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ISOLATION OF P700-CHLOROPHYLL-PROTEIN COMPLEX FROM A BLUE-GREEN ALGA BY A NONDETERGENT METHOD

Cinnia Huang and Donald S. Berns

Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201

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SUMMARY

A procedure has been developed for the isolation of a P700-chlorophyll-protein complex from a blue-green alga by a nondetergent method. The use of a Sepharose 4B column in conjunction with sucrose density-gradient centrifugation allows the separation of this complex from three other chlorophyll a-containing fractions which have higher ratios of carotenoids to chlorophyll a. Isoelectric focusing results in a single band with an isoelectric point at 4.5. Analysis of the major (green) fraction reveals the presence of P700. The absorption and emission spectra of this complex are reported.

The existence of two photosystems acting in series for photosynthesis in blue-green algae was first suggested by Hill and Bendall in 1960 (1). The phycobiliproteins function as accessory pigments in photosystem II, whereas chlorophyll a and carotenoids serve the equivalent light-harvesting function in photosystem I. The phycobiliproteins are easily extracted from the membranes with aqueous solvents, and their functions and properties have been studied extensively (2-4).

Knowledge of the chlorophyll-protein complexes in higher plants and algae has been extended by studies on isolated fractions of the photosynthetic apparatus (5-9). Dietrich and Thornber (10) have shown that one chlorophyll-protein complex of a blue-green alga contains P700 in a ratio of 1 reaction center molecule per 100 light-harvesting chlorophyll molecules. A number of reports in the last few years indicate resolution of additional chlorophyll-protein complexes from various sources (11-13). The pigment and protein compositions in the complexes have been reported

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by many authors (9, 13-15). As indicated in the reviews by Thornber (16) and Boardman (17), the use of a detergent, whether sodium dodecyl sulfate (SDS; 9), digitonin (18), Triton X-100 (19), or Deriphat 160 (6), is required to fractionate the chlorophyll-protein complexes.

This report describes a new procedure for the isolation of four pigment-protein complexes from a blue-green alga, Phormidium luridum, by a nondetergent method. One of these complexes appears to be analogous to the P700-chlorophyll-protein previously observed by Thornber (9). Investigation of a P700-chlorophyll-protein complex of the simpler blue-green alga by this new procedure would allow us to learn more about the organization of chlorophyll a and carotenoids in vivo.

EXPERIMENTAL

Procedure for Isolation of P700-Chlorophyll-Protein Complex (Fig. 1): After harvest the P. luridum cells are mixed with 50 mM Tris buffer at pH 8.0 and lysed by lysozyme in the cold room overnight. The cell suspension is then frozen and thawed at room temperature. The suspension of broken cells is poured onto a Sepharose 4B column, which is eluted with 50 mM Tris (pH 8.0) buffer. The chlorophyll-containing fractions (yellow-green) are eluted first, followed by the phycobiliprotein fractions (blue). The yellow-green fractions are pooled and then concentrated with Ficoll. The concentrated solution is layered onto a 15-60% sucrose gradient in 50 mM Tris buffer. After centrifugation at 39,000 rpm for 2 h in a Beckman SW41 rotor the separated bands are collected. The fastest-sedimenting band appears in such a way that a minor orange portion is adjacent to the major green portion. This orange-green band is layered on a second 15-60% sucrose gradient and centrifuged for 48 h. A complete separation between the orange and green fractions is obtained. The green fraction is used for the following experiments.

Isoelectric Focusing: Isoelectric focusing is performed in a 110-ml LKB Ampholine column (Type 8101) with a sucrose gradient and

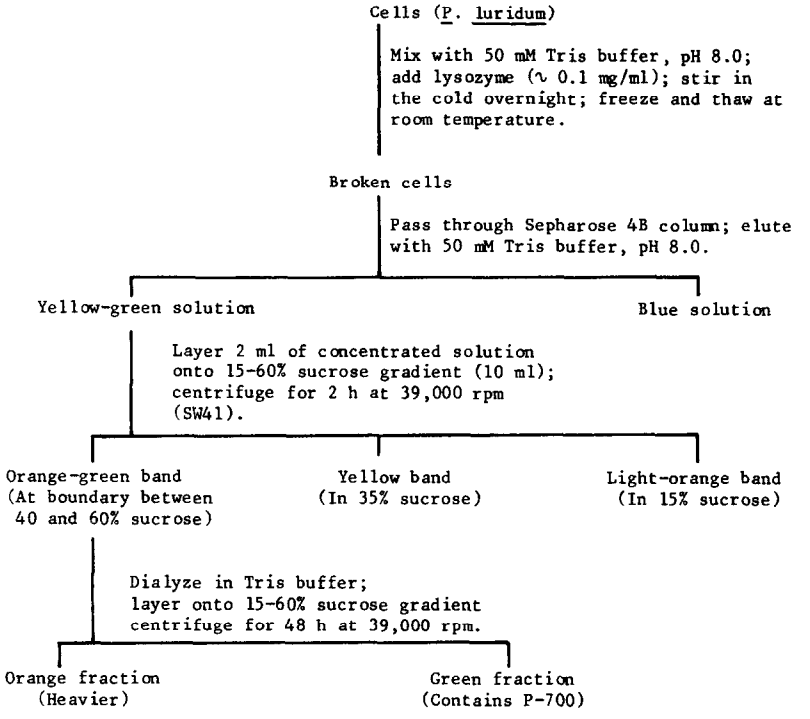


Figure 1: Procedure for isolation of P700-chlorophyll-protein complex.

pH 3.5-10 Ampholine. The green solution is focused in one band. About 15 drops of solution are collected in each tube. The absorbance of each fraction at 437 nm is measured, and the pH of each tube is determined on a digital pH-meter (Radiometer PHM63).

Absorption and Fluorescence Emission Spectra: Absorption spectra are taken on a Perkin-Elmer 320 spectrophotometer in 50 mM Tris buffer. Fluorescence spectra are measured at room and liquid-nitrogen temperatures on a Perkin-Elmer MPF-44A fluorescence spectrophotometer equipped with an R777 HTV photomultiplier in the ratio mode.

RESULTS

The elution pattern from the Sepharose 4B column chromatographic analysis of the broken cells is shown in Fig. 2. The yellow-green fractions eluted first, followed by the blue fractions. The spectra of whole aglal

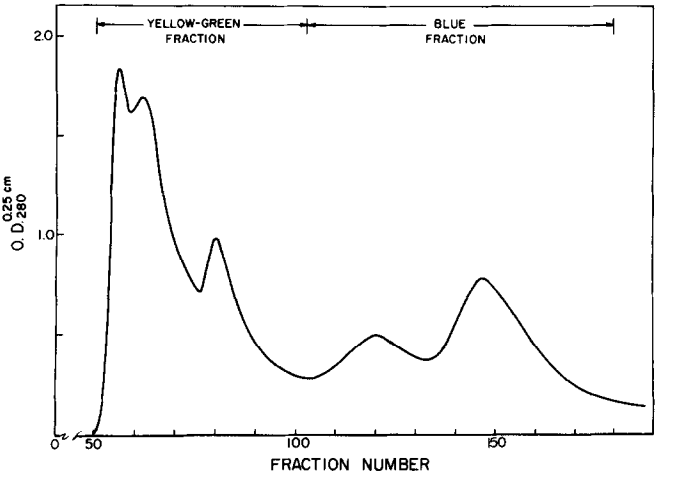


Figure 2: Gel filtration of cell suspension (2 ml) of P. luridum on a column (2.7 x 20 cm) of Sepharose 4B with 50 mM Tris buffer, pH. 8.0. Absorbance at 280 nm.

cells and of the yellow-green and blue fractions after Sepharose 4B column chromatography are shown in Fig. 3. The blue-green alga contains at least two major pigment-protein complexes, phycobiliproteins and chlorophyll-protein (Fig. 3A). The bands at 677 and 437 nm (Fig. 3B) are due to chlorophyll a. The red absorption bands at 615 and 650 nm (Fig. 3C) are due to phycocyanin and allophycocyanin respectively.

The yellow-green fractions were layered onto a 15-60% sucrose gradient and centrifuged for 2 h in the SW41 rotor at 39,000 rpm. Three pigmented bands were observed. The heaviest is composed of two fractions, orange and green. In a second sucrose-gradient centrifugation these two bands were completely separated. The lighter (green) fraction is of interest; its spectrum is shown in Fig. 4. The bands at 437, 418, 380, and 338 nm in the Soret region are due to chlorophyll a; the shoulder at about 490 nm is probably contributed by the carotenoids in the complex. The red absorption maximum is at 677 nm.

The green fraction was further purified by isoelectric focusing. A single band with a major absorption peak at 437 nm appeared at pH 4.5.

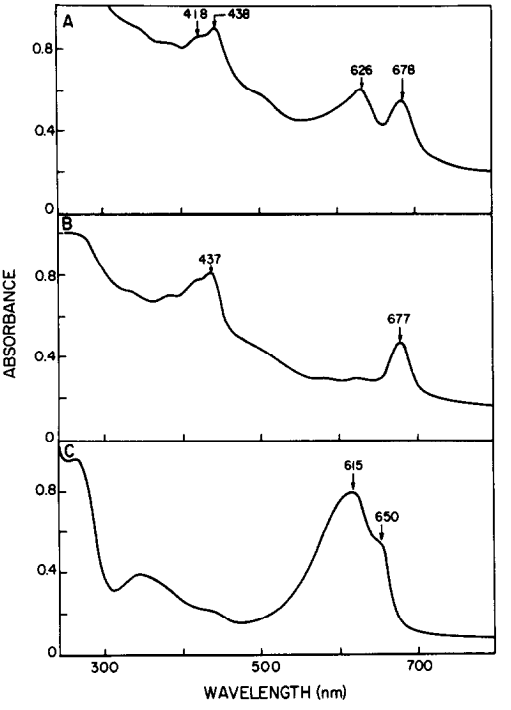


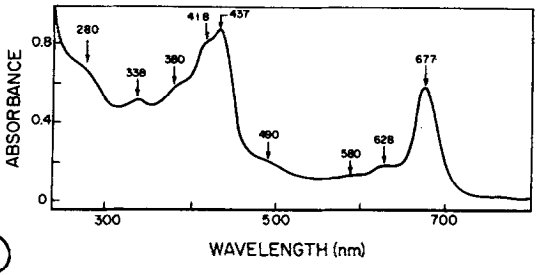
Figure 3: Absorption spectra of (A) whole P. luridum cells, (B) the yellow-green fraction, and (C) the blue fraction after Sepharose 4B column chromatography.

No further purification has been carried out on this fraction.

The room-temperature fluorescence spectrum (Fig. 5A) of the isolated chlorophyll-protein complex shows a maximum emission at 685 nm. Oxidation or reduction of this complex results in a decrease or increase of fluorescence respectively (Fig. 5A). When the fluorescence spectrum is measured at liquid-nitrogen temperature, the maximum is at approximately 720 nm, and a shoulder appears at 690 nm (Fig. 5B). These results are consistent with the presence of a photoreaction center, P700 (10).

DISCUSSION

The main purpose of this report is to describe a new procedure developed in this laboratory for the isolation of a P700-chlorophyll-protein complex from a blue-green alga, P. luridum, by a nondetergent method. The use of lysozyme and of freezing and thawing resulted in the



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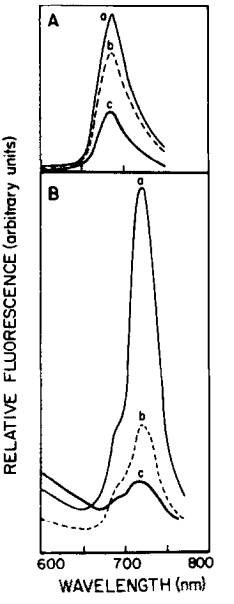

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Figure 4: Absorption spectrum of green fraction after second sucrose density-gradient centrifugation.

Figure 5: Fluorescence emission spectra of P700-chlorophyll-protein complex measured at (A) room temperature and (B) liquid-nitrogen temperature. The curves show spectra of samples which have been (a) reduced with 10 mM sodium dithionite, (b) left untreated, or (c) oxidized with 10 mM potassium ferricyanide.

release of pigment-protein complexes without utilizing detergents, such as SDS, digitonin, or Triton X-100. Four chlorophyll-protein fractions were obtained, and the major (green) fraction was subjected to analysis for characteristics associated with the P700 reaction center.

The use of phosphate buffer causes pheophytinization of chlorophyll even in the absence of SDS. Pheophytinization causes the 418 nm band to become dominant in the Soret region and also shifts the 677 nm peak to 664 nm. Tris buffer (50 mM) at pH 8.0 was therefore used through the entire purification.

The spectra of the four chlorophyll-containing fractions are similar in the Soret region, except that the intensities of the 437 nm and 490 nm bands are different. The red absorption bands are also different for each fraction and are shifted toward the shorter wavelength. These

results suggested the presence of different ratios of chlorophyll to carotenoids and different environments of the chlorophyll molecules within the complex.

The major (green) fraction was treated with 10 mM sodium dithionite or 10 mM potassium ferricyanide for reduction or oxidation respectively. The results (Fig. 5) were similar to those reported by Dietrich and Thornber (10). Preliminary electron-spin resonance experiments to test for P700 activity indicated the presence of P700 in this fraction. There does not appear to be any detectable photosystem II activity in this complex. We have had some success in preliminary experiments using 0.2% SDS gel electrophoresis to demonstrate that the chlorophyll and carotenoid complexes contain several protein subunits of varying molecular weights.

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