WATER SOLUBLE SPINACH CHLOROPLAST CHLOROPHYLL-PROTEIN COMPLEXES PREPARED WITHOUT ADDED DETERGENTS OR ORGANIC SOLVENTS[+]

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Two water soluble chlorophyll-protein complexes were obtained from spinach chloroplast thylakoids without the aid of added detergents or organic solvents. One complex contained the pigments chlorophyll a & b and the quinones vitamin K_1, tocopherolquinone, and a plastoquinone while the other contained in addition a second plastoquinone and the pigments lutein, violaxanth and neoxanthin. Both complexes showed a tendency to aggregate during purification; however, a crude mixture of these two complexes had an average apparent sedimentation coefficient ($S_{20,w}$) of 5.6 S. The chlorophyll absorption spectrum of the combined complexes was very similar to that of the chlorophylls in vivo. The red absorption maximum for chlorophyll a was at 679 mμ and that for chlorophyll b was at 652 mμ.

A number of investigators have attempted to separate physically the two photosystems through the use of detergents such as digitonin (Anderson and Boardman, 1966: Anderson et al., 1966), sodium dodecyl sulfate (SDS) (Ogawa et al., 1966), and Triton X-100 (Vernon et al., 1967). The detergents were found to disrupt the chloroplast thylakoids into two main fractions. Based on composition and biological activity, one fraction has been associated with photosystem I and the other with photosystem II. The two fractions were separated by differential centrifugation after digitonin or Triton X-100 treatment or by disc gel electrophoresis after SDS treatment. "Heavy particles" (Photosystem II) sedimented by centrifugal forces of 10,000 xg for 30 min., had a chlorophyll a to chlorophyll b ratio of about 2, were enriched in Mn

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and xanthophyll, and showed Hill activity. "Light particles" (Photosystem I) sedimented by centrifugal forces of 144,000 x g for 60 min., had a chlorophyll a to chlorophyll b ratio of about 6, were enriched in P700 and β-carotene, and photoreduced NADP in the presence of sodium ascorbate and 2,6-dichlorophenol-indophenol.

The present paper presents results which indicate that we have isolated both photosystems I and II in a water soluble form without the aid of added detergents or organic solvents.

Methods and Materials

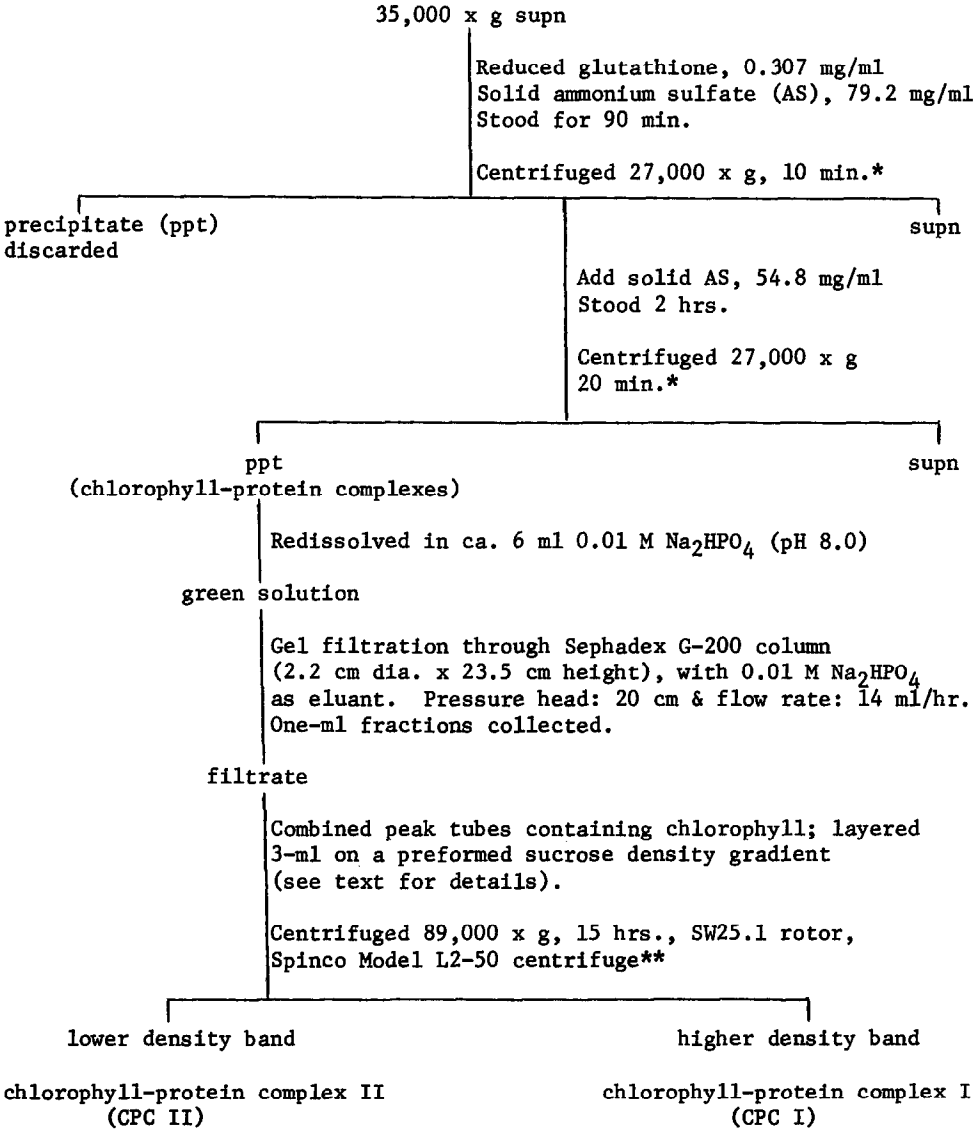
Isolation and Purification of the Chlorophyll-Protein Complexes

