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ISOLATION AND PROPERTIES OF PARTICLES CONTAINING THE REACTIONCENTER COMPLEX OF PHOTOSYSTEM II FROM SPINACH CHLOROPLASTS

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SUMMARY

By density gradient centrifugation of the $80000 \times g$ supernatant of digitonin-treated spinach chloroplasts two main green bands and one minor green band were obtained. The purification and properties of the particles present in the main bands, which were shown to be derived from Photosystem I and Photosystem II, have been described previously; those of the particles in the minor fraction will be described in the present paper.

After purification, these particles show Photosystem II activity but are devoid of Photosystem I activity. They have a high chlorophyll *a*/chlorophyll *b* ratio and are enriched in β-carotene and cytochrome b_{559}. At liquid nitrogen temperature, photoreduction of C550 and photooxidation of cytochrome-b_{559} can be observed. At room temperature, cytochrome b_{559} undergoes slight photooxidation.

These properties indicate that this particle may be the reaction-center complex of Photosystem II. It is suggested that, *in vivo*, the Photosystem II unit is made up of a reaction-center complex and an accessory complex, the latter being found in one of the main green bands of the density gradient.

INTRODUCTION

Fragmentation of chloroplasts by detergents, sonic oscillation, or French press treatment, and differential or density gradient centrifugation of the fragmented material, has been used widely in recent years for the physical separation of the two photosystems of photosynthesis[1]. Although heavy membranous fractions rich in Photosystem II were obtained from chloroplasts, isolation of small photoactive Photosystem II particles, completely uncontaminated by Photosystem I, presented considerable difficulties. Photosystem I, on the other hand, could be isolated quite easily as a small particle in a pure state free from Photosystem II.

In previous papers[2,3] we have shown that density gradient centrifugation of the $80000 \times g$ supernatant of chloroplasts treated with a high concentration of digitonin produced a separation into four distinct, coloured bands as illustrated in Fig. 1. From the top downward we obtained a green band with an absorption

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

maximum at 674 nm (F_{III}), a light pink band containing the cytochromes *f* and b_6 (molar ratio 1 : 2), a minor green band with an absorption maximum at 675 nm (F_{II}), and a main, bluish-green coloured band with an absorption maximum at 679 nm (F_I). The photochemical activities, pigment composition and other properties of F_I and F_{III} indicated that these bands contained particles derived from Photosystem I and Photosystem II, respectively. It was found, however, that the Photosystem II activity of F_{III} (photoreduction of 2,6-dichlorophenolindophenol (DCIP) by 1,5-diphenylcarbazide) was relatively low, particularly in comparison with the activity of F_{II}. In addition to Photosystem II activity, F_{II} showed a substantial Photosystem I activity (photoreduction of $NADP^+$ by ascorbate–DCIP). But owing to the small yield of F_{II}, we were unable to decide whether this fraction consisted of an association of Photosystem I and Photosystem II particles, the association somehow stabilizing the Photosystem II activity, or whether the Photosystem I activity of F_{II} was due to contamination by F_I.

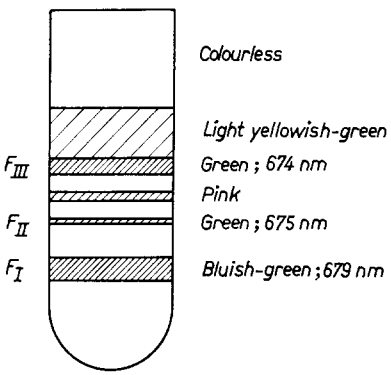


Fig. 1. Location of the four coloured bands in the gradient tube after density gradient centrifugation of the 80 000 × *g* supernatant of digitonin-treated chloroplasts.

In the present paper an improved, large-scale method for the isolation of F_{II} is described. This method, involving a modified digitonin-incubation medium and the use of a zonal rotor, enhances both the yield and the purity of the particles. It is shown that under these conditions the Photosystem II activity in the gradient is mainly localized in F_{II}, and that the Photosystem I activity in this band is due to overlap of the F_I zone. Further purification of F_{II} was accomplished by repeated density gradient centrifugation and chromatography of the particles on a DEAE-cellulose and a hydroxylapatite column. The properties of the purified F_{II} particles, which showed a high Photosystem II activity but no Photosystem I activity, indicate that they may represent a reaction-center complex of the Photosystem II unit. Although there are some differences in properties, the particles may be similar to the Triton-subchloroplast-fragment containing Photosystem II (TSF-2a) particles recently obtained by Vernon and co-workers[4–6] from Triton-treated chloroplast preparations. It is suggested that *in vivo* the photosystem II unit consists of an association of the two upper green bands of the density gradient, F_{II} representing the reaction-center complex and F_{III} the accessory complex.

