PHYCOBILISOMES OF *PORPHYRIDIUM CRUENTUM*

I. Isolation

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ABSTRACT

A procedure was developed for the isolation of phycobilisomes from *Porphyridium cruentum*. The cell homogenate, suspended in phosphate buffer (pH 6.8), was treated with 1% Triton X-100, and its supernatant fraction was centrifuged on a sucrose step gradient. Phycobilisomes were recovered in the 1 M sucrose band. The phycobilisome fraction was identified by the characteristic appearance of the phycobilisomes, and the absorbance of the component pigments: phycoerythrin, R-phycocyanin, and allophycocyanin Isolated phycobilisomes had a prolate shape, with one particle axis longer than the other. Their size varied somewhat with their integrity, but was about 400–500 A (long axis) by 300–320 A (short axis). Phycobilisome recovery was determined at six phosphate buffer concentrations from 0.067 M to 1.0 M. In 0.5 M phosphate, phycobilisome yield (60%) and preservation were optimal. Such a preparation had a phycoerythrin 545 nm/phycocyanin 620 nm ratio of 8.4. Of the detergents tested (Triton X-100, Tween 80, and sodium deoxycholate), Triton X-100 gave the best results Freezing of the cells caused destruction of phycobilisomes.

INTRODUCTION

The function of phycobiliproteins as photosynthetic accessory pigments has been well documented and accepted (6, 10, 28). While it was known that they were localized in the chloroplasts of red algae (7, 18), the exact location within the chloroplast was not known In an examination of *Porphyridium cruentum*, Gantt and Conti (14, 15) found unidentified granules on the stroma side of the photosynthetic lamellae. These granules were proposed to be sites of phycobiliproteins because they were only found in algae which contained these types of accessory pigments. However, not until the isolation (16) of these granules was it possible to show that they contained phycoerythrin and phycocyanin; accordingly, they were named phycobilisomes (phycobiliprotein containing bodies) Morphological identification of phycobilisomes by electron microscopy has since been made in a number of red algae (5, 9, 12, 17, 19–23, 26, 30), and in several blue-green algae (see reference 11, bibliography).

The earlier isolation procedure for phycobilisomes had the limitation that glutaraldehyde was necessary to keep the phycobilisomes intact. The morphology of the phycobilisomes and their absorbance spectra could be examined, but further detailed analysis of phycobilisome components was prevented by irreversible fixation. To overcome this limitation the isolation procedure was revised. In developing the new procedure the main considerations were: (*a*) to isolate intact phycobilisomes which could be dissociated at will, (*b*) to select meaningful purity criteria, and (*c*) to obtain phycobilisomes in substantial amounts which would allow a detailed analysis of their components and an understanding of the structural relationship

of these components This communication describes the rationale and details for the isolation of phycobilisomes without fixation.

MATERIALS AND METHODS

Culture Conditions

P. cruentum Naegeli was originally obtained from Dr. M. B. Allen when she was at the Kaiser Foundation at Richmond, Calif. Cells were grown axenically in culture flasks in artificial seawater medium according to Jones et al. (21). 1 liter of medium was routinely inoculated with 100 ml from an existing 12–14 day old culture Cultures were continuously illuminated with a bank of fluorescent lamps (Westinghouse F40D) at an incident intensity of 300 ft-c and maintained at 20°–25°C. They were agitated on a gyratory shaker and aerated with a stream of 1% CO_2 and 99% air.

Growth of Cultures

For monitoring of the cultures, 10- or 20-ml samples were withdrawn through a separate port on the culture vessel to avoid contamination. Cell counts were made with a hemocytometer on samples which were also used for chlorophyll and phycoerythrin determinations. The results are plotted in Fig. 1. For the isolation of phycobilisomes, cells were routinely harvested from cultures grown for 5–12 days. These cultures were in the log phase of growth and had a constant phycoerythrin/chlorophyll ratio.

Chemical Analyses

Chlorophyll determinations were made according to the method of Arnon (2). For monitoring of the cultures (0–15 days), phycoerythrin extraction was carried out as follows: cells were rinsed and suspended in 0.067 M phosphate (Na_2HPO_4 and KH_2PO_4) buffer, pH 6 8. They were then broken by sonication (setting of 4 with a maximum acoustical output of 4 dc amp, Branson sonifier) for 1 min and deoxycholate was added to a final concentration of 0.4%. Chloroplast membranes and other cell debris were removed by centrifugation at 100,000 *g* for 60 min.

