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Chlorophyll Composition and Photochemical Activity of Photosystems Detached from Chloroplast Grana and Stroma Lamellae

(chloroplast fractions, ultrastructure, and spectra/forms of chlorophyll)

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ABSTRACT **A stroma fraction that has photosystem 1 activity and grana lamellae fractions that have activities for both photosystems were isolated by differential centrifugation of a needle valve homogenate. Subsequent fractions, corresponding to photosystems 1 (F-1D) and 2 (F-2D) were isolated by digitonin treatment of the grana lamellae (P-10K) and compared with respect to their chlorophyll composition and electron transport activities.**

Fraction F-2D from grana lamellae having photosystem 2 activity is primarily active in photosystem 2 and contains only the four major forms of chlorophyll *a* with a predominance of chlorophyll *a* 677 nm. This fraction differs from the original grana membranes in the absence of the long-wavelength form of chlorophyll *a* and in the widening of the absorption band of chlorophyll *a* 682 nm from 10.9 to 15.6 nm.

Photosystem 1 particles from grana and stroma both have high photosystem 1 activity but differ from each other in the proportions of the four major forms of chlorophyll *a*. The short-wavelength forms of chlorophyll *a* and also chlorophyll *b* 650 nm in particles from grana lamellae comprise relatively more total area than these same forms in the particles from stroma. In addition, the fraction corresponding to photosystem 1 from grana lamellae is not shifted to the long-wavelength side of the main absorption maximum, as compared to the photosystem 2 particles from grana and the original grana membrane fraction; this is usually observed in fractions that have photosystem 1 activity. Furthermore, the longest wavelength form of chlorophyll *a* in the photosystem 1 particles from grana is at 700 nm, while in the same fraction from stroma, it is at 706 nm.

The half-width of the four main forms of chlorophyll *a* and both forms of chlorophyll *b* in the photosystem 1 fraction from grana is narrower than that of the corresponding forms in the same fraction from stroma. This may indicate a different packing of pigment molecules that are aggregated on the surface of membranes of these two fractions.

A method developed by Michel and Michel-Wolwertz (1) for partially separating two photochemical systems demonstrated that passage of chloroplasts through a needle valve followed by sucrose gradient centrifugation separates system 1 and system 2 activities. Sane *et al.* (2) and Jacobi and Lehmann (3) proposed that photosystem 1 fragments prepared in this way originate from stroma lamellae and possibly from the ends of grana membranes, whereas the fraction enriched in photosystem 2 originates from the grana regions. From these studies, it was also concluded that stroma lamellae contain photosystem 1, while grana contain photosystems 1 and 2. Goodchild and Park (4) suggested that the photosystem 1 fraction obtained by incubation with digitonin also originates from stroma lamellae. Boardman (5) agrees with the conclusion that the initial action of digitonin is to separate stroma lamellae from grana.

Huzisige *et al.* (6) and Arnon *et al.* (7) achieved a further separation and purification of photosystem 2 from digitonin fragments enriched in photosystem 2 by treating them with Triton X-100. Boardman (5) also demonstrated conclusively that photosystems 1 and 2 can be released from grana fragments prepared by incubation with digitonin by treatment with Triton X-100. According to him, there were no significant differences between photosystem 1 particles obtained from stroma and grana membranes either in chlorophyll *a/b* ratio or in P700 content.

Recently, Arntzen *et al.* (8) have further fractionated press-treated particles with digitonin. They were able to fractionate the grana membranes into 60% photosystem 2 and 40% photosystem 1 particles on a chlorophyll basis. Photosystem 1 particles obtained from stroma or grana membranes were quite similar with regard to electron transport activity, P700 content, ultrastructure appearance, and ultrafiltration characteristics. However, photosystem 1 fragments of stroma did not recombine with the photosystem 2 fraction of grana and reconstitute electron transport activity from diphenylcarbazide → $NADP^+$ as did photosystem 1 fractions of grana (8). Further, the sample of stroma photosystem 1 had higher chlorophyll *a/b* ratio and higher content of P700 than grana lamellae fractions (8). In addition, on the basis of the P700 content of stroma and grana lamellae, Sane *et al.* (2) suggested that the photosystem 1 of stroma has a smaller photosynthetic unit than grana photosystem 1.