METHODS

Chlorophylls *a* and *b* were measured spectrophotometrically by the method of Arnon[7]. When the chlorophyll *a*/chlorophyll *b* ratio was 7 or higher, the spectrofluorimetric method of Boardman and Thorne[8] was used.

The reaction mixture for $NADP^+$ photoreduction (Photosystem I activity) contained, in a final volume of 3 ml, 2 μmoles of $NADP^+$, 10 μmoles of ascorbate, 0.1 μmole of DCIP, 0.2 mg of ferredoxin, 40 transhydrogenase units of ferredoxin–$NADP^+$ reductase (NADPH:ferredoxin oxidoreductase, EC 1.6.99.4), 0.1 mg of plastocyanin, 150 μmoles of Tris buffer (pH 7.0), and 20 μg of chlorophyll. The three enzymes were prepared as described previously[9]. $NADP^+$ photoreduction was measured by the increase in absorbance at 340 nm.

Photosystem II activity was determined by monitoring the photoreduction of DCIP at 590 nm. The reaction mixture contained, in a total volume of 3 ml, 20 μmoles of phosphate buffer (pH 6.0), 0.3 μmole of DCIP, 3 μmoles of 1,5-diphenylcarbazide, and 20 μg of chlorophyll. A correction was made for the slight reduction that occurred in darkness.

The activity measurements were performed at 20 °C with a Unicam SP 1800 B spectrophotometer, adapted for side illumination with red light (630–700 nm) of intensity $2 \cdot 10^5$ ergs$\cdot$cm$^{-1}\cdot$s^{-1}. The actinic light was obtained from a Philips 100 W Tungsten halogen reflector lamp, type 6834, and passed through a 1-cm layer of running water, two infrared blocking filters (Balzer, type Calflex C and Calflex B_1/K_1), and a red filterglass RA-64 (Saale-Glas GmbH, Jena, DDR). The light was introduced into the sample compartment by a light pipe (Corning flexible fiber optics, $^1/_4$ inch diameter, 2 ft length). Light intensity at the sample position was measured with an Isco, model SR, spectroradiometer. $NADP^+$ reduction was performed with a Corning-5840 filter and DCIP reduction with a 600-nm short-wavelength pass filter (Optics Technology) placed in front of the phototube.

Absorption spectra were recorded with a Cary, model 14 R, spectrophotometer, fluorescence spectra with a Perkin–Elmer, type MPF-2 A, fluorescence spectrophotometer.

Oxidized-*minus*-reduced difference spectra, light-*minus*-dark difference spectra, and light-induced absorbance changes of cytochrome b_{559} were measured in an Aminco-Chance dual-wavelength spectrophotometer, type 4-8450, fitted with a side-illumination attachment.

Light-*minus*-dark difference spectra at liquid nitrogen temperature were measured by means of a slightly modified low-temperature attachment. The sample and reference cuvettes (optical path length 1 mm) each contained 0.5 ml of a suspension of subchloroplast particles (about 1 mg chlorophyll/ml) in a solution containing 62 parts by volume of glycerol and 38 parts of 0.01 M Tris buffer (pH 7.0). The suspension was frozen by slowly immersing the cuvette assembly into liquid nitrogen in an optical Dewar. The cell compartment was flushed with dry nitrogen gas in order to prevent condensation of water vapor. Actinic light was provided by the illumination attachment described above, except that the Saale-Glas filter was replaced by a Schott interference filter (AL 645 nm) and the light was introduced at an angle of 45 °C onto the sample cell. A 600-nm short-wavelength pass filter (Optics Technology) was placed in front of the phototube. The reference cell was

shaded from the actinic light by a piece of black felt. Following illumination ($3 \cdot 10^4$ ergs$\cdot$cm$^{-2}\cdot$s^{-1} for 30 s), the felt was removed and the light-*minus*-dark difference spectrum recorded. For light-induced absorbance changes of cytochrome b_{559} at room temperature a combination of a Corning 4-76 filter and a 600-nm short-wavelength pass filter was used to screen the photomultiplier from the actinic light.

The pigment composition of the subchloroplast particles was determined by the method of Hager and Bertenrath[10], and protein by the method of Lowry *et al.*[11].

Digitonin was obtained from Fisher (digitonin certified), 1,5-diphenylcarbazide from Merck, and $NADP^+$ from Boehringer.

RESULTS

Preparation of F_{II} particles

Chloroplasts were prepared by homogenizing 750 g of sliced spinach leaves for 1 min in a Sorvall Omnimixer (at maximum speed) in 0.05 M Tris–HCl buffer (pH 7.8) containing 0.4 M sucrose (1 ml/g spinach leaves). The homogenate was filtered through nylon cloth (40-μm pores) and centrifuged for 1 min at $200 \times g$. The sediment was discarded and the supernatant centrifuged for 7 min at $2000 \times g$ in a 12×100 ml rotor in an MSE Mistral 6 L refrigerated centrifuge. The chloroplast pellet was washed once with the Tris–sucrose solution, and resuspended in 0.05 M Tris buffer (pH 7.0) containing 2% NaCl. All operations were performed at 2–5 °C.