Phycoerythrin content was assayed at various steps of the phycobilisome isolation by using the absorption at 545 nm to obtain the relative phycoerythrin content. Calculation of the phycoerythrin content was made by using the extinction coefficient of $E^{1\%}_{1cm545nm} = 87.8$ as determined by Neufeld (25) after subtracting the background absorption at 700 nm from 545 nm.

Protein content was determined according to the method of Lowry et al. (24), using bovine serum albumin as standard. Precautions had to be taken against the interference of Triton and sucrose

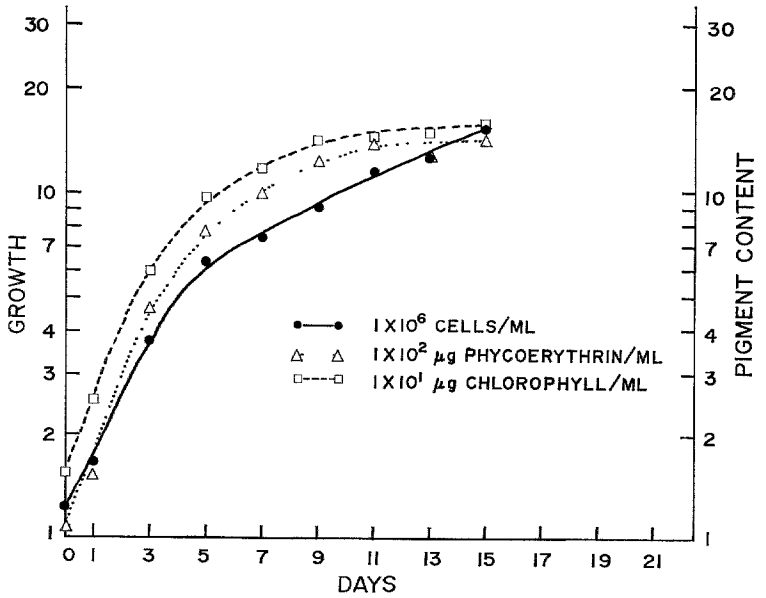


FIGURE 1 Growth and pigment content of *P. cruentum* grown under continuous illumination (300 ft-c). Cultures for phycobilisome isolations were used in the log phase of growth (5–12 days) when the chlorophyll and phycoerythrin ratio remained constant.

Samples of the cell homogenate were taken for protein determinations before Triton was added. Since Triton remained at the top of the sucrose step gradient it did not interfere with determinations of the phycobilisome fraction. It was found that a depression of the color development was caused by sucrose. Therefore, protein determinations on the phycobilisome fraction (1 M sucrose) were made either after the removal of sucrose or with a correction to account for the suppression of the Folin reagent color development by sucrose For the release of phycobilisomes from the photosynthetic membranes three detergents were tested Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.), Tween 80 (Atlas Chemical Industries Inc., Wilmington, Del.), and sodium deoxycholate (Mann Research Labs, Inc , New York). The detergents were added with rapid mixing after the cells had been broken in the French pressure cell. After 15–20 min the cell homogenate was centrifuged and the isolation proceeded as described in the Results. Controls were treated identically except that no detergent was present. Sodium deoxycholate and Tween 80 were tested at a final concentration of 0 5 and 1 0%, while the Triton X-100 concentrations were 0 1, 0.3, 0 5, 0 7, 1 0, 1.5, and 2.0% Phycobilisome recovery was assayed by the absorption of phycoerythrin (545 nm) in the 1 M sucrose band. Chlorophyll absorption at 675 nm was used as an indication of membrane contamination.

For preparation of sucrose step gradients the sucrose was dissolved in the appropriate phosphate buffer (pH 6.8) concentrations (0 067, 0.17, 0.33, 0.5, 0 75, 1 0 M). The step gradients were prepared by layering the various sucrose concentrations into centrifuge tubes (for the 50.1 Beckman rotor) with a pipette. The standard gradient consisted of 2 M, 1 M, and 0.75 M sucrose in proportions of 3:12:3 ml, respectively. 1 ml of sample was layered on the gradient.