Chloroplasts were prepared from mature leaves of greenhouse grown Spinacea oleraceae by the method of Pon (1967). Chloroplasts from 2.4 kg of spinach leaves were washed five times by alternate resuspension and centrifugation using as wash medium, 0.01 M disodium phosphate (pH 8, 150 ml). Prior to each centrifugation, the suspension was made to 0.1 M NaCl. As a pellet, the chloroplasts were frozen and stored for at least three weeks in the freezer compartment of a refrigerator (-10 to -12°C). If enough time for aging is not allowed, little or no yield of the chlorophyll-proteins is obtained. The frozen chloroplasts were thawed at between 0 to 5°C and re-suspended in 0.01 M disodium phosphate (pH 8, 150 ml). Solid sodium chloride (5.84 mg/ml) was added and the suspension was centrifuged at 35,000 x g for 90 min. in a Sorvall RC-2B refrigerated centrifuge. The resulting clear, green supernatant (35,000 x g supn) contained the chlorophyll-protein complexes.

A detailed flow diagram of the purification procedure is given in figure 1. The sucrose density gradient was formed by layering the following solutions into a centrifuge tube (cellulose nitrate - 1 in.dia. x 3 in. length): 3 ml of 46% sucrose, 4 ml of 42% sucrose, 5 ml each of 41%, 40%, 39% and 38% sucrose, and 3 ml of sample (bottom to top).

Protein Assays

Protein was assayed by its absorbance at 280 mμ or by the Folin-phenol



* A Sorvall SS-34 rotor and a Sorvall RC 2B centrifuge were used unless otherwise noted.

**The bottoms of the centrifuge tubes were punctured and 15-drop fractions were collected.

Figure 1. Flow diagram for the purification of the chlorophyll-protein complexes. All operations were performed at, or near 0°C.

method of Lowry et al. (1951). The amount of protein determined from its absorbance at 280 mμ is based on the assumption that 1-mg protein/ml gives an optical density of 1.0 for a 1-cm light path. In the Lowry assay, bovine serum albumin was used as the standard and the chlorophylls, carotenoids, and quinones were found to not interfere with the measured absorbance at 750 mμ of the assay.

