Characterization of a Partially Purified Photosynthetic Reaction Center from Spinach Chloroplasts*

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ABSTRACT: Treatment of freeze-dried spinach chloroplasts with 15% acetone in hexane at 0° removes carotenoids and the majority of the chlorophyll, but leaves the P700 in the membrane residue. Subsequent treatment of the residue with the detergent Triton X-100 solubilizes a small rod-to-ellipsoidal-shaped particle (about 150 × 60 Å) which contains P700 in the ratio of one P700 per 30 chlorophyll molecules and is thought to represent the basic structural unit of photosystem 1. The particles contain small amounts of cytochromes *f* and b_6, but no β-carotene. Titration with ferri–ferrocyanide redox buffers reveals an E_0' of +480 mV. The particles so obtained, called high P700 (HP700) particles, exhibit a light-induced bleaching at 698 nm which represents the oxidation of P700. A secondary band is observed in the light-induced spectrum at 680 nm, and this band appears to be produced by a red shift of some accessory chlorophyll molecule closely associated with P700. Chemical oxidation of the particle with ferricyanide produces a similar difference spectrum. The kinetics of P700 bleaching reveal a markedly slower rate of oxidation in the light compared with a photosystem 1 particle prepared by the action of Triton X-100 alone (TSF-1).

This difference most likely reflects a lowered efficiency of energy transfer between the residual light-harvesting chlorophyll and the reaction center P700. This conclusion is supported by the nature of the HP700 fluorescence at −196°. Compared with TFS-1 preparations, the HP700 particle shows decreased fluorescence at 730 nm (related to P700) and an increased fluorescence at 680 nm. The HP700 particle exhibits a light-induced electron paramagnetic resonance spectrum whose kinetics resemble those of bleaching at 698 nm. The particles photoreduce nicotinamide–adenine dinucleotide phosphate slowly with added crude ferredoxin and ascorbate–2,6-dichlorophenolindophenol under anaerobic conditions.

The first evidence for a reaction center chlorophyll in chloroplasts was obtained by Kok (1956) who showed a light-induced absorbance change at 700 nm. Kok (1961) obtained partial purification of this chlorophyll by extracting chloroplasts with acetone, which removed about 85% of the light-harvesting chlorophyll but rendered the residual membrane resistant to detergent treatment.

Detergents do solubilize native chloroplasts into pigment–protein complexes (Ogawa *et al.*, 1966; Thornber *et al.*, 1967), or fragments (Anderson and Boardman, 1966; Vernon *et al.*, 1966) which correspond to photosystems 1 and 2 in terms of composition and photochemical activities. The isolation and properties of such fragments obtained with Triton X-100 have been reported by this laboratory. The photosystem 1 fragment obtained with Triton (TSF-1) reduced NADP at high rates (Vernon *et al.*, 1966) and was enriched in the reaction center chlorophyll P700, chlorophyll a, and β-carotene (Vernon *et al.*, 1966, 1967).

Recently Reed and Clayton (1968) isolated bacterial reaction centers (P870) free from light-harvesting chlorophyll by treating chromatophores from a carotenoidless strain of *Rhodopseudomonas spheroides* with Triton X-100. The availability of such a reaction center preparation has enabled some definitive studies to be performed on the nature of P870 in bacterial photosynthesis (Sauer *et al.*, 1968; Zankel *et al.*, 1968). On the assumption that the successful isolation of bacterial reaction centers was dependent upon the use of a carotenoidless strain, we have investigated the effects of prior carotenoid extraction from plant membranes on subsequent fractionation with Triton X-100 and have obtained highly purified P700 reaction centers from several plant species (Vernon *et al.*, 1969). The properties of such fragments from *Anabaena* were reported previously (Ogawa and Vernon, 1969). We report herein the characterization of particles highly enriched in P700 (called "high P700 particles" or HP700) isolated from spinach chloroplasts.

Experimental Procedures

All operations were performed at 0°. Chloroplasts were isolated from 800 g of deribbed spinach leaves (*Spinacea oleracea*) by grinding in STN solution (0.25 M sucrose–0.02 M Tricene[1] buffer (pH 8)–0.01 M NaCl), filtering through 12 layers of gauze, and centrifuging at 2500*g* for 10 min. The chloroplasts were washed successively in 1 l. each of 10 mM NaCl–0.75 mM EDTA (pH 8)–2 mM Tricene and centrifuged at 25,000*g* for 20, 30, and 60 min, respectively. Following the three washings, the chloroplast material was resuspended in minimal volume and freeze dried thoroughly. The bulk of the chlorophylls and carotenoids was extracted by resuspend-

* From the Charles F. Kettering Research Laboratory, Yellow Springs, Ohio 45387. *Received May 7, 1969.* This investigation was supported in part by Research Grant GB-8434 (L. P. V.) from the National Science Foundation. H. Y. Y. on leave from the University of Hawaii. Supported in part by Special Research Fellowship 1-F3-GM10260 from the National Institute of General Medical Sciences, U. S. Public Health Service.