To the chloroplast suspension (containing about 400 mg of chlorophyll) a 4% solution of digitonin in Tris–NaCl was added in such a manner that the ratio mg digitonin/mg chlorophyll was 5 and the final concentration of digitonin in the suspension 1.3%. The suspension was stirred for 1 h, and then successively centrifuged at $1000 \times g$ (2 min) and $30000 \times g$ (1 h) in an MSE high-speed 18 centrifuge. The $30000 \times g$ supernatant, containing soluble proteins and most of the solubilized stroma material and cytochromes b_6 and f[2,3], was discarded. The $30000 \times g$ sediment, which was relatively rich in the photosystems derived from the grana lamellae and in cytochrome b_{559}, was resuspended in 0.05 M Tris buffer (pH 7.0) containing 2% NaCl and incubated overnight with 1.3% digitonin (5 mg digitonin per mg chlorophyll). A high NaCl concentration as well as a relatively low digitonin/chlorophyll ratio appeared to be essential for giving a good yield of F_{II}. At the end of the incubation period the suspension was centrifuged for 1 h at a maximum relative centrifugal force of $100000 \times g$ in an MSE Superspeed 65 centrifuge (8×50 ml angle rotor), and the top layer (about 5 ml) and sediment were discarded. The clear supernatant (about 80 ml, containing 20–25 mg of chlorophyll) was layered on to a sucrose gradient of 10 to 30% (w/w; 1400 ml; linear with respect to the radius), containing 0.05 M Tris buffer (pH 7.0), 2 mM EDTA, 5 mM $MgCl_2$, and 0.5% digitonin, and centrifuged for 64 h at a maximum relative centrifugal force of $45000 \times g$ in a B XV Titanium zonal rotor in an MSE Superspeed 65 centrifuge. The sucrose density gradient was prepared by means of a gradient-forming device (MSE Automatic variable gradient former). After centrifugation, the rotor contents were displaced by pumping in a dense sucrose solution (45%). The displaced gradient was monitored by ultraviolet and visible (440 nm) absorbance and collected in fractions for further analysis.

Fig. 2 represents the distribution of the Photosystem I and Photosystem II activities over the different gradient fractions. It is seen that the Photosystem II activity was concentrated in the middle green band, and that the Photosystem I activity previously found in this band may be due to some overlap of the blue-green zone (F_I). Likewise, the low Photosystem II activity observed in F_{III} could be due to contamination by F_{II}. Apart from that, the present method enhanced not only the yield but also the purity of the F_{II} particles.

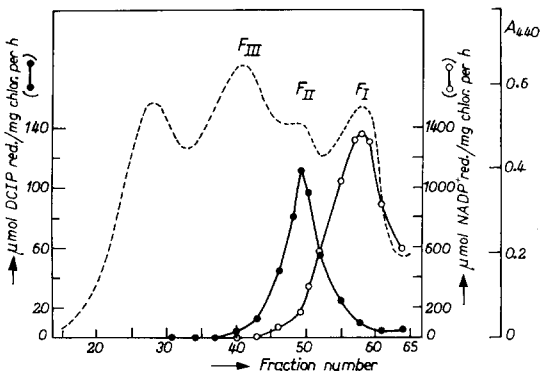


Fig. 2. Photosystem I and Photosystem II activities of the different fractions obtained from the zonal rotor after sucrose density gradient centrifugation of subchloroplast particles. -------, absorbance at 440 nm.

The fractions with a high Photosystem II activity, which appeared to be characterized by an absorption peak at 674–675 nm (in contrast to F_I, which showed a max. absorbance at 678–679 nm) and by the absence of an absorption peak at 651 nm (characteristic of F_{III}), were combined and passed through a Sephadex G-25 column in order to remove excess sucrose. Subsequently the F_{II} fraction was concentrated to 18 ml with an Amicon ultrafiltration cell, model 401, using a Diaflo ultrafiltration membrane, type UM-10 (Amicon Corporation, Cambridge, Mass.), and centrifuged through a linear sucrose gradient in the SW 27 rotor of a Beckman, model L 2, centrifuge, operated at a maximum relative centrifugal force of $90000 \times g$ for 40 h. The sucrose gradient was prepared by means of a linear gradient mixing device (Buchler Instr., Inc.), which was loaded with 34 ml of 10% sucrose and 34 ml of 30% sucrose, each sucrose solution containing 0.01 M Tris buffer (pH 7.0), 5 mM $MgCl_2$, 2 mM EDTA, and 0.5% digitonin. 3-ml samples were layered carefully at the top of the sucrose gradient. After centrifugation, the major green band, localized in the middle of the tube, was recovered by siphoning through a narrow steel needle. The yield at this stage was about 4 mg of chlorophyll.