It is well known that chlorophyll *a* exists *in vivo* as several forms with distinct absorption spectra (9). It is generally believed that two photosystems function in green plant photosynthesis. One photoreaction (photosystem 2) is closer to the O_2 evolving step and mainly utilizes visible light of wavelength shorter than 680 nm absorbed by such active pigments as chlorophyll *b*, and certain forms of chlorophyll *a*. The other (photosystem 1) is closer to NADP reduction and is mediated preferentially by the longer wavelength forms of chlorophyll *a*. When the photosystems have been partially

Abbreviations: P-10K, fraction consists of grana membranes; P-144K, fraction from stroma lamellae corresponding to photosystem 1; F-1D and F-2D, digitonin fractions from grana lamellae corresponding to photosystems 1 and 2, respectively.

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physically separated from each other, absorption spectra that show the relative amounts of the different pigments in each fraction may be used to indicate the degree of separation. The results reported here support the views of Sane *et al.* (2) and of Arntzen *et al.* (8) that system 1 preparations from grana and those from stroma lamellae are different from each other in some properties, such as their pigment composition.

MATERIALS AND METHODS

Preparation of Grana and Stroma Lamellae. Chloroplasts were isolated from spinach leaves purchased at the local market by blending in phosphate buffer (pH 7.8)—0.05 M phosphate, 0.4 M sucrose, and 0.01 M NaCl. The pellet was suspended in a solution containing 150 mM KCl and 50 mM Tricine–KOH buffer (pH 7.8) and forced through a needle valve three times at 12,500 lbs/in² (800 kg/cm²) (1). The resulting suspension of disrupted chloroplasts was centrifuged at 3000 × *g* for 5 min to remove unbroken chloroplasts, and the supernatant was then centrifuged at 10,000 × *g* for 30 min. The sediment containing grana membrane particles was suspended in the KCl–Tricine buffer and centrifuged for 5 min at 3000 × *g* to remove large or aggregated particles. The supernatant was used as the grana membrane preparation (P-10K) (10). The supernatant from the 10,000 × *g* fraction was centrifuged at 60,000 × *g* for 30 min, and the sediment was discarded. The photosystem 1 particles in this supernatant were all sedimented at 144,000 × *g* for 60 min (P-144K) and comprised the stroma lamellae system 1.

Digitonin Fractionation. The grana membrane fraction (P-10K) (0.25–0.3 mg of chlorophyll per ml) was made 1% in digitonin and incubated at 4° for 30 min with gentle stirring. After this period, 3 ml of the mixture was fractionated by centrifugation at 60,000 × *g* for 45 min on a 30-ml linear sucrose density gradient [sucrose 12.5–55% (w/v)–0.05 M Tricine buffer (pH 7.8), containing 0.15 M KCl]. Two distinct green bands were formed. The uppergreen band with about 22% sucrose, containing the system 1 preparation from grana, was called (F-1D), while the lower band at about 41% sucrose, containing system 2 from grana, was called (F-2D).

TABLE 1. *The photochemical activity and chlorophyll a/b ratio of several chloroplast fractions from grana and stroma lamellae*

Fraction	Chlorophyll *a/b* ratio	Photosystem 2 activity DPC → DCIP (μmol per mg of chlorophyll/hr)	Photosystem 1 activity Cytochrome *c* oxidation (μmol per mg of chlorophyll/hr)
Homogenate*	3.1	32	29
P-10K (grana membranes)	2.7	43	23
P-144K	8.1	3	53
F-1D	4.6	6	174
F-2D	2.2	38	10

Medium for photosystem 1 assay contained: 40–60 μM reduced horse-heart cytochrome *c*, 8 μM 3-(3,4-dichlorophenyl)-I,I-dimethylurea, 40 μM methyl viologen, 4 mM potassium cyanide, about 2 μM plastocyanin, 8 μg chlorophyll/ml, and 0.05 M Tricine (pH 7.5), in a total volume of 2.5 ml.