Electron Microscopy

Phycobilisome preparations were negatively stained at pH 7.0 with 1% ammonium molybdate or 2%

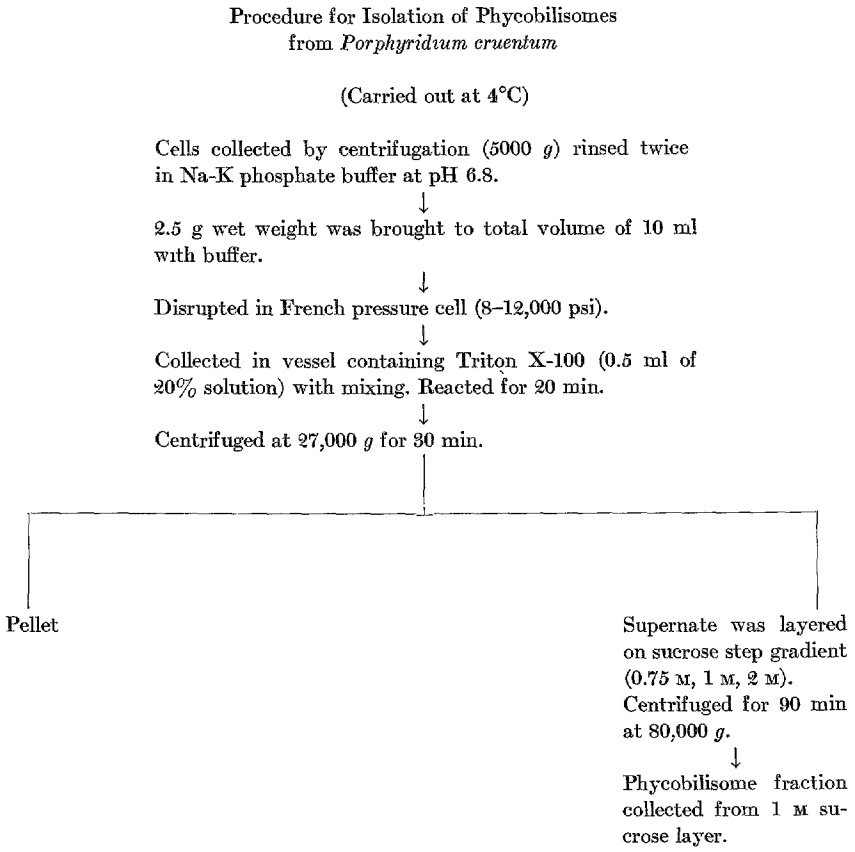


FIGURE 2 Outline of phycobilisome isolation procedure. Time interval from cell breakage to collection of phycobilisomes was about 150 min. The same buffer concentration (0.067, 0.17, 0.33, 0.50, 0.75, or 1.00 M phosphate) was used in all steps of the isolation.

phosphotungstic acid. The latter gave the best image quality, especially after dialysis against 1 mM phosphate buffer. Before dialysis, phycobilisomes were fixed in 4% glutaraldehyde for 1 hr to avoid dissociation in the low molarity phosphate buffer. Grids were examined with a Philips EM-300, and photographic records were made on Kodak Electron Microscope film at magnifications of 80,000–125,000.

RESULTS

Isolation and Characterization of Phycobilisomes

ISOLATION PROCEDURE: An outline of the standard phycobilisome isolation procedure is presented in Fig. 2. The entire isolation procedure was carried out at 4°C and all solutions were buffered at pH 6.8 with Sörensen's phosphate buffer (0.067, 0.17, 0.33, 0.50, 0.75, or 1.0 M). Cells were pelleted by centrifugation at 5000 *g* and rinsed twice with the appropriate buffer. The pellet (wet weight 2.5 g) was resuspended in buffer to a final volume of 10 ml, then disrupted in a French pressure cell (8–12,000 psi). The disrupted sample was collected in a vessel containing Triton X-100 (0 5 ml of 20% to a final 1%) and mixed by stirring for 15–20 min It was then centrifuged for 30 min at 27,000 *g*. The pellet (of the 27,000 *g* centrifugation consisted of large cell fragments and starch) was analyzed for phycobilisomes, phycoerythrin, and chlorophyll when appropriate and then discarded. 1 ml of the supernatant fraction was layered on each tube of the sucrose step gradient and centrifuged in an angle rotor at 80,000 *g* for 90 min The phycobilisomes migrated into the 1 M sucrose layer as a purple band (at the lowest phosphate concentrations some pelleting of phycobilisomes also occurred at the side of the tube in the 1 M sucrose layer).