The F_{II} particles were then adsorbed on a DEAE-cellulose column (Whatman DE-22; 5 cm × 2.5 cm), equilibrated with 0.01 M Tris buffer (pH 7.0) containing 0.2% digitonin. The column was successively washed with 0.01 M Tris buffer + 0.2% digitonin (50 ml) and 0.075 M Tris buffer + 0.2% digitonin, which eluted traces of F_I and F_{III} from the column. Washing with the latter solution was continued until the eluate was colourless. The F_{II} particles were eluted with 0.1 M Tris buffer, containing 0.1 M NaCl and 0.2% digitonin. The yield after DEAE-cellulose chromato-

graphy was about 2 mg of chlorophyll. The particles were subjected to gel filtration on a Sephadex G-25 column, in order to remove excess salt, and then applied to a column of hydroxylapatite (4 cm × 2.5 cm), equilibrated with 10 mM phosphate buffer, pH 7.0, containing 0.2% digitonin. The column was washed with 0.01 M and 0.5 M phosphate buffer, containing 0.2% digitonin (each 30 ml), and elution of F_{II} was achieved with 1 M phosphate buffer + 0.2% digitonin. Excess of phosphate could be removed from the effluent by passage through a Sephadex G-25 column.

The purification procedure is summarized in Table I. It is seen that the purified particles did no longer show any Photosystem I activity, whereas the Photosystem II activity had been increased. Chromatography of the particles on a hydroxylapatite column removed some colourless protein and enhanced the ratios of chlorophyll *a* to chlorophyll *b* and of β-carotene to chlorophyll. However, this step did not increase the activity, and involved a considerable loss in the yield (approx. 40% of the chlorophyll).

TABLE I

PURIFICATION OF THE REACTION-CENTER COMPLEX OF PHOTOSYSTEM II (F_{II} PARTICLES)

Fraction	*Rate (μmoles reduced/mg chlorophyll per h)*		
	Chlorophyll a/ chlorophyll b	*$NADP^+$ photoreduction by ascorbate–DCIP*	*DCIP photoreduction by 1,5-diphenylcarbazide*
F_{II} fraction from zonal rotor	3.4	200	100
F_{II} fraction from second density gradient centrifugation	4.2	150	130
Eluate DEAE-cellulose column	7.2	0	220
Eluate hydroxylapatite column	25	0	200

The purified F_{II} particles were stored in the dark at 2 °C. Under these conditions the loss in activity was 50% in about 4 weeks. The particles retained their activity for a longer period after freeze–drying.

Properties of F_{II} particles

Generally, the photochemical activity of the purified particles was between 200 and 250 μmoles DCIP reduced per mg chlorophyll per h, using 1,5-diphenylcarbazide as an electron donor. No reduction was observed with water as an electron donor, indicating that water oxidation was completely blocked. The Photosystem II activity was inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), but the DCMU sensitivity of the particles was somewhat less than is usually observed with chloroplasts. The reaction was inhibited to the extent of 70% in the presence of 20 μM DCMU. The pH optimum of the DCIP photoreduction was 6.0. Ferricyanide was reduced at a very high rate (3000 μmoles per mg chlorophyll per h), but this

reaction was complicated by a rapid dark reaction. Moreover, the photoreduction of ferricyanide was found to be resistant to DCMU.

As shown in Table I, the particles did not show any Photosystem I activity, as measured by $NADP^+$ photoreduction in the presence of the ascorbate–DCIP couple. They were also unable to catalyze the disproportionation of 1,5-diphenylcarbazone, a reaction that was found to be specifically dependent on Photosystem I[12,13]. The latter reaction was measured by the absorbance change at 485 nm in the presence of plastocyanin. The absence of P700 in F_{II} particles was proved by measuring the oxidized-*minus*-reduced difference spectrum as described by Yamamoto and Vernon[14].

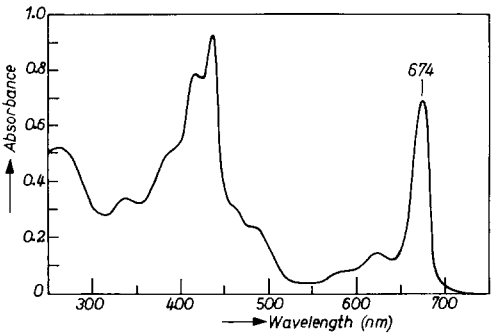


Fig. 3. Absorption spectrum of F_{II} particles.

The absorption spectrum of the particles is shown in Fig. 3; the absorption maximum is located at 674 nm. The fluorescence spectra at room temperature and at liquid nitrogen temperature are shown in Fig. 4. The maxima are located at 681 nm and at 685 nm, respectively, which is characteristic of Photosystem II. However, the emission band at 695 nm, which is usually observed in the low temperature emission spectra of spinach chloroplasts and of chloroplast fragments enriched in Photosystem II[15,16], was lacking in that of the F_{II} particles. This does not support the previous suggestion[17,18] that the fluorescencence at 695 nm may have originated from the trapping center for photosystem II.