Medium for photosystem 2 assay contained: 20 μM 2,6-dichloroindophenol (DCIP), 400 μM 1,5-diphenylcarbohydrazide (DPC), and 8 μg chlorophyll/ml in 0.05 M Tricine (pH 7.5), in a total volume of 2.5 ml.

* Needle valve chloroplast fraction.

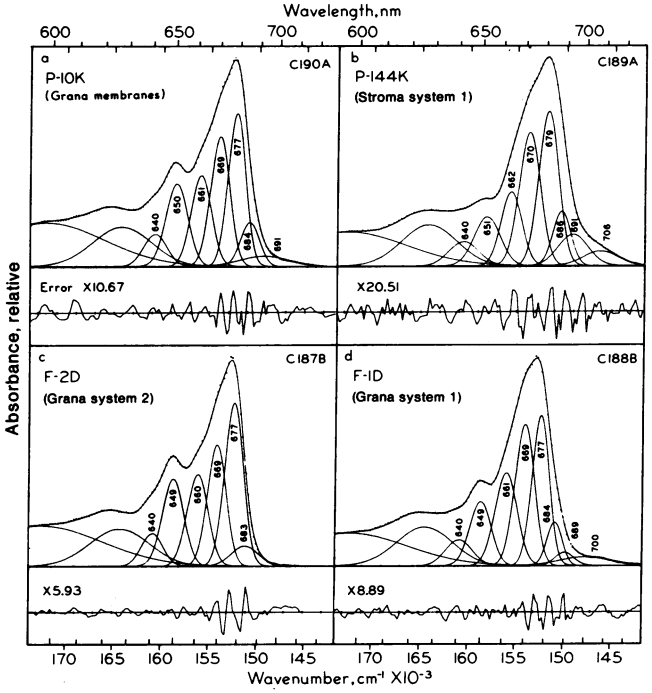

Fig. 1. Curve analyses of the absorption spectra of chloroplast fractions from stroma and grana lamellae measured at −196°. The parameters of the components are given in Table 2.

Other Assays. Chlorophyll *a/b* ratios were determined by Arnon's method (11). The light-saturated reaction rates of 2,6-dichloroindophenol reduction (photosystem 2 activity) and of cytochrome *c* oxidation (photosystem 1 activity) were measured as described by Brown (12). Absorption spectra at −196° were recorded in the spectrophotometer described in ref. 13. Curves were analyzed on digitized spectra, as described by French *et al.* (14).

RESULTS AND DISCUSSION

Chlorophyll *a/b* ratios, chlorophyll composition (by curve analysis), and the activities of photochemical systems 1 and 2 of these four (P-10K, P-144K, F-1D, and F-2D) fractions were investigated. The photochemical activities and chlorophyll *a/b* ratios of the various membrane fractions are shown in Table 1. The stroma lamellae (P-144K) are deficient in photosystem 2 activity and have a higher chlorophyll *a/b* ratio than the original.

The grana membrane fraction (P-10K) shows both activities. Digitonin treatment of grana membranes allowed the isolation of purified photosystem 1 (F-1D) and greatly enriched photosystem 2 (F-2D) fractions. The F-1D fraction had a higher chlorophyll *a/b* ratio than either the French press homogenate or the grana membrane fraction (P-10K), although the values were not as high as those for the stroma lamellae fractions. Fraction F-2D had a very low chlorophyll *a/b* ratio, as shown in Table 1.

TABLE 2. *Peak wavelengths, halfwidths, and areas of spectral components representing different forms of chlorophyll in fractions detached from grana membranes and in the system 1 fraction from stroma membranes*