[1] Abbreviations used are: Tricene, *N*-tris(hydroxymethyl)methylglycine; PMS, phenazine methosulfate; DPIP, 2,6-dichlorophenolindophenol.

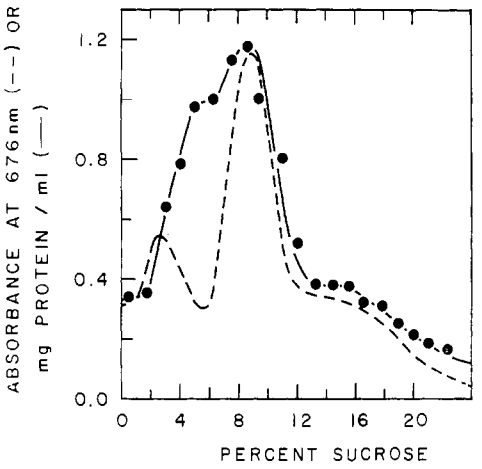

FIGURE 1: Distribution of chlorophyll and protein in sucrose density gradient fractions. Absorbance at 676 nm was obtained with a continuous flow cell. Protein was determined by the Lowry method (Lowry *et al.*, 1951).

TABLE I: Composition of HP700 Particles from Spinach Chloroplasts.[a]

Component	Amount
Chlorophyll a to chlorophyll b	4.0
Chlorophyll	100 mμmoles
P700	3.3 mμmoles
P700 to chlorophyll	1:30
Cytochrome *f*	0.9 mμmole
Cytochrome b_6	1.2 mμmoles
Protein	3.2 mg

[a] Chlorophyll concentrations and the chlorophyll a to b ratio were determined by the method of Vernon (1960). The extinction coefficients used were 6×10^4 M^{-1} cm^{-1} for P700 at 698 nm and 2×10^4 M cm^{-1} for cytochrome *f* at 554 nm and cytochrome b_6 at 565 nm in their difference spectra, reduced minus oxidized. Protein was determined by the Lowry method (Lowry *et al.*, 1951). The values given are the averages of four preparations.

ing the dried chloroplast materials in 240 ml of 15% acetone in hexane (precooled to −18°), and the suspension was centrifuged at 5000*g* for 2 min. This step was repeated seven times, using a glass homogenizer to thoroughly resuspend the fragments on the first and fourth extractions. After the last extraction, the fragments were resuspended in cold hexane, divided into four equal portions, and centrifuged at 5000*g* for 2 min, and the pellets so obtained were stored at −70° until used.

The following describes the further treatment of one-fourth of the above preparation. The residual hexane was removed by evaporation under nitrogen, yielding a light green powder which was homogenized in 60 ml of 0.05% Triton X-100 in Tricene buffer to extract additional chlorophyll and cytochromes, and centrifuged at 39,000*g* for 30 min. The resulting pellet was homogenized with 16 ml of 5% Triton X-100 in Tricene buffer. This extracted the HP700 particles which were separated from insoluble brown materials by centrifugation (39,000*g* for 30 min). Portions (3 ml) of this extract were layered on discontinuous sucrose gradients (2–20% in 2% steps) and centrifuged at 131,000*g* for 20 hr in a Spinco Model L2-65 ultracentrifuge with SW27 rotor. The P700 particles were located in the 8% layer and the yield of P700 was approximately 60%, based on an assumed initial content of 1 P700 to 500 chlorophylls in chloroplasts. Photosystem 1 fragments produced by the action of Triton on chloroplasts (not extracted with solvents) were prepared as described previously (Vernon *et al.*, 1966). These fractions formally referred to as PD-10 are now called TSF-1 (Triton subchloroplast fraction 1) to identify their photochemical activity.

