the activity of the P-D10 fraction in this reaction, with either trimethylhydroquinone or hydrazobenzene as the donor molecule. The mechanism of the chlorophyll a-sensitized reaction involves a primary reduction of trimethylbenzoquinone (through oxidation reactions some trimethylbenzoquinone always accompanies the trimethylhydroquinone added) by the photoexcited chlorophyll a, followed by a chemical reduction of chlorophyll+ by trimethylhydroquinone3 (28, 29). Further support for this mechanism is shown in Fig. 4, both for the chloroplast fragment and for chlorophyll a solubilized by Triton X-100. Regardless of whether trimethylhydroquinone or hydrazobenzene was used as the donor molecule, for the chloroplast particle, the reaction was markedly stimulated by the addition of trimethylbenzoquinone. Addition of Triton X-100 to the particles allowed a much faster reaction to proceed, most likely caused by chlorophyll which had been solubilized by the detergent. As shown in Fig. 4B, chlorophyll a catalyzes a much faster reaction than does the chloroplast fragment. A marked stimulation by trimethylbenzoquinone is shown when hydrazobenzene is the donor. The data in Fig. 4B are interesting, since in this case different molecules are used for the acceptor (trimethylbenzoquinone) and donor (hydrazobenzene) functions of the photo-reaction. This allows a clearer separation of the function of these molecules in this facile photo-reaction.

**Heat Stability**—The stability of the P-D10 particle toward heating is shown by the data in Fig. 5. The NADP photoreduction activity was most labile, and was not observed after heating to 40° for 5 min. The methyl red reaction was less sensitive, but it was destroyed by heating to 50°. Least sensitive was the reaction involving cytochrome c and trimethylhydroquinone, which showed only moderate loss of activity. These data are in agreement with the requirements for the three reactions, since

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**Fig. 3.** Stimulation of cytochrome c photooxidation aerobically by plastocyanin (PC) with chloroplast particles (P-D10). The reaction mixture contained, in 3.0 ml, 1.7 mM sodium azide, 0.05 M phosphate buffer (pH 7.0), 1 mg of reduced cytochrome c (horse heart) and chloroplast particles equivalent to 33 µg of chlorophyll.

**Fig. 4.** Cytochrome c photoreduction by chloroplast fragments (P-D10) or purified chlorophyll a with trimethylhydroquinone or hydrazobenzene as electron donors. All reactions were performed under anaerobic conditions. A, the reaction mixtures of 3.0 ml contained 2 µg of cytochrome c (horse heart), 0.05 M phosphate buffer, pH 7.0, chloroplast particles (P-D10) equivalent to 33 µg of chlorophyll, and, where indicated, 67 µM trimethylbenzoquinone (TMQ), 67 µM trimethylhydroquinone (TMQH), 133 µM hydrazobenzene (HAB), and 0.1% Triton X-100. B, the reaction mixtures contained 2 µg of cytochrome c, 0.05 M phosphate buffer, pH 7.0, 20 µg of purified chlorophyll a, 0.1% Triton X-100, and, where indicated, 33 µM trimethylbenzoquinone and 33 µM hydrazobenzene.

**Fig. 5.** Photochemical activities of heated chloroplast particles (P-D10). The reaction conditions were those given for Figs. 1, 2, and 4. Ascorbate-DC1 was the donor system used, and for cytochrome c (Cyt c) photoreduction no trimethylbenzoquinone was added with trimethylhydroquinone (TMQH). The particles were heated for 5 min at the designated temperatures.

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3 The chlorophyll+ is formed from photoexcited chlorophyll when it donates an electron to an acceptor molecule.
NADP photoreduction requires the addition of three enzymes and would be most sensitive to structural alterations produced by heating. The cytochrome c photoreduction reaction is readily catalyzed by isolated chlorophyll a alone, and the portion of the reaction caused by nonorganized chlorophyll should be stable to heating. It appears that some chlorophyll on the chloroplast fragment is still accessible to these reagents even following extensive heating.