	Chlorophyll *b*		Chlorophyll *a*					
	Cb 640	*Cb* 650	*Ca* 662	*Ca* 670	*Ca* 677	*Ca* 684	*Ca* 692	*Ca* 705
Grana membranes, P-10K								
Wavelength, nm	640.4	649.8	660.8	669.4	677.3	683.9	690.7	—
Width, nm	11.7	11.4	10.8	10.5	9.9	10.9	29.9	—
Area, as percent of total *a* or *b*	29	71	22	28	32	11	7	—
System 1 from stroma, P-144K								
Wavelength, nm	640.6	650.9	661.8	670.2	679.2	685.7	691.3	705.8
Width, nm	15.1	13.6	11.1	11.7	11.3	10.4	17.7	21.4
Area, as percent of total *a* or *b*	36	64	15	30	32	9	9	5
System 2 from grana, F-2D								
Wavelength, nm	640.0	649.1	660.1	668.9	677.0	682.7	—	—
Width, nm	10.8	11.0	11.2	10.3	10.5	15.6	—	—
Area, as percent of total *a* or *b*	26	74	25	30	38	7	—	—
System 1 from grana, F-1D								
Wavelength, nm	640.0	649.4	660.8	669.3	676.8	683.8	688.7	699.7
Width, nm	13.4	11.1	11.8	10.6	9.7	8.8	11.0	31.1
Area, as percent of total *a* or *b*	33	67	23	31	30	7	3	6

The low temperature absorption spectra of the chloroplast fractions are shown in Fig. 1. The spectra have been matched with component curves. The peak wavelength, bandwidth, and percentage of total area of each Gaussian component curve are given in Table 2. The region around 680 nm is much more strongly absorbed by P-144K than by P-10K particles, and the height of chlorophyll *b* is much greater in P-10K. The present results agree remarkably well with the conclusions of Michel and Michel-Wolwertz (1) and French *et al.* (15). Fig. 1*b* shows a curve analysis of fraction P-144K. The major chlorophyll *a* components are at 662, 670, 679, and 685 nm. There are also two longer wavelength chlorophyll forms at 691 and at 705 nm. Fig. 1*d* shows a curve analysis of fraction F-1D that is greatly enriched in photosystem 1 from the grana membranes. This fraction has high system 1 activity but differs from the P-144K preparation of system 1 of stroma in the relative proportions of the four major forms of chlorophyll *a*. The shorter wavelength forms of chlorophyll *a* (chlorophylls *a* 662 and *a* 670) and also chlorophyll *b* 650 comprise relatively more total area (see Table 2) than these same pigment forms in fraction P-144K. In addition, fraction F-1D does not have a shift to the long-wavelength side of the main maximum in the absorption spectrum, compared to fraction F-2D and the grana membranes, as is usually observed in fractions that have enriched photosystem 1 activity, and in particular, in fraction P-144K. Furthermore, the longest wavelength form of chlorophyll in fraction F-1D is chlorophyll *a* 699.7, while in fraction P-144K, it is chlorophyll *a* 705.8. Such detergents as Triton increase the activity of chloroplast fractions for cytochrome *c* oxidation. This treatment also changes the absorption spectrum (12). It is possible that digitonin treatment also leads to the same result. This suggests that the long-wavelength bands are aggregates of shorter wavelength components that may be disrupted by the detergent treatment. On the other hand, our preliminary work revealed that a digitonin treatment of fraction P-144K does not lead to changes in the absorption spectrum.

Analysis of the halfwidth of the main forms of chlorophyll *a* and chlorophyll *b* in fraction F-1D (Table 2), gave bands relatively narrower than the corresponding forms of these pigments in fraction P-144K. In addition, fraction F-1D has more chlorophyll *b* than P-144K. Probably, the pigments in fraction F-1D from grana membranes have different packing into aggregates than the corresponding forms of pigment that are on the surface of the stroma lamellae. Thus, in all probability, photosystem 1 preparations from stroma and grana membranes have different pigment compositions. This could be one of the reasons why Arntzen *et al.* (8) observed that the photosystem 1 fraction of stroma lamellae prepared by digitonin treatment was not capable of recombining with the photosystem 2 fraction of grana.

From Fig. 1*c* and Table 2 it is evident that fraction F-2D contains only four major forms of chlorophyll *a* with a predominance of chlorophyll *a* 677. This fraction differs from the original fraction in the absence of the long-wavelength form chlorophyll *a* 691 and in the widening of band chlorophyll *a* 682 from 10.9 to 15.6. These particles may be suitable from the point of view of pigment composition for studying P-680 as a possible reaction center of photosystem 2.

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