Paper Chromatography of Pigments and Quinones

Pigments and quinones were extracted at room temperature from the chlorophyll-protein complexes with 80% (v/v) aqueous acetone and then with ether (Jeffery, 1961). Care was taken to work in a darkened room with diffused light for visibility. Separation of the pigments was achieved by the two dimensional paper chromatography method of Jeffery (1961) while the quinones were separated by the method of Lichtenthaler (1964).

All spectrophotometric measurements were carried out using a Cary Recording Spectrophotometer, Model 15 and using 1-cm light path cuvettes.

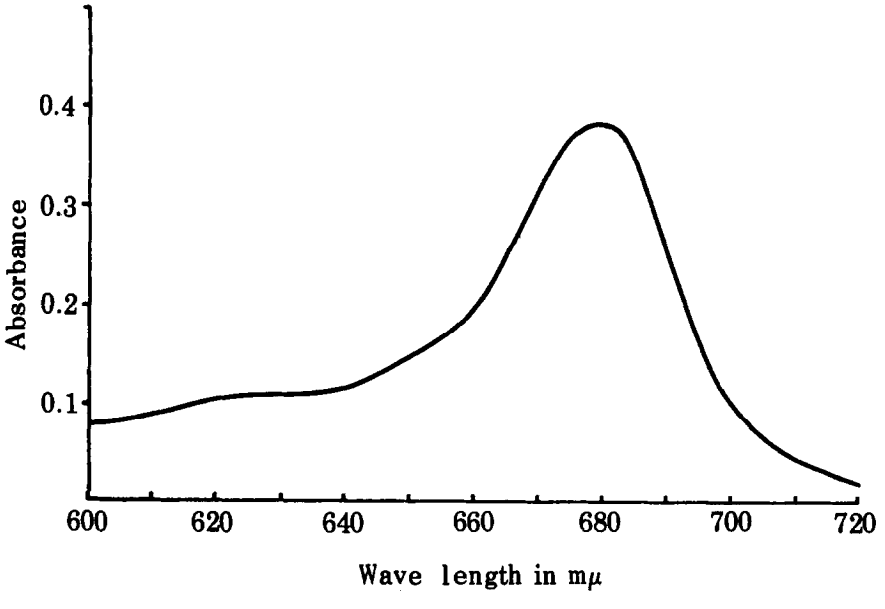


Figure 2. Absorption spectrum of the 35,000 x g supn. The solvent was 0.01 M Na_2HPO_4 (pH 8.8) + 0.1 M NaCl and the work was done at room temperature.

Results

The absorption spectrum of the 35,000 x g supn is given in figure 2. The red absorption band for chlorophyll a has its maximum at 679 mμ while that for chlorophyll b is at 652 mμ. The spectrum obtained is qualitatively identical to that obtained by Thomas (1962) for the chlorophylls in whole leaves. A slight shift (2 mμ) of the chlorophyll a red-band maximum toward shorter wavelengths was observed when the proteins were fractionated with ammonium sulfate.

Figure 3 shows a typical centrifuge tube obtained from the sucrose density gradient centrifugation step. The lower band (higher density)

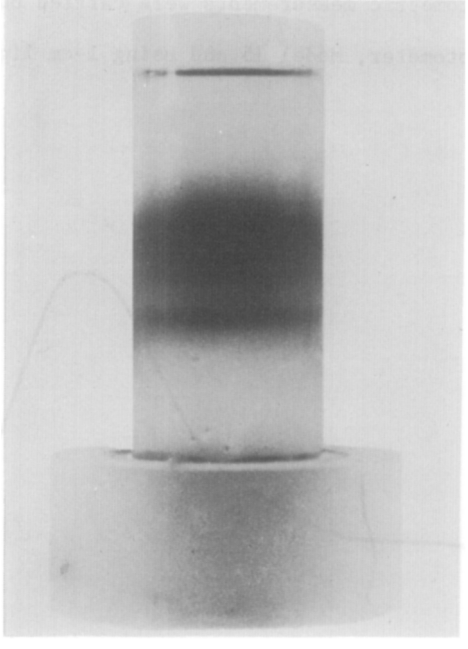


Figure 3. Centrifuge tube showing separation of CPC I and CPC II on a preformed density gradient. The total volume per tube was 30 ml and the tubes were centrifuged in an SW 25.1 rotor at 88,900 x g for 10 hrs. at 10°C. The upper band is CPC II and the lower band is CPC I.

was designated as CPC I and the upper band (lower density) was designated as CPC II. Based on chlorophyll, the amount of CPC II is about 3.6 times greater than CPC I. Preliminary results from equilibrium sucrose density gradient centrifugation based on the method of Meselson et al. (1957) gave a density of 1.151 for CPC II and 1.156 for CPC I.