Table II shows that the purified particles had a very high chlorophyll *a*/chlorophyll *b* ratio and that they were enriched in β-carotene. Per mg of chlorophyll they

TABLE II

PIGMENT COMPOSITION OF F_{II} PARTICLES

Pigment	*moles/100 moles chlorophyll a*
Chlorophyll *a*	100
Chlorophyll *b*	4
β-Carotene	12.5
Lutein	2.5

contained about 10 mg of protein. As some chlorophyll was lost during chromatography of the particles on a hydroxylapatite column, we cannot exclude the possibility that the increase of the chlorophyll *a*/chlorophyll *b* ratio in this purification step was due to the fact that the chlorophyll *b* was less tightly bound in the complex than chlorophyll *a*.

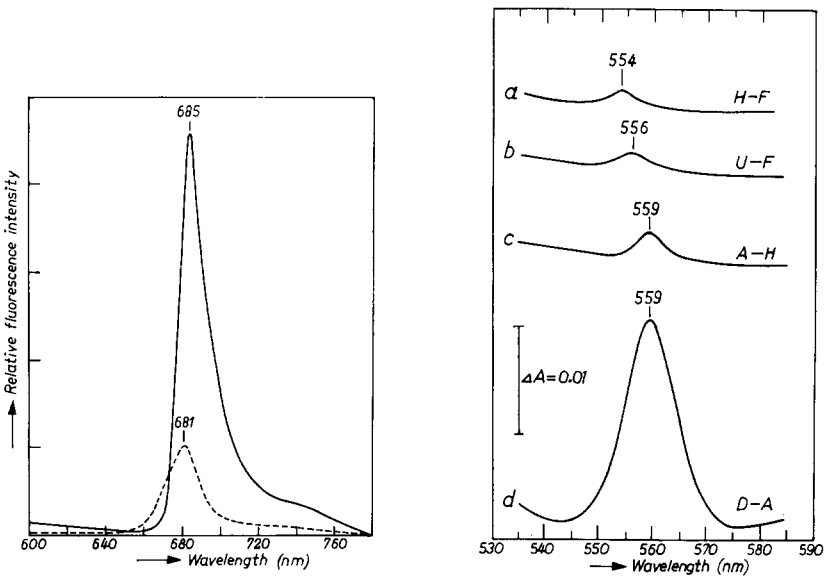


Fig. 4. Fluorescence emission spectrum of F_{II} particles at room temperature (-------) and at liquid nitrogen temperature (———). Wavelength for fluorescence excitation 440 nm. Chlorophyll concentration 5.8 μg/ml.

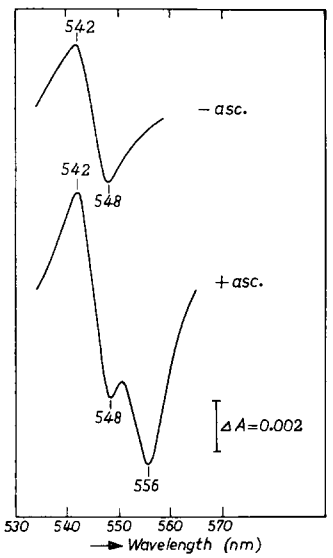
Fig. 5. Oxidized - *minus* - reduced difference spectra of F_{II} particles. The chlorophyll concentration was 42 μg/ml and the path length 1 cm. Where indicated potassium ferricyanide (0.16 mM), hydroquinone (0.4 mM), ascorbate (1.6 mM) or a few grains of dithionite were added. H−F, hydroquinone *minus* ferricyanide; U−F, untreated *minus* ferricyanide; A−H, ascorbate *minus* hydroquinone; D−A, dithionite *minus* ascorbate.

Although traces of cytochrome *f* and possibly also of cytochrome b_6 were present, the major cytochrome was cytochrome b_{559}, the ratio chlorophyll/cytochrome b_{559} being 40–50. Fig. 5, curve a, represents the hydroquinone reduced-*minus*-ferricyanide oxidized difference spectrum, which indicates that some cytochrome *f* was still present in the particles. As the band of solubilized cytochromes *f* and b_6 was located very close to the F_{II} band in the sucrose density gradient it appeared to be very difficult to get rid of all the cytochrome *f*. The small amount of this cytochrome in the particles (less than 1 mole per 600 moles of chlorophyll) suggests, however, that cytochrome *f* was not associated with F_{II} and had no essential function in its photochemical activity. The ascorbate reduced-*minus*-hydroquinone reduced (curve c), and the dithionite reduced-*minus*-ascorbate reduced (curve d) difference spectra show a peak at 559 nm, characteristic of cytochrome b_{559}. The latter curve demonstrates that little or no cytochrome b_6 is present in the preparation.