Results

Composition and Structure of HP700 Particles. Although solvent extraction of freeze-dried chloroplasts with cold 15% acetone in hexane removed about 90% of the chlorophylls and all the carotenoids, the majority of the P700 remained in the extracted residue. Treatment of this residue with Triton X-100 solubilized some components which were resolved by sucrose density gradient centrifugation into several bands, as indicated in Figure 1. The material concentrating at 2% sucrose was mainly solubilized chlorophyll and varied in magnitude depending on the extent of chlorophyll extracted in earlier steps. A protein band (yellow) at 4% sucrose contained cytochromes *f* and b_6, and a diffuse (light green) band peaking around 14% sucrose contained partially fragmented membranes.

The HP700 particles concentrated as a well-defined band at 8% sucrose. Electron microscopic examination showed (Figure 2A) a uniform preparation composed of very small particles which were rod-to-ellipsoidal-shaped (about 150 × 60 Å). Few irregularly shaped particles and no large fragments were evident. No difficulties were encountered in obtaining such particles from numerous preparations and similar preparations in composition and appearance were obtained when freeze-dried TSF-1 particles (Vernon *et al.*, 1966) were used as the starting material for solvent extraction. The appearance of the HP700 particles contrasted sharply with TSF-1 (Figure 2B) which showed a mixture of some large fragments as well as the typical stranded material.

The composition of the HP700 particles is shown in Table I. On the basis of chlorophyll content, a 14-fold purification of the P700 reaction center from chloroplasts was obtained. The small amount of cytochromes present appears to be part of the particles rather than contaminants from the protein fraction, since no further separation was obtained by chromatography on Bio-Gel A-50m. The spectrum of the HP700 particles (Figure 3) shows a chlorophyll b shoulder at 465 nm and absence of carotenoid absorption around 500 nm. The lack of carotenoids was also confirmed by ether extraction of a saponified methanol extract of the particles. A comparison of the spectra for the TSF-1 and HP700 particles shows there is relatively more chlorophyll b in the latter, which is reflected in the lower chlorophyll a to chlorophyll b ratio. Earlier, Kok (1961) partially purified P700 using 72% acetone to remove about 85% of the chlorophylls. From his data it is possible to calculate a ratio of P700 to chlorophyll of approximately 1:75, which is about the same as the ratio we