Composition and Physical Properties

Absorption Spectra—The small chloroplast fragment described in the papers of Boardman and Anderson (10, 11) and Wessels (12) resembles the one we have investigated in its ability to catalyze NADP photoreduction. The particle described by these investigators, prepared by digitonin treatment, had a significantly higher chlorophyll a to chlorophyll b ratio than the original chloroplast, indicating that chlorophyll b was either removed during the treatment, or that the particle isolated was a structural entity which contained relatively less chlorophyll b. The same situation applies in the present investigation. Fig. 6 shows the absorption spectra obtained for two of the heavier fractions and the lighter P-D10 particle which is active in NADP photoreduction. These spectra clearly show the different chlorophyll b contents of the two particles, which are also manifest in a difference spectrum obtained with two preparations. The chlorophyll a to chlorophyll b ratios for the P-1 and P-D10 fractions are 2.0 and 5.7, respectively. Although the P-1 fraction has relatively more chlorophyll b, its absorption maximum in the red is located about 2 mμ farther toward the red than that of the P-D10 particle. This difference in location of the maxima must reflect different types of binding of the chlorophyll, at least in part, within the two particle types. Analysis of the absorption spectra shows increased absorption in the 700 mμ region for the P-D10 particle. This is also apparent as a minimum in the difference spectrum. This indicates the accumulation of a long wave length form of chlorophyll a in P-D10, which would agree with the data presented below on absorption changes and ESR properties of the P-D10 fraction. All data indicate an accumulation of P700, the reaction center chlorophyll of Pigment System 1.

Light-induced Absorbance Change—Examination of the P-1 and P-D10 particles for their light-induced absorbance changes related to P700 photoreduction gave the data shown in Fig. 7. Both particles were examined at 430 and 705 mμ, since absorp-
The reason for the unusual distribution of activity for made, the NADP photoreduction activity for some reason was photoreduction. P-l fragment. These data indicate that the P700 activity (as presented in Table III).

In one (not reported in this paper) of a total of 15 preparations made, the NADP photoreduction activity for some reason was concentrated in the P-8 fraction. In this case the P-8 fraction also showed the greatest light-induced absorbance change at 430 mp. The reason for the unusual distribution of activity for NADP photoreduction in this one case is not known, but it did allow a direct correlation to be made between an active Pigment System 1 (NADP photoreduction) and the 430- and 705-mp absorbance changes. By way of comparison, the 430- and 705-mp changes in the P-D10 fraction reported in Fig. 7 are themselves 4- to 5-times greater than those observed for digitonin-treated (22, 30) or sonically treated 4 spinach chloroplasts.

**Pigment Composition**—The cytochrome composition of the active particle, P-D10, was investigated by extracting the chlorophyll with acetone and determining the difference spectrum of the intact, unextracted P-D10 particle. The cytochrome content was calculated by the use of 19,680 as the difference molar extinction coefficient at 555 mp. Numerical data are presented in Table III.

The relative proportion of cytochrome, P700, and total chlorophyll was determined for a P-D10 preparation. These data allow calculation of a ratio of about 140 to 200 chlorophyll molecules per cytochrome or P700, which indicates some concentration of the cytochrome and P700 in this preparation over that found in chloroplasts (31, 32).

Two of the chloroplast fragments have been analyzed for their pigment content. The separated particles were extracted with a (2:1) petroleum ether-methanol mixture, and the pigments were transferred to petroleum ether by washing with aqueous NaCl (33). The pigments were separated by thin layer chromatography, with Kieselguhr G (Merck 8129) as the supporting

$^4$ B. Ke, S. Katoh, and A. San Pietro, manuscript in preparation.

![Graph](https://example.com/graph.png)

**Fig. 8.** Reduced minus oxidized difference spectrum of cytochrome in acetone-extracted chloroplast particle (P-D10). Cytochrome content was calculated by the use of 19,680 as the difference molar extinction coefficient at 555 mp. Numerical data are presented in Table III.

**Table III. Composition of P-10 particle**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>10 mg</td>
</tr>
<tr>
<td>Cytochrome f</td>
<td>3 mmol/mg</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>410 mmol/mg</td>
</tr>
<tr>
<td>P700</td>
<td>≥2 mmol/mg</td>
</tr>
</tbody>
</table>

**Table IV. Pigment composition of the P-1 and P-D10 particles**

<table>
<thead>
<tr>
<th>Component</th>
<th>Per 100 mmol of total chlorophyll</th>
<th>Per mg of protein</th>
<th>Per 100 mmol of total chlorophyll</th>
<th>Per mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>67</td>
<td>84</td>
<td>85</td>
<td>18</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>35</td>
<td>41</td>
<td>15</td>
<td>3.2</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>6</td>
<td>7.5</td>
<td>16</td>
<td>3.4</td>
</tr>
<tr>
<td>Lutein</td>
<td>16</td>
<td>20</td>
<td>6</td>
<td>1.3</td>
</tr>
<tr>
<td>Neoxanthin</td>
<td>2</td>
<td>2.5</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Violaxanthin</td>
<td>3</td>
<td>3.8</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Protein</td>
<td>0.8 mg</td>
<td>4.7 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll a/chlorophyll b</td>
<td>2.0</td>
<td>5.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
This is also true for each individual pigment, as shown in Columns b of Table IV. Little is known concerning the individual proteins on these two particles. The cytochromes remain with the P-D10 particle, but the other enzymes of the photosynthetic electron transfer system have apparently been removed, since they must be added back to the system to make it functional in NADP reduction. The difference in the total protein content of the two particles must be related to the presence of other proteins, perhaps structural protein or carboxydiismutase. Further work is needed on this important problem. Since we are concerned primarily with the photochemical reactions of the particles, more emphasis has been placed on the pigments which are involved. The distribution of the pigments suggests that particles representative of Pigment System 1 and Pigment System 2 have been obtained, with only the Pigment System 1 particle retaining activity. Since the enzymatic components of Pigment System 2 are unknown, however, the designation of the P-1 and P-3 particles as inactive Pigment System 2 particles is only tentative.