Results from ultracentrifugation studies showed that the chlorophyll-proteins have a strong tendency to aggregate during purification. To obtain an average sedimentation coefficient of the chlorophyll-proteins, sedimentation velocity studies were conducted using the 35,000 x g supn. Interference from contaminating proteins was eliminated by using a 436 mμ filter with a special camera lens in the absorption optics system of a Spinco Model E analytical ultracentrifuge. An apparent average $S_{20,w}$ value of 5.6 S was obtained, using 0.87 cc/g for the average apparent specific volume.

Table I

Pigment and quinone content of the chlorophyll-protein complexes; (+) = present and (-) = absent.

Compound	Sample		
	Frozen-thawed chloroplast thylakoids**	CPC I	CPC II
Chlorophyll a		+	+
Chlorophyll b		+	+
Lutein		-	+
Violaxanthin		-	+
Neoxanthin		-	+
Plastoquinone$_1$*		+	+
Plastoquinone$_2$*		-	+
Vitamin K_1		+	+
Tocopherolquinone*		+	+
Chlorophyll a:b ratio	2.8	3.8	1.8

**Only chlorophyll a:b ratio was determined.

*Results did not permit an unequivocal identification of the form of the quinone in question.

A list of the pigments and quinones extracted from the two pigment-protein complexes by 80% (v/v) aqueous acetone is given in Table I. CPC I was found to contain vitamin K_1, tocopherolquinone, a plastoquinone, and the chlorophylls a & b. CPC II contained in addition to the above a second plastoquinone and the pigments lutein, violaxanthin, and neoxanthin. A more specific identification of the quinones must await further work.

The chlorophyll a to chlorophyll b ratios given in Table I for frozen-thawed thylakoids and CPC's were calculated by the method of Arnon (1949).

The values obtained are 3 for thylakoids, 2 for CPC II, and 4 for CPC I. This suggests a possible one to one relationship between CPC I and CPC II.

Discussion

Chemical and physical evidence indicate that we have isolated the minimal structural unit of photosystems I and II. The chemical composition and the chlorophyll a to chlorophyll b ratio given in Table I for CPC I agree with that of the "heavy particles" while that given for CPC II agree with that of the "light particles". The relatively small average sedimentation coefficient of 5.6 S favors the chlorophyll-proteins having a relatively small molecular weight. The diffusion coefficient has not been measured but assuming a normal diffusion coefficient for a 5.6 S lipoprotein, the average molecular weight would be about 50,000. Considering the results from chemical analysis, it would be highly unlikely for the value of 50,000 to be a whole number multiple of a smaller molecular weight unit.

The absorption spectrum given in figure 2 indicates that the chlorophylls of the chlorophyll-proteins are in an environment very similar to that in vivo. Qualitatively, the spectrum is essentially identical to that obtained by Thomas (1962) for the chlorophylls in whole leaves. The slight shift of the red maximum toward shorter wavelengths upon purification is contrary to what one would expect if aggregation of the chlorophyll-proteins was solely due to interactions of chlorophyll molecules. Exactly what is taking place during the aggregation process is still unknown.

Not only is this the first time that chlorophyll-protein complexes have been isolated from spinach chloroplasts without the aid of added detergents or organic solvents, it is the first time that such small sized components of the chloroplasts have been isolated with spectroscopic properties similar to that of the in vivo chlorophylls. If the present day concept of two photosystems is correct, then a study of the two chlorophyll-proteins may provide answers to questions concerning the structure, composition, and size of the structural units of photosynthesis. Work along these lines are currently under progress.

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