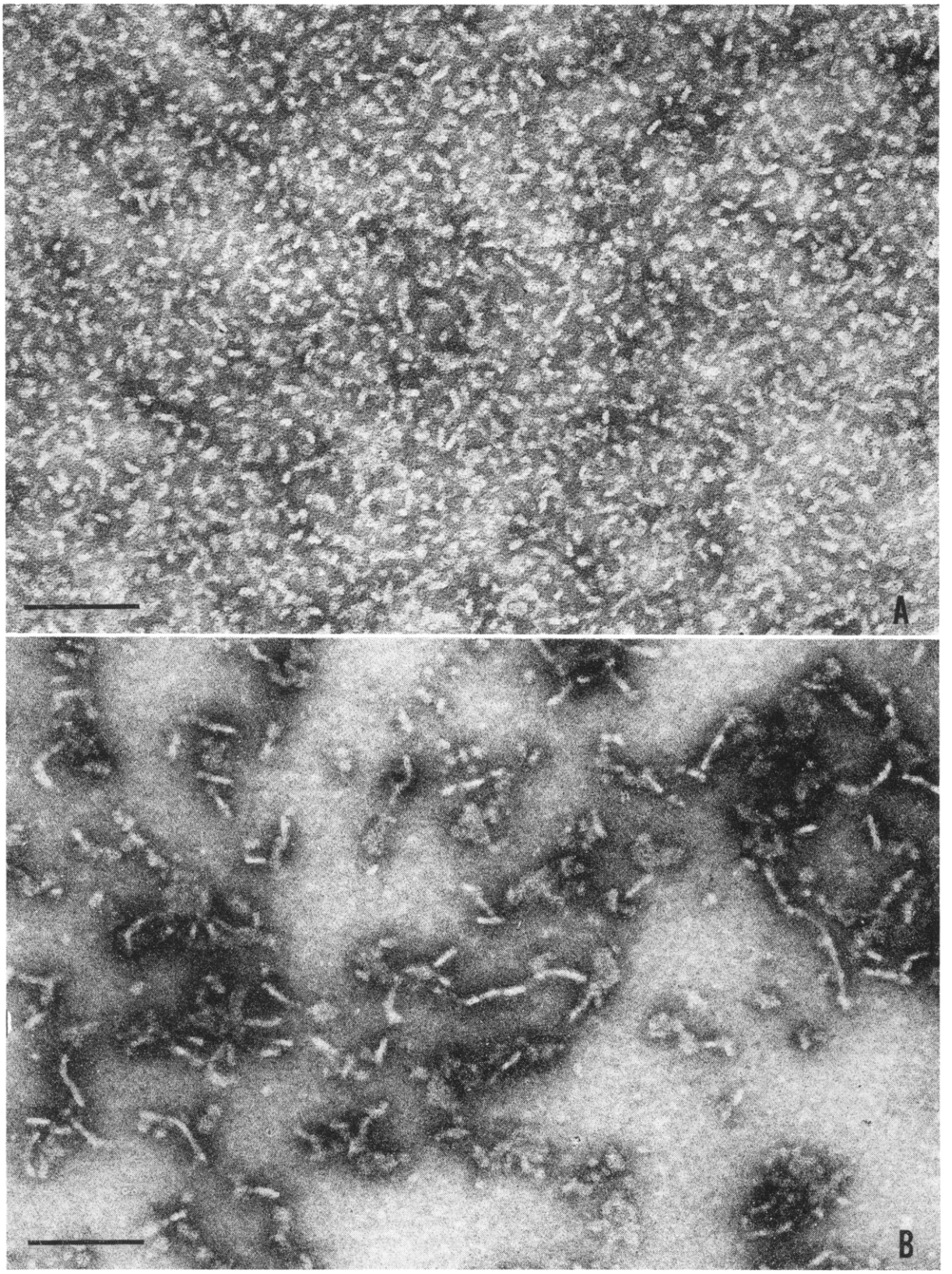

FIGURE 2: Electron micrograph of chloroplast HP700 particles (A) and a TSF-1 preparation (B) from EDTA-treated chloroplasts (Vernon *et al.*, 1968). The preparations were either dialyzed against 2 mM Tricene buffer (pH 8) or pelleted to remove sucrose and Triton X-100, negatively stained with phosphotungstic acid at pH 5.9 in 5 mM $MgCl_2$, and examined with a Phillips EM200 electron microscope. The bar represents 1000 Å.

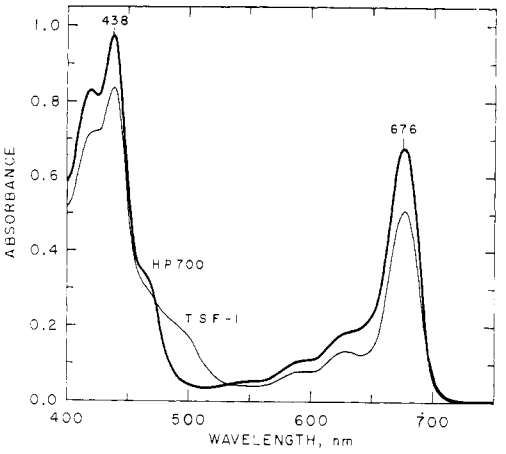

FIGURE 3: Absorption spectra of HP700 particles (heavy line) and TSF-1 preparation (light line) from spinach chloroplasts. The spectrum was obtained with a Cary 14 spectrophotometer, using particles suspended in 0.05 M phosphate buffer (pH 7.2).

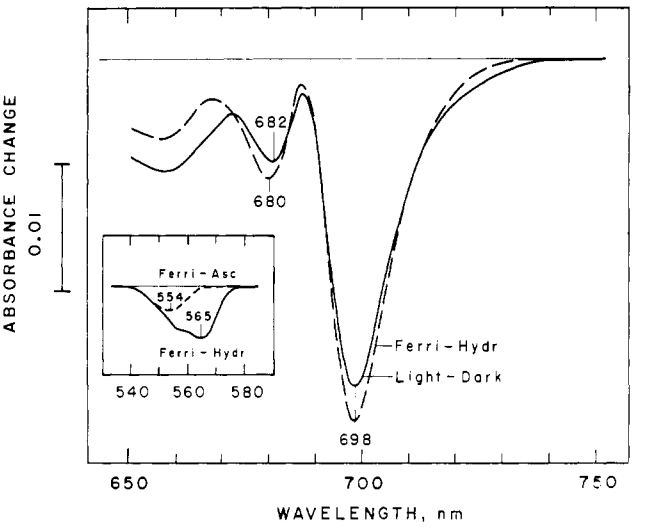

FIGURE 4: Difference spectra of HP700 particles in the chlorophyll and cytochrome regions. The spectra were obtained with a Phoenix dual-wavelength spectrophotometer modified for actinic illumination. For the light-minus-dark spectrum, the photomultiplier was shielded with Corning filter CS 2-58 and the sample was illuminated with light passing a blue actinic light (Corning filter CS 5-60) at 1.5 $\times$ 10^4 ergs cm^{-2} sec^{-1}. For chemically induced difference spectra, ferricyanide (Ferri), sodium ascorbate (Asc), and sodium hydrosulfite (Hydr) were added in the solid form in excess. The particles were suspended in 0.05 M phosphate buffer (pH 7.2) and the chlorophyll concentration was 14 μg/ml (0.8 absorbance at 676 nm).