The difference spectra indicate that most of the cytochrome b_{559} was in the low-potential form; only a small part of this cytochrome (10–15%) could be reduced by ascorbate. Curve b, which represents the untreated-*minus*-ferricyanide oxidized difference spectrum, shows that the cytochrome b_{559} was mainly present in the oxidized state in F_{II} particles. Only a few per cent of this cytochrome seemed to be present in the reduced state.

At liquid-nitrogen temperature a photoreduction of C550 was observed, as shown by the light-*minus*-dark difference spectrum represented in Fig. 6. When ascorbate was added to the particle suspension prior to freezing, the photoreduction of C550 was accompanied by a photooxidation of cytochrome b_{559} (bleaching at 556 nm). Though a quantitative estimation of the amount of cytochrome b_{559} that was oxidized at 77 °K was not possible because of the variable intensification of the bands on freezing, the amount of ascorbate-reducible cytochrome b_{559}, measured at room temperature, seemed to be sufficient to account for the photooxidation observed at low temperature. The fact is that the absorbance change due to photo-oxidation of cytochrome b_{559} at 77 °K was equal to, or somewhat lower than, the height of the absorption peak at 559 nm in the ascorbate reduced-*minus*-hydroquinone reduced difference spectrum at room temperature.

At room temperature, only a very small light-induced absorbance change was observed at 559 nm (Fig. 7), which could be correlated with the photooxidation



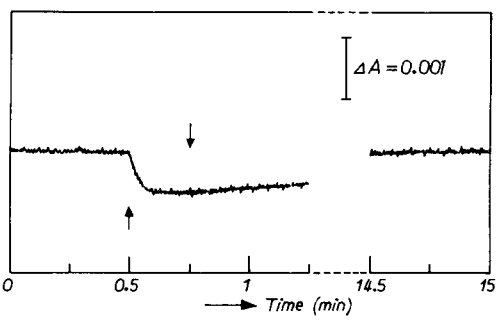


Fig. 6. Light - *minus* - dark difference spectrum of F_{II} particles at liquid-nitrogen temperature. The chlorophyll concentration was 1.4 mg chlorophyll/ml and the path length 1 mm. The band width was 0.8 nm. The lower curve was obtained when ascorbate was added to the particle suspension prior to freezing.

Fig. 7. Light-induced absorption change at 559 nm in F_{II} particles at room temperature. The light intensity was 10^4 ergs·cm^{-2}·s^{-1}, and the illumination time 15 s. Reference wavelength 540 nm. The chlorophyll concentration was 50 μg/ml, the path length 1 cm. Upward arrow, actinic light on; downward arrow, actinic light off.

of only a few per cent of the cytochrome b_{559} content of the particles. This is not surprising as it was shown that this cytochrome was mainly present in the oxidized state. In the dark the absorbance change was slowly reversed. Measurements at 554 and 563 nm (with 540 nm as reference wavelength) indicated that the light-induced absorbance change was not due to cytochrome *f* or to cytochrome b_6. In the presence of 1,5-diphenylcarbazide no absorbance change was observed. No indication was found for a photoreduction of cytochrome b_{559}, either in the presence or in the absence of 1,5-diphenylcarbazide, nor was it found either under anaerobic or under aerobic conditions.

DISCUSSION

In previous papers[2,3,19–22] we reported that the two main green bands obtained by density gradient centrifugation of the $80000 \times g$ supernatant of digitonin-treated spinach chloroplasts, F_I and F_{III}, were derived from Photosystem I and Photosystem II, respectively. F_I showed a high Photosystem I activity and was rich in chlorophyll *a*, β-carotene and P700. F_{III}, on the other hand, had a low chlorophyll *a*/chlorophyll *b* ratio, was rich in xanthophylls, and did not contain any P700. This fraction exhibited only a low Photosystem II activity, however, particularly in comparison with the activity of a minor green band, denoted F_{II}, and the activity was completely lost after chromatographic purification of the particles on a DEAE-cellulose column. The present isolation procedure permitted us to conclude that the Photosystem II activity in the gradient was in fact localized in F_{II}, and that the Photosystem I activity previously found in this band was due to some overlap of the F_I zone. Likewise, the low Photosystem II activity observed in F_{III} could be due to contamination by F_{II}. Further purification of F_{II} completely removed the Photosystem I activity while enhancing the Photosystem II activity.