The pigmented zones on the sucrose step gradient when examined from top to bottom were as follows: the aqueous layer at the top consisted of a translucent green to olive–colored band; below that, the 0.75 M sucrose band was clear orange to pink; and finally the zone in the otherwise colorless 1 M sucrose layer which contained the phycobilisomes was deep purple. When the pigmented fractions were collected for examination, electron microscopy showed that phycobilisome presence correlated positively with the purple zone in the 1 M sucrose.

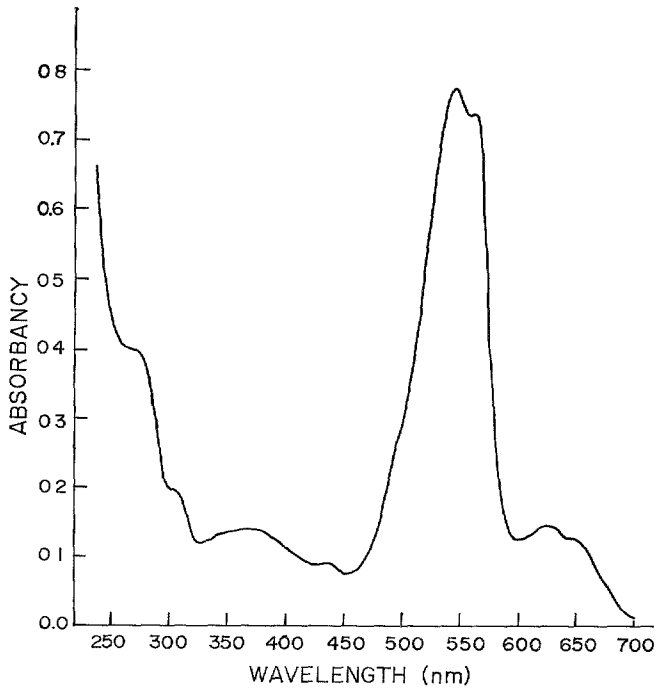


FIGURE 3 Absorption spectrum of isolated phycobilisome preparation in 1 M sucrose, 0.067 M phosphate buffer pH 6.8. The major double peak at 545–563 nm is phycoerythrin. Phycocyanin absorbs at 620–625 nm, and allophycocyanin at 650 nm The small peak at 440 nm is due to chlorophyll (1 μg chlorophyll/450 μg protein).

CHARACTERISTICS OF PHYCOBILISOMES. Phycobilisomes were identified spectrophotometrically and by morphological appearance in the electron microscope The absorption spectrum of a phycobilisome preparation appears in Fig. 3. Phycoerythrin represents the major peak with absorption at 545 and 563 nm, and a shoulder at 500 nm. Phycocyanin at 620–625 nm is the second