have observed for TSF-1 preparations (Vernon *et al.*, 1968). Kok (1961) also observed that the extracted residue was not solubilized by detergents. We have confirmed this observation, and chose the present method of extraction since it leaves a residue which can be solubilized by Triton X-100. Hexane appears to be important for this effect, since freeze-dried chloroplasts extracted with acetone were not solubilized by Triton unless rinsed with hexane. Presumably the hexane replaces lipids and pigments removed by the solvents and prevents strong protein–protein interactions which are resistant to solubilization by the detergent.

Physical Properties. The HP700 preparation shows the absorbance changes expected for P700 in the 700-nm region, as well as another change of lower magnitude around 680 nm (Figure 4). The chemical and light-induced difference spectra were very similar, and in both cases the main band was skewed and the change was maximal at 698 nm. Since the P700 was isolated in a partly oxidized form (about 70%), addition of ascorbate was required for the production of a maximum light-minus-dark absorbance change at 698 nm. However, the magnitude for the light-induced change was always less than the chemically induced change, owing to slow kinetics of P700 bleaching (shown below) and a partial oxidation of the "dark" reference sample by the measuring beam.

A smaller light-induced absorbance change centering around 680 nm usually accompanied the P700 change (Vernon *et al.*, 1969; Kok, 1961; Döring *et al.*, 1968). This change is different from large chemically induced changes in this region which are generated by nonspecific oxidation of light-harvesting chlorophyll a-680 (Vernon *et al.*, 1967; Kok, 1961; Horio and San Pietro, 1964). In the HP700 particles the magnitude of the chemically induced change at 680 nm was about equivalent to the light-induced change owing to the removal of the bulk chlorophyll which might be chemically oxidized by ferricyanide.

The chemically induced difference spectra in the cytochrome region (Figure 4, insert) show a cytochrome *f* band at 554 nm, and a cytochrome b_6 band at 565 nm. Both cytochromes were in the oxidized form on the isolated particle.

The kinetics of the light-induced absorbance change at 698 nm and the related electron paramagnetic resonance signal are shown in Figure 5. That P700 was isolated in a partially oxidized form is shown by the large electron paramagnetic resonance signal observed in the dark, and by the lesser extents of the light-induced absorbance change and electron paramagnetic resonance signal without additions. The increased extents of the light-induced signals with ascorbate present reflect the more complete reduction of P700 prior to actinic illumination. The further addition of PMS increased the recovery rates in the dark, and consequently decreased the extent of the signals in the light by virtue of promoting faster turnover of P700. This correlation between absorbance change and electron paramagnetic resonance signal is consistent with observations reported previously with spinach TSF-1 particles (Vernon *et al.*, 1967). In the present case, however, the kinetics of the light-induced changes were considerably slower (for a comparison of P700 kinetics of TSF-1 and HP700, see Vernon *et al.*, 1969), probably owing to less efficient energy transfer from the remaining chlorophylls to the reaction center.

Titration of P700 with ferricyanide–ferrocyanide redox buffers gave the data shown in Figure 6. The experimental points were in excellent agreement with the theroetical curve for a one-electron process of $E_0' = +480$ mV. Kok (1961) found an $E_0' = +430$ mV for P700 and Cusanovich *et al.* (1968) reported $E_{m7.5} = +489$ mV for bacterial P883. In the present study, the extent of reduction was determined by difference spectra from chemically oxidized or reduced samples. It was not possible to determine the degree of reduction by light-dependent assay owing to a relatively fast back-reaction from ferrocyanide.