The high chlorophyll *a*/chlorophyll *b* ratio and the enrichment in β-carotene of the F_{II} particles are remarkable. The high, DCMU-sensitive, Photosystem II activity and the enrichment in β-carotene indicate that these particles could represent the reaction-center complex of Photosystem II. Okayama and Butler[23] have recently shown that β-carotene is essential for the primary photochemical activity of Photosystem II, namely the photoreduction of C550 and the photooxidation of cytochrome b_{559} at 77 °K (see also refs 24 and 25). These reactions were first demonstrated by Knaff and Arnon[26–28], and confirmed by Erixon and Butler[29], Boardman *et al.*[30] and Bendall and Sofrova[31].

Erixon and Butler[32] showed that C550 acted as if it were the primary electron acceptor of Photosystem II and that cytochrome b_{559} was oxidized by the primary electron donor. The observation that F_{II} particles contain a large amount of cytochrome b_{559} and show a photoreduction of C550 and a photooxidation of cytochrome b_{559} at liquid nitrogen temperature strongly supports our assumption that these particles represent the reaction-center complex of Photosystem II.

The cytochrome in the particles was found to be mainly present in the oxidized state. This may be due to the fact that digitonin, like a variety of other disruptive treatments[23,24,33–40], converts the high-potential form of cytochrome b_{559} to a low-potential, autooxidizable form. Actually, most of the cytochrome b_{559} was present in the dithionite-reducible form; only a small part of it was reducible by

ascorbate and none by hydroquinone. However, the amount of ascorbate-reducible cytochrome b_{559} was sufficient to account for the photooxidation observed at 77 °K. Several groups[30,31,41] have suggested that it is the high-potential cytochrome b_{559} in chloroplasts that is photooxidized by Photosystem II at low temperature. Erixon *et al.*[39], on the other hand, demonstrated that cytochrome b_{559} in Tris-washed chloroplasts was oxidized at lower potentials and over a wider range of potentials than in intact chloroplasts. It is conceivable, therefore, that in F_{II} particles the ascorbate-reducible part of cytochrome b_{559} is oxidized by the primary electron donor of Photosystem II at 77 °K (*cf.* ref. 40).

Recently, Vernon *et al.*[4,5] and Ke *et al.*[6] isolated small particles with Photosystem II activity from Triton-treated chloroplast preparations, which were named TSF-2a particles and which were suggested to represent the reaction-center complex of the Photosystem II unit. In several respects the TSF-2a particles resemble the F_{II} particles described in this paper. The photochemical activity, the absorption and fluorescence spectra, and the cytochrome b_{559} content are similar. The higher chlorophyll *a*/chlorophyll *b* ratio and the higher β-carotene content of the F_{II} particles could be ascribed to the different isolation and purification procedure, However, the primary photochemical activity of the two types of Photosystem II particle is completely different. In TSF-2a particles no photooxidation of cytochrome b_{559} was observed at 77 °K, even when they were pre-reduced chemically. Absorbance changes at 550 nm, due to photoreduction of C550, have not been reported for these particles. At room temperature the cytochrome b_{559} in TSF-2a particles underwent a DCMU-resistant photoreduction, even in the absence of an electron donor such as 1,5-diphenylcarbazide. The F_{II} particles, on the other hand, did not show any photoreduction of cytochrome b_{559} under a variety of conditions. On the contrary a very slight photooxidation was observed, which was slowly reversed in the dark.

The observation that the primary photochemical activity of F_{II} particles resembles that of Photosystem II in chloroplasts suggests that these particles are in a more native state than the TSF-2a particles. It is possible that a structural derangement has occurred in the TSF-2a particles, as a result of the more rigorous detergent treatment, which severely inhibits the photooxidation of cytochrome b_{559}. A more efficient photooxidation of cytochrome b_{559} in the F_{II} particles, resulting in a faster cycling of electrons around Photosystem II *via* cytochrome b_{559}, could then have prevented us from observing the photoreduction of this cytochrome at room temperature.

In conclusion, the properties of the F_{II} particles strongly support the suggestion that these particles represent the reaction-center complex of the Photosystem II unit. *In vivo* the Photosystem II unit may consist of an association of this reaction-center complex and an accessory complex. Probably the upper green band of the density gradient (F_{III}), which is characterized by a low chlorophyll *a*/chlorophyll *b* ratio and a low β-carotene content, contains the accessory complex of Photosystem II.