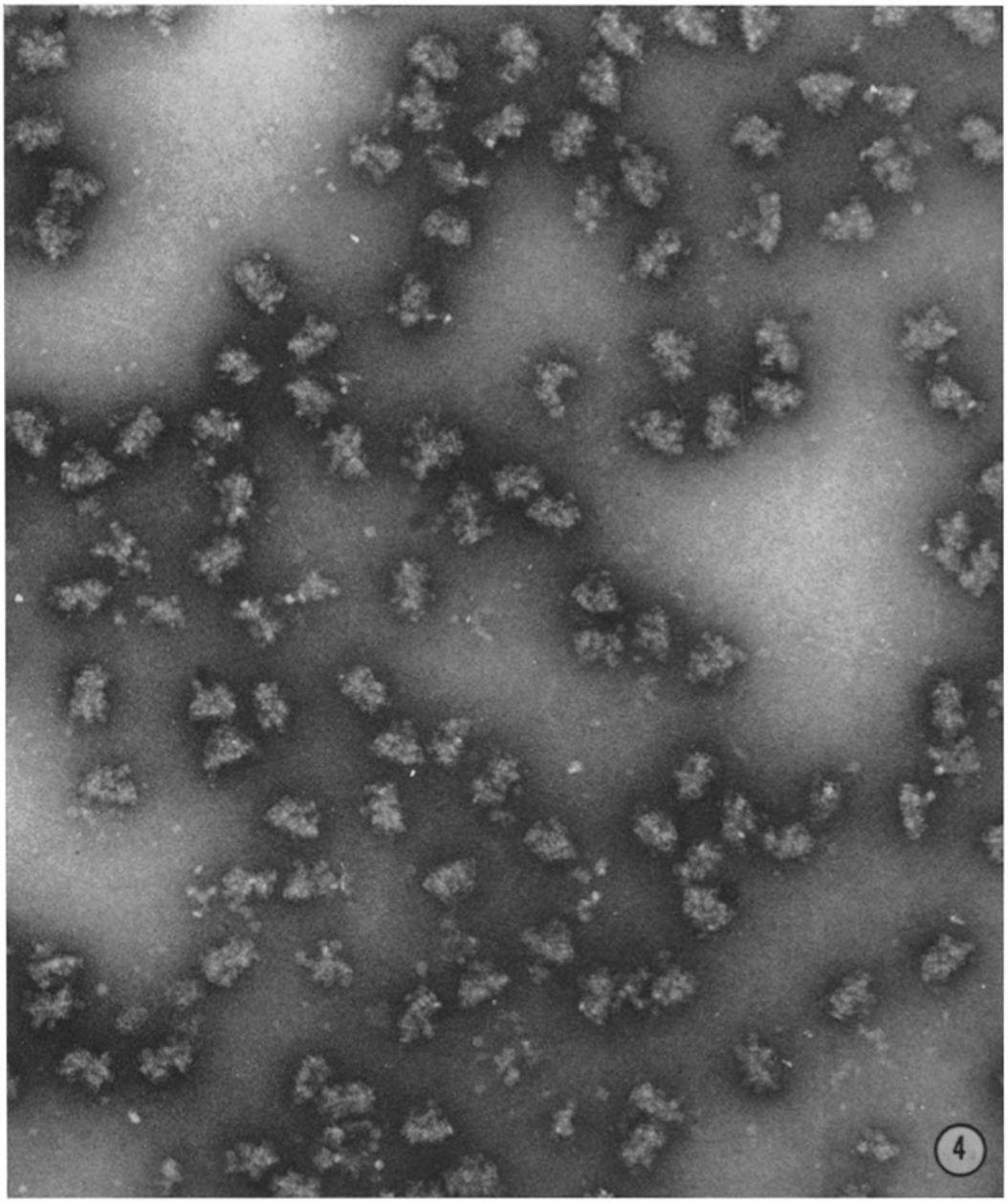


FIGURE 4 Electron micrograph of a phycobilisome preparation isolated according to the procedure outlined in Fig. 2 and stained with phosphotungstic acid. The phycobilisomes appear as aggregates of smaller units which are loosely packed, causing irregular surface outlines The small units in the background are presumed to be subunits of dissociated phycobilisomes. See Fig. 3 for absorption spectrum. × 152,000.

major peak although smaller. The presence of allophycocyanin is shown by absorption at 645–650 nm Absorption at 440 nm and/or 675 nm is an indication of the presence of chlorophyll. It is evident from the absorption in the ultraviolet region that there is no gross contamination by other proteins, nucleic acids, or Triton. The absorption of 260–275 nm is due to the phycobiliproteins, scatter, and perhaps a small amount of Triton (< 0 005%). Analysis of phycobilisomes on acrylamide gels with Coomassie blue staining revealed no contaminating protein bands.