The fluorescence yield of the HP700 particles was low at

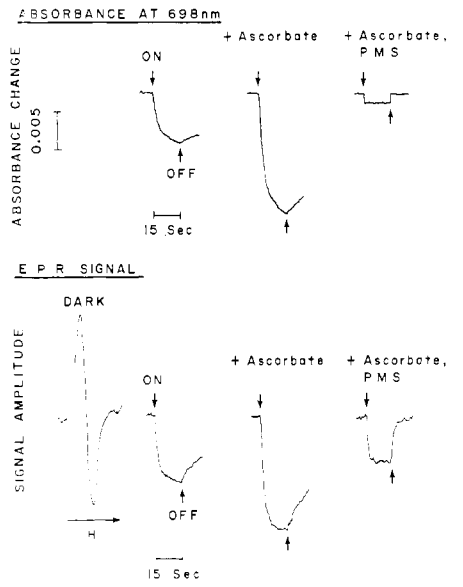

FIGURE 5: Kinetics of light-induced absorbance change at 698 nm and electron paramagnetic resonance signal for HP700 particles. The kinetics of the light-induced absorbance changes were measured with the instrument described in Figure 4 in the converging beam mode with the reference wavelength at 740 nm. Electron paramagnetic resonance kinetics were determined with a Varian Model 4500 electron spin resonance spectrometer with accessory equipment described previously (Vernon *et al.*, 1967). Settings of 12-gauss modulation, 1000 amplitude, and 3×10^{-4} response were used. The red actinic light (Corning filter CS 2-58) gave 1×10^{4} ergs cm^{-2} sec^{-1}. The particles were suspended in 0.05 M phosphate buffer (pH 7.2) and where indicated the mixture also included 1 mM sodium ascorbate and 10 μM PMS. The chlorophyll concentration was 13 μg/ml for the light-induced absorbance change and 228 μg/ml for the electron paramagnetic resonance study.

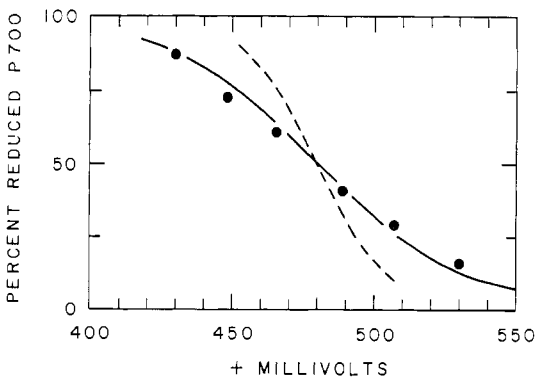

FIGURE 6: Redox titration of P700 in the HP700 particles at pH 7. The percentage of reduced P700 in various millimolar ferri–ferrocyanide mixtures was determined from the difference in absorbance at 698 nm from samples fully oxidized with ferricyanide or fully reduced with ferrocyanide. Samples were freshly diluted with buffer for each determination since at pH 7 turbidity developed after several minutes. The dashed line represents a calculated two-electron change and the solid line is for a one-electron change, both with $E_0' = +480$ mV. An $E_0' = 430$ mV was used for the ferro–ferricyanide redox system. The HP700 particles were suspended in 0.05 M phosphate buffer, pH 7, and the chlorophyll concentrations were 15 μg/ml.

25°, and was greatly enhanced at −196° (Figure 7). The properties of the HP700 fluorescence were markedly different from those of TSF-1 at −196°. The HP700 fluorescence spectrum did not show the large 730-nm peak at liquid nitrogen temperatures which is characteristic of photosystem 1 preparations and has been attributed to P700 fluorescence (Ke and Vernon, 1967), but did show a large peak at 680 nm with indications of other partly resolved components around 674 and 696 nm.

Zankel *et al.* (1968) have shown that the 900-nm fluorescence from bacterial reaction centers was from reduced P870 and oxidation of the reaction center, P870, prevented the fluorescence. The addition of hydrosulfite to HP700 particles to maintain P700 in a completely reduced state did not change the 730-nm fluorescence at −196°, but did produce a slight increase in the 695-nm region. Thus, it is unlikely that the low fluorescence at 730 nm at −196° was due to the state of oxidation of P700. Considering the slow kinetics of P700 bleaching in the light (Figure 5), it is probable that energy transfer from residual light-harvesting chlorophylls to P700 was poor so that excitation energy contained in the light-harvesting chlorophylls gave low fluorescence yields from these shorter wavelength chlorophylls.

The HP700 fragments from *Anabaena* also show reduced low-temperature fluorescence at 730 nm and a shoulder around 695 nm, but show no fluorescence at 680 nm (Ogawa and Vernon, 1969). Since *Anabaena* has no chlorophyll b, it is possible that in chloroplast HP700 particles, the 680-nm fluorescence at low temperatures derives from chlorophyll b.