REFERENCES

1 Boardman, N. K. (1970) *Annu. Rev. Plant Physiol.* 21, 115–140
2 Wessels, J. S. C. and van Leeuwen, M. J. F. (1971) in *Energy Transduction in Respiration and Photosynthesis* (Quagliariello, E., Papa, S. and Rossi, C. S., eds), pp. 537–550, Adriatica Editrice, Bari
3 Wessels, J. S. C. and Voorn, G. (1972) in *Proc. 2nd Int. Congress on Photosynthesis Research* (Forti, G., Avron, M. and Melandri, A., eds), Vol. I, pp. 833–845, Dr. W. Junk, The Hague
4 Vernon, L. P., Shaw, E. R., Ogawa, T. and Raveed, D. (1971) *Photochem. Photobiol.* 14, 343–357
5 Vernon, L. P., Klein, S., White, F. G., Shaw, E. R. and Mayne, B. C. (1972) in *Proc. 2nd Int. Congress on Photosynthesis Research* (Forti, G., Avron, M. and Melandri, A., eds), Vol. I, pp. 801–812, Dr. W. Junk, The Hague
6 Ke, B., Vernon, L. P. and Chaney, T. H. (1972) *Biochim. Biophys. Acta* 256, 345–357
7 Arnon, D. I. (1949) *Plant Physiol.* 24, 1–15
8 Boardman, N. K. and Thorne, S. W. (1971) *Biochim. Biophys. Acta* 253, 222–231
9 Borchert, M. T. and Wessels, J. S. C. (1970) *Biochim. Biophys. Acta* 197, 78–83
10 Hager, A. and Bertenrath, T. (1962) *Planta* 58, 564–568
11 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, A. J. (1951) *J. Biol. Chem.* 193, 265–275
12 Shneyour, A. and Avron, M. (1971) *Biochim. Biophys. Acta* 253, 412–420
13 Vernon, L. P. and Shaw, E. R. (1972) *Plant Physiol.* 49, 862–863
14 Yamamoto, H. Y. and Vernon, L. P. (1969) *Biochemistry* 8, 4131–4137
15 Boardman, N. K., Thorne, S. W. and Anderson, J. M. (1966) *Proc. Natl. Acad. Sci. U.S.* 56, 586–593
16 Ke, B. and Vernon, L. P. (1967) *Biochemistry* 6, 2221–2226
17 Krey, A. and Govindjee (1964) *Proc. Natl. Acad. Sci. U.S.* 52, 1568–1572
18 Krey, A. and Govindjee (1966) *Biochim. Biophys. Acta* 120, 1–18
19 Wessels, J. S. C. (1965) *Biochim. Biophys. Acta* 109, 614–616
20 Wessels, J. S. C. (1966) *Biochim. Biophys. Acta* 126, 581–583
21 Wessels, J. S. C. (1968) *Biochim. Biophys. Acta* 153, 497–500
22 Wessels, J. S. C. (1969) in *Progress in Photosynthesis Research* (Metzner, H. ed.), Vol. I, pp. 128–136, Laupp, Tübingen
23 Okayama, S. and Butler, W. L. (1972) *Plant Physiol.* 49, 769–774
24 Sofrova, D. and Bendall, D. S. (1972) in *Proc. 2nd Int. Congress on Photosynthesis Research* (Forti, G., Avron, M. and Melandri, A., eds), Vol. I, pp. 561–567
25 Butler, W. L., Erixon, K. and Okayama, S. (1972) in *Proc. 2nd Int. Congress on Photosynthesis Research* (Forti, G., Avron, M. and Melandri, A., eds), Vol. I, pp. 73–80
26 Knaff, D. B. and Arnon, D. I. (1969) *Proc. Natl. Acad. Sci, U.S.* 63, 956–962
27 Knaff, D. B. and Arnon, D. I. (1969) *Proc. Natl. Acad. Sci. U.S.* 63, 963–969
28 Knaff, D. B. and Arnon, D. I. (1969) *Proc. Natl. Acad. Sci. U.S.* 64, 715–722
29 Erixon, K. and Butler, W. L. (1971) *Photochem. Photobiol.* 14, 427–433
30 Boardman, N. K., Anderson, J. M. and Hiller, R. G. (1971) *Biochim. Biophys. Acta* 234, 126-136
31 Bendall, D. S. and Sofrova, D. (1971) *Biochim. Biophys. Acta* 234, 371–380
32 Erixon, K. and Butler, W. L. (1971) *Biochim. Biophys. Acta* 234, 381–389
33 Boardman, N. K. and Anderson, J. M. (1967) *Biochim. Biophys. Acta* 143, 187–203
34 Plesnicar, M. and Bendall, D. S. (1970) *Biochim. Biophys. Acta* 216, 192–199
35 Hind, G. and Nakatani, H. Y. (1970) *Biochim. Biophys. Acta* 216, 223–225
36 Wada, K. and Arnon, D. I. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 3064–3068
37 Okayama, S., Epel, B. L., Erixon, K., Lozier, R. and Butler, W. L. (1971) *Biochim. Biophys. Acta* 253, 476–482
38 Cramer, W. A., Fan, H. N. and Böhme, H. (1971) *J. Bioenerg.* 2, 289–303
39 Erixon, K., Lozier, R. and Butler, W. L. (1972) *Biochim. Biophys. Acta* 267, 375–382
40 Cox, R. P. and Bendall, D. S. (1972) *Biochim. Biophys. Acta* 283, 124–135
41 Cramer, W. A. and Böhme, H. (1972) *Biochim. Biophys. Acta* 256, 358–369