An electron micrograph of a typical phycobilisome preparation stained with phosphotungstic acid is shown in Fig. 4 The individual phycobilisomes have a prolate shape, where one axis is longer than the other. They are all of the same size range (Table I and Fig. 5) and are composed of aggregated subunits Their outlines suggest a certain plasticity. If they were compact and rigid structures they would not exhibit the irregular surface outline Preparations sometimes contained a few small vesicles similar to the smallest ones observed in Fig. 7, which are believed to be con-

TABLE I

Assessment of Phycobilisome Size As a Function of Buffer Concentration, Day of Isolation, and Fixation

Condition	Culture age	Phosphate buffer, pH 6 8	Long axis (mean ± SD)	Short axis (mean ± SD)	Number of PBS measured
	days	*M*	*A*	*A*	
Fixed immediately	3	0.067	470 ± 36	324 ± 24	100
Fixed immediately	11	0.067	470 ± 39	318 ± 27	100
Fixed immediately	7 and 11	0.067	460 ± 51	315 ± 37	400
Fixed after isolation	7 and 11	0 067	388 ± 50	300 ± 18	500
Fixed after isolation	8 and 12	0 50	459 ± 36	304 ± 27	400
Unfixed (stored as pellet 24 hr)	12	0 50	436 ± 35	283 ± 27	100

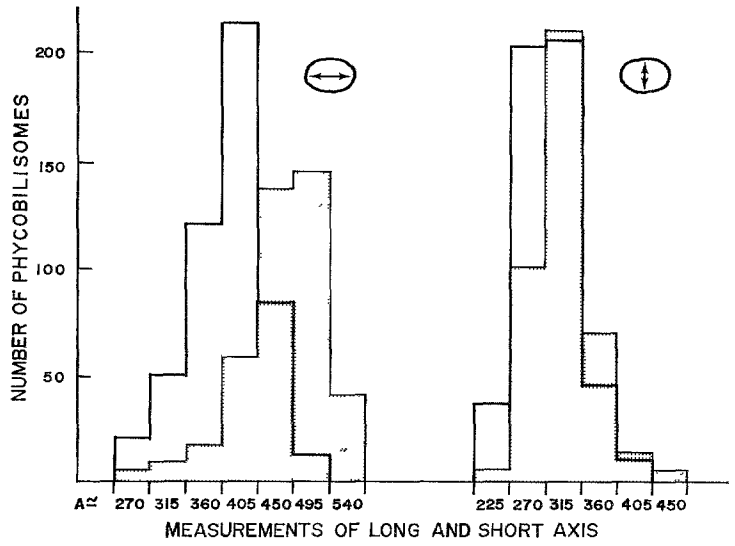


FIGURE 5 Size distribution of phycobilisomes isolated in 1 M sucrose, 0.067 M phosphate buffer pH 6.8. The long (↔) and short (↕) axes of phycobilisomes were measured and expressed in Angstrom units. The shaded area represents the size range of phycobilisomes fixed immediately upon cell breakage (400 measured). The area outlined by the heavy black line is of phycobilisomes fixed 150 min after cell breakage and isolation by centrifugation through a discontinuous sucrose gradient (500 measured).

taminating membrane vesicles. The small units in the background of Fig. 4 resemble phycoerythrin and phycocyanin particles in size (100 A or less) and shape It is assumed that they are products of dissociated phycobilisomes.

PHYCOBILISOME DIMENSIONS: The phycobilisome size was used to assess their integrity under various conditions of isolation. Because it is difficult to assign three-dimensional parameters to particles which have been removed from their natural orientation on the membrane, reference can only be made to their long and short axes (Table I and Fig 5).

The largest and presumably the most intact phycobilisomes were obtained with 0 50 M phosphate or when preparations (0.067 M) were fixed immediately upon cell breakage. As seen in Table I the long axis of intact phycobilisomes was 459–470 A and their short axis was 304–324 A. This is presumed to be their maximum size under these conditions Phycobilisome size in cells from 3-day cultures was the same as those from 11-day cultures; therefore, the age of the culture does not appear to affect phycobilisome size.