As shown in Table II, the HP700 particles reduced NADP slowly with added crude spinach ferredoxin and ascorbate–DPIP, a system geared for NADP reduction by photosystem 1. Crude ferredoxin was required for activity (expt A), and aerobic conditions inhibited the activity almost completely (expt B). Experiment B was conducted with a sensitive spectrophotometer set for 0.1-absorbance full-scale response. Nonspecific absorbance change at 340 nm was not observed with NADP omitted. The higher rate of NADP reduced in

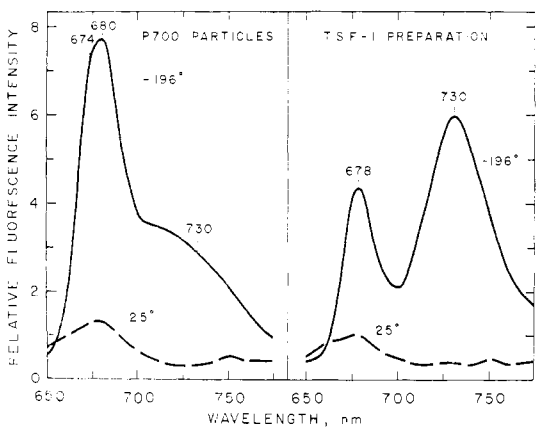

FIGURE 7: Fluorescence spectra of HP700 particles and a TSF-1 preparation at 25° (dashed lines) and −196° (solid lines). The preparations were illuminated with 430-nm light from the front in a 0.15-mm path cell. The fluorescence emitted from the back of the cell was resolved with a Baush & Lomb monochrometer blazed at 1 μ and detected with a RCA 7102 photomultiplier cooled with Dry Ice. The preparations were suspended in 0.05 M phosphate buffer, pH 7.2, and the Chl concentrations were 30 μg/ml.

TABLE II: NADP Reduction by HP700 Particles from Spinach Chloroplasts.[a]

Conditions	NADP Reduced (μmoles/hr per mg of chlorophyll)
Experiment A	
Ascorbate, DPIP, NADP, anaerobic	0
Plus crude ferredoxin	8.3
Experiment B	
Ascorbate, DPIP, NADP, crude ferredoxin, anaerobic	3.2
As above, aerobic	0.4
Minus NADP, anaerobic	0

[a] The reactions were run in 0.1 M Tricene buffer (pH 7.5) in Thunberg cuvets. Anaerobic conditions were obtained by flushing four times with Argon. When present the reaction mixture contains 5 mM sodium ascorbate, 50 μM DPIP, per 400 μM NADP, and 0.15 ml of crude spinach ferredoxin ml (San Pietro and Lang, 1958). In expt A, a modified Spectronic 505 spectrophotometer with red actinic light (Corning filter CS 2-58) of 7×10^5 ergs cm^{-2} sec^{-1} was used and the chlorophyll concentration in HP700 particles was 28 μg/ml. In expt B, a dual-wavelength spectrophotometer with red actinic light (Corning filter CS 2-64) at 9×10^4 ergs cm^{-2} sec^{-1} was used, and the chlorophyll concentration was 24 μg/ml.

the complete system of expt A was probably due to the higher actinic light intensity used.

Discussion

Although previous experiments performed in this laboratory by Garcia *et al.* (1966a,b) with photosynthetic bacteria showed that the detergent Triton X-100 readily split the photosynthetic membrane into two easily separable fractions, the one containing the reaction center also contained considerable light-harvesting bacteriochlorophyll. By using Triton X-100 on chromatophores of a carotenoidless mutant of photosynthetic bacteria, Reed and Clayton (1968) and Gingras and Jolchine (1969) have obtained reaction center complexes free of the light-harvesting bacteriochlorophyll, showing the importance of the presence of carotenoids upon the degree of interaction of this detergent with the bacteriochlorophyll. Although not conclusive the present study supports this concept, since the use of Triton X-100 alone on spinach chloroplasts yields a particle with a P700 to chlorophyll ratio of 1:85, while the HP700 particle described here has a ratio of 1:30. The difference could derive from the prior removal of either carotenoids or other lipids. A recent report by Thornber *et al.* (1969) states that in the case of the bacterium *R. viridis*, the use of the ionic detergent sodium dodecyl sulfate alone on wild-type cells is sufficient to remove the light-harvesting bacteriochlorophyll *b* and produce a reaction center complex from chromatophores of this bacterium. This detergent is a little more active than is Triton X-100, and can interact with the light-harvesting bacteriochlorophyll even when it is complexed with the carotenoids in the chromatophore membrane.