**ESR and Fluorescence Spectra**—The various fractions obtained by treatment with Triton X-100 were examined for their ESR spectra by means of the technique previously described (21). From the data of Fig. 9 it is apparent that the P-D10 fraction shows a highly magnified ESR signal by comparison with chloroplasts or the other fractions. At the low chlorophyll concentrations used, the chloroplasts did not give a detectable signal; thick suspensions of chloroplasts (not shown here) gave the usual light-induced ESR signal characteristic of chloroplasts (21). The addition of DCU, which markedly enhances the ESR spectra of chloroplasts (not shown here) gave the usual light-induced ESR signal characteristic of chloroplasts (21). The magnitude of the light-induced ESR signal of the P-D10 fraction allowed a low sensitivity to be used (12 gauss modulation and 630 amplitude setting) as well as a low chlorophyll concentration. The peak to peak separation was 11 gauss. The chlorophyll concentrations in milligrams per ml for the various fractions were: chloroplasts (1.4), P-1 (3.3), P-3 (1.8), and P-D10 (0.49). At these low chlorophyll concentrations the chloroplasts did not give a detectable signal; thick suspensions of chloroplasts (6 mg of chlorophyll per ml) exhibited the usual response observed previously for chloroplasts (20). When present, ascorbate (ASC) was 3 nm; PMS, 20 μm, DCI (DPIP), 5 μm and plastocyanin (PC), 1 μm.

![ESR and Fluorescence Spectra](image)

**Fig. 9.** Light-induced ESR spectra of the various particles obtained with Triton X-100 treatment. The magnitude of the signal observed with the P-D10 fraction allowed a low sensitivity to be used (12 gauss modulation and 630 amplitude setting) as well as a low chlorophyll concentration. The peak to peak separation was 11 gauss. The chlorophyll concentrations in milligrams per ml for the various fractions were: chloroplasts (1.4), P-1 (3.3), P-3 (1.8), and P-D10 (0.49). At these low chlorophyll concentrations the chloroplasts did not give a detectable signal; thick suspensions of chloroplasts (6 mg of chlorophyll per ml) exhibited the usual response observed previously for chloroplasts (20). When present, ascorbate (ASC) was 3 nm; PMS, 20 μm, DCI (DPIP), 5 μm and plastocyanin (PC), 1 μm.

![Kinetics of light-induced ESR signal of P-D10 particle](image)

**Fig. 10.** Kinetics of light-induced ESR signal of P-D10 particle from spinach chloroplasts. The ESR spectrometer was set at the field strength which gave maximal signal amplitude. The concentrations of reagents were the same as for Fig. 9.

than that observed in the presence of DCU, but with the detergent present a signal was observed in the dark also.

The kinetics of the light-induced ESR signal of the P-D10 particles is shown in Fig. 10. In contrast to that observed with chloroplasts, the decay of the ESR signal with the particle alone is slow, requiring about 6 sec to decay to half the value observed in the light. The rise of the signal was rapid, and was limited by the response of the instrument. Adding ascorbate and PMS resulted in a marked stimulation of signal decay. A similar response was observed when ascorbate DCI and plastocyanin were added. In the latter case the signal height in the light was lower than that observed for the particle alone (see Fig. 9), but the same stimulation of the decay was noted. In the case of ascorbate-PMS, the light-induced signal height was slightly decreased also from the control system. In all cases the rise of the signal was fast, being limited by the instrument response.

A weak fluorescence was observed for the P-D10 particle. By comparison, it was less than one-fifth that of a comparable preparation (equal chlorophyll) of P-1 particles. The maximum of the emitted light was at 687 μm at room temperature. These preparations were also examined for their ability to give delayed light by means of the apparatus described by Clayton (39). Whereas the P-1 particles gave a delayed light that was less than half that observed for fresh chloroplasts, the P-D10 particle gave no detectable delayed light. These properties of the P-D10 particle are consistent with the idea that it is a purified Pigment System 1 preparation.