Phycobilisomes isolated in 0.067 M phosphate buffer decreased in size during the isolation (150 min) from 460 to 388 A on their long axis, and from 315 to 300 A on their short axis. As seen in Fig 5 the two size populations show a considerable overlap The short axis decreased proportionately less than the long axis. Dissociation was at a minimum when the isolating buffer was 0.50 M phosphate. Even after storage (24 hr) of phycobilisome pellets (4°C) the average long axis was 436 A and the short axis was 283 A

Factors Affecting Phycobilisome Isolation

BUFFER CONCENTRATION: It was established that free phycoerythrin (300,000 molecular weight) could not penetrate into the 1 M sucrose layer under the conditions of isolation. Therefore, only phycoerythrin belonging to phycobilisomes was present in this layer, and thus was used as an indicator of phycobilisome recovery. Free phycoerythrin resulting from dissociated phycobilisomes was always present in the upper part of the gradient Phycobilisome recovery was dependent on the buffer concentration as is shown in Table II. In the 0 067 M phosphate buffer system the recovery of phycoerythrin as phycobilisomes was 40% of the total, while 35% was present as free phycoerythrin, and 25% remained in the pellet At 0.17 M and 0.33 M phosphate buffer the phycobilisome recovery increased to 53%, while the free phycoerythrin decreased to 23% and the phycoerythrin in the pellet was essentially the same The optimum buffer concentration was 0 50 M because at this phosphate concentration the phycobilisome recovery was the greatest at 60%, and free phycoerythrin accounted for only 15%. When the phosphate concentration was increased above 0.50 M the phycobilisome recovery decreased.

Cell breakage and release of phycobilisomes from the photosynthetic membranes by the use of detergents was the same at buffer concentrations of 0.067–0.50 M phosphate, because the relative amount of phycoerythrin recovered in the pellet and supernate was always about 25% and 75%, respectively (Table II). Examination of the pellets by electron microscopy revealed the presence of large membrane fragments with phycobilisomes attached which had the same dimensions as those recovered in the 1 M sucrose layer. For complete phycoerythrin extraction of these pellets, repeated washing with distilled water was necessary.

The increase of phycoerythrin in the phycobilisome fraction was also manifested by the increase in the phycoerythrin$_{545}$/phycocyanin$_{620\ nm}$ ratio (Table II), and a concomitant decrease in the free phycoerythrin. This, along with electron microscope examination, showed that the phycobili-

TABLE II

Percentage of Phycoerythrin Contained in the Various Fractions of the Phycobilisome Isolation Procedure at Six Buffer Concentrations

	Cell homogenate		Supernatant fraction after sucrose gradient sedimentation	
Phosphate buffer, pH 6 8	Pellet	Supernatant fraction	0 0–0 75 M	1 M (phycobilisome fraction)
M	%	%	%	% (*PE/PC*)*
0.067	25	75	35	40 (6.6)
0 170	26	74	22	52 (7.4)
0.330	24	76	23	53 (7.7)
0.500	25	75	15	60 (8 4)
0.750	29	71	14	57 (8 3)
1 000	50	50	10	40 (8.5)

The supernatant fractions from the cell homogenates were sedimented in sucrose gradients. The yields are expressed as per cent of the total cell homogenates.

* Phycoerythrin$_{545nm}$/phycocyanin$_{620nm}$ ratio of phycobilisome fraction in 1 M sucrose.