A prior communication from this laboratory reports on the isolation of HP700 particles from photosynthetic membranes of four plant species: spinach, bush bean, *Euglena* and the blue-green alga, *Anabaena variabilis*. These particles all had closely similar ratios of P700 to chlorophyll, and showed similar light-induced or ferricyanide-induced difference spectra. Because of the occurrence of the 680-nm absorbance change in these preparations, and because this absorbance change occurred in concert with the changes at 698 nm, the 680-nm changes were assigned to a red shift of a chlorophyll associated with the reaction center chlorophyll P700. The 680-nm change does not appear to be a secondary band of the P700 molecule, since the shapes of the main bands in the difference spectra in all cases were skewed. This is discussed in more detail in the paper by Vernon *et al.* (1969).

Thornber (1969) has recently described a chlorophyll–protein complex obtained from the blue-green alga, *Phormidium luridum*, by use of the detergent sodium dodecyl sulfate. Similar complexes have been prepared from several photosynthetic tissues, and these authors consider a common chlorophyll–protein complex is basic to all photosynthetic systems. From the amino acid analysis, the monomeric protein unit would be of mol wt 35,000 and would contain five chlorophyll molecules. It is difficult to relate these data to those given in this paper, since the majority of the chlorophyll has been removed by solvent extraction in our investigation, and the molecular weight of the HP700 complex has not been determined. The constancy of the ratio of P700 to chlorophyll in all HP700 preparations examined to date (Vernon *et al.*, 1969) speaks for some basic protein–chlorophyll complex in the various plant systems. A significant difference between the chlorophyll–protein complexes obtained by Thornber (1969) and the particles prepared in this study is the lack of a demonstrable P700 in the former. This is destroyed by the action of sodium dodecyl sulfate. In the case of the particle obtained with Triton X-100, some residual NADP photoreduction activity remains, which indicates that the structural integrity of the reaction center has been retained to the extent necessary to allow the interaction of the reaction center with the added ferredoxin. The slow initial rate of the light-induced bleaching at 698 nm, coupled to the fluorescence properties of the particles, indicates that the structure of the original photosynthetic unit has been altered to the extent that the transfer of excitation energy from the residual light-harvesting chlorophyll to the P700 is less efficient than in the original chloroplast or in the TSF-1 preparations (see also the paper by Vernon *et al.*, 1969).

The HP700 particle has the properties one would expect for the reaction center of the plant system, including the light-induced absorbance change and electron paramagnetic resonance signal associated with the oxidation of P700. The oxidation potential, E_0', of the P700 was determined to be +480 mV, which contrasts to the value of +430 mV obtained by Kok (1961) for his partially purified P700 preparations. The reason for this difference is not immediately apparent. Because of the difficulty of working with preparations made by Kok's procedure (the material is not solubilized by Triton X-100), we have not attempted to repeat the redox measurement on preparations prepared according to the direction of Kok.

Although the activity is low, the HP700 particle does retain NADP photoreduction activity, which attests to the gentleness of Triton X-100 in its action on the photosystem 1 apparatus. An interesting feature of the NADP photoreduction reaction is the sensitivity of the reaction to oxygen. Although the NADP photoreduction reactions carried out with chloroplasts and with TSF-1 preparations usually proceed faster in the absence of oxygen, the oxygen inhibition noted in Table II is severe. This could be related to the low rate of NADP photoreduction under anaerobic conditions.

All evidence indicates that there are small amounts of both cytochrome *f* and cytochrome b_6 on the HP700 particles we have obtained. Treatment of such particles by chromatography on Bio-Gel A-50m does not remove the cytochromes. Their low concentration, however, shows that the detergent does succeed in removing the majority of the cytochromes from the membrane system, which agrees with data previously reported (Vernon *et al.*, 1968; Wessels, 1968).

All spinach HP700 preparations to date show a high degree of uniformity in the electron microscope. By contrast to the TSF-1 preparations, which contain stranded material as well as considerable aggregated material, the HP700 preparations contain particles which are discrete and fairly uniform in size. Thus we conclude that these rod-shaped particles could represent the basic structural units of spinach photosystem 1 which are embedded in and are a component of the photosynthetic membrane. Their *in vivo* size and shape, however, may be slightly different. Because of the uniformity of this preparation, and the fact that the majority of the chlorophyll has been removed from the particles, they offer an opportunity to study the photoreactions of photosystem 1 of plants in some detail.

Acknowledgments

The authors gratefully aknowledge the many fruitful discussions and helpful suggestions offered by Dr. T. Ogawa. We also thank Dr. D. Raveed for the electron micrographs and Dr. B. Mayne for use of the fluorometer. We also appreciate the technical help of Miss D. Limbach in some phases of this work.

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