**DISCUSSION**

Treatment of spinach chloroplasts with Triton X-100 produces small fragments of differing types which are separable by high speed centrifugation. All available data show that the smallest particle contains a functional Pigment System 1, which in most respects resembles that found in the intact chloroplast. The individual properties are discussed below.
**Photochemical Properties**—The detergent treatment utilized to produce the active P-D10 particle effectively removes from the chloroplast a particle which has the same enzymatic requirements for NADP photoreduction as does the intact chloroplast, indicating that the basic structural characteristics of the active site have not been changed. Moderate heating suffices to inactivate the particle, which further indicates that little structural modification has taken place during the detergent treatment.

Other photochemical reactions representative of the Pigment System 1 system are shown by the P-D10 particle. Methyl red reduction does not show an absolute requirement for, but is stimulated by plastocyanin. This reaction, of course, does not require ferredoxin and ferredoxin-NADP reductase, since the methyl red reacts directly with the particle. This reaction is also prevented by heating the particle, but it is more resistant than the NADP photoreduction activity. Its destruction by heating indicates that the ascorbate-DC1 couple does not interact directly with the chlorophyll in the particle, but reacts through some other component which becomes inactivated or inaccessible through heating. That the chlorophyll on the particle is accessible to added reagents is indicated by the fact that in the case of another photochemical reaction, the photoreduction of ferricytochrome c by trimethylhydroquinone heating the chloroplast at 70° for prolonged periods does not cause more than 40% inhibition. This latter reaction is catalyzed by isolated chlorophyll a alone, which explains the insensitivity to heating observed for this photoreaction. (Methyl red is also photoreduced by chlorophyll a solubilized by Triton X-100, but at a much slower rate.) It is possible that the trimethylhydroquinone-cytochrome c photoreduction utilizes the bulk chlorophyll, while the methyl red and NADP photoreactions require a functional reaction center. This would be consistent with the heat inactivation of the latter two reactions. Covering up or altering the reaction center would prevent these reactions from proceeding.

The rates of the photochemical reactions reported for the P-D10 particle are not maximal, since high concentrations of plastocyanin were not used. Only in the case of NADP photoreduction was plastocyanin employed at concentrations greater than 1.0 μM, and in this case greatly increased rates were observed with higher plastocyanin concentrations. Even at concentrations as high as 13.7 μM, the reaction was not saturated under the conditions employed. The availability of the P-D10 particle, and its ready interaction with plastocyanin, opens the way for more intensive study of the role of this important compound in the photochemistry of Pigment System 1.

**Composition**—The photoactive particle contains those components which are generally associated with Pigment System 1:1. It has a higher chlorophyll a content, it contains cytochromes, and it has a functional P700. The depletion of chlorophyll b agrees with previously published data on a particle obtained through the action of digitonin (10-19), and again indicates that chlorophyll b functions at the Pigment System 2 system which is related to oxygen evolution. The cytochrome spectrum obtained for extracted particles does not resolve cytochrome f and cytochrome b4, but the observed broad maximum at 557 μM indicates that both cytochromes are retained on the particle. This would be consistent with the function of cytochrome f near to P700 and the fact that cytochrome b4 is held tightly and has not previously been removed and purified from chloroplasts.

The carotenoids show some fractionation between the different particle types. β-Carotene is the major pigment (other than chlorophyll) in the P-D10 particle, while lutein has this role in the P-1 particle. It has been suggested, without any sound evidence, that the xanthophylls may play a role in oxygen evolution. The present data, which show a general correlation of the oxygenated carotenoids with the particle most nearly representative of Pigment System 2, are in agreement with this concept, but certainly are not definitive in themselves. These data are in agreement with those reported for particles fractionated with sodium dodecyl sulfate (9) and digitonin (40).

The presence of P700, the reaction center chlorophyll (32), is shown by absorbance changes at 705 and 430 μM and by an ESR signal which is associated with P700 (40). Both of these physical responses, which are related to P700, are much greater for the P-D10 particle than for intact chloroplasts or the other particles. This indicates a functional and facile photochemical system in the P-D10 particle. When all of these data are related to the high rates of NADP photoreduction observed, the logical conclusion is that the particle isolated and studied is a small unit which contains a highly functional Pigment System 1 when external electron donor systems such as ascorbate-DC1 and ascorbate-TPD are used. The necessary structural relationship pertaining to the reaction site is maintained, and is easily destroyed by heating. The increased activity for NADP photoreduction, when compared with chloroplasts, most likely results from a better accessibility of the electron donor system to the chloroplast particle. This could result from the removal of lipid material in the detergent treatment, thus releasing different subunits of the lamellar membrane of the chloroplast.

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