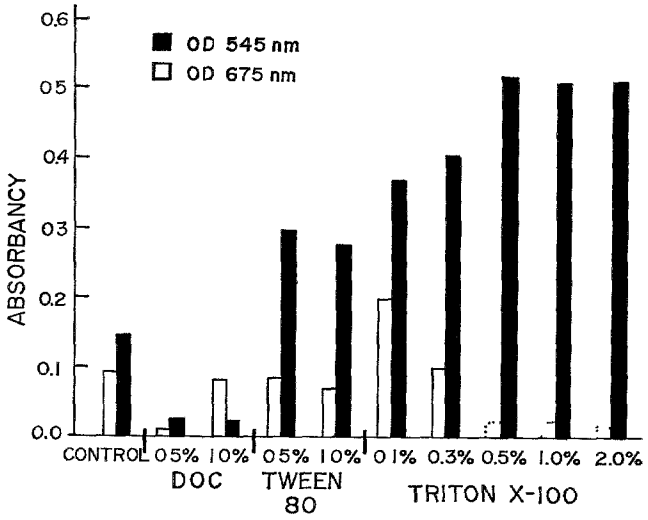


FIGURE 6 Effect of detergents on the release of phycobilisomes, as measured by the phycoerythrin recovered in the phycobilisome fraction. For procedure, see Materials and Methods and section on detergent treatment in Results. The black bars represent the phycoerythrin absorption at 545 nm. White bars represent a peak at 675 nm indicating chlorophyll. At concentrations of 0.5–2.0% Triton, neither a peak nor shoulder was present at 675 nm; therefore, absorption due to chlorophyll is doubtful and the lines are dotted. The control lacked detergent. These experiments had the same initial cell concentration and were done in 0.067 M phosphate, pH 6.8. DOC, sodium deoxycholate.

somes are more compact and somewhat larger at higher (0.5 M) than at lower (0.067 M) phosphate buffer concentrations. While the phycoerythrin/phycocyanin ratio can vary to some extent from one experiment to the next, the trend was always the same. For example, at a phosphate concentration of 0 067 M the ratio ranged from 6 to 7; at 0 5 M and above, it was always above 8. The conclusion that a high phycoerythrin/phycocyanin ratio reflects a greater phycobilisome integrity is also supported by controlled dissociation studies. When phycobilisome preparations were partially dissociated, and the free phycobiliproteins were removed, it was found that the "cores" dropped from an initial 7 to a final 4.5 phycoerythrin/phycocyanin ratio.

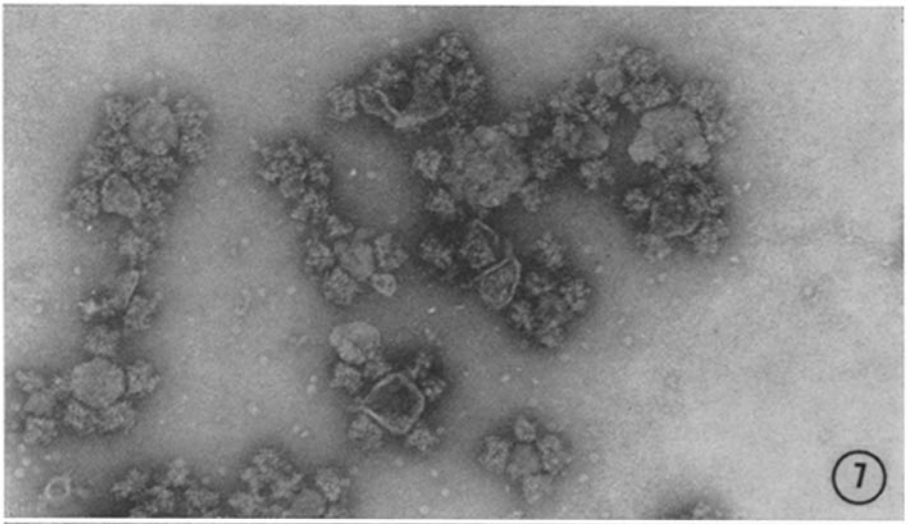
EFFECTS OF DETERGENTS: Phycobilisomes were released from the photosynthetic membranes only by detergent treatment. The effectiveness of three detergents (Triton X-100, Tween-80, and sodium deoxycholate) was tested under identical conditions in 0.067 M phosphate buffer at pH 6 8. After a reaction time of 20 min the isolation progressed as outlined in Fig. 2. Recovery of phycobilisomes in the 1 M sucrose band was again determined by phycoerythrin absorption at 545 nm (Fig. 6). Chlorophyll absorption was monitored at 675 nm. The same fractions were used for spectral

FIGURES 7, 8, 9 Phycobilisome fractions recovered in the 1 M sucrose band, stained with phosphotungstic acid (1%, pH 7.0).

FIGURE 7 Control preparation lacking detergent treatment.

FIGURE 8 Preparation treated with 0.1% Triton X-100. Only a few phycobilisomes were released from the membranes.

FIGURES 9 Treated with 1.0% Tween. Most phycobilisomes are unattached but some membranes are still present. Comparison of these electron micrographs with Fig. 6 shows that when membrane content is high, so is chlorophyll content. Chlorophyll and membrane contamination is very low in preparations treated with 1.0% Triton (Figs. 4 and 6) as compared with the above figures. × 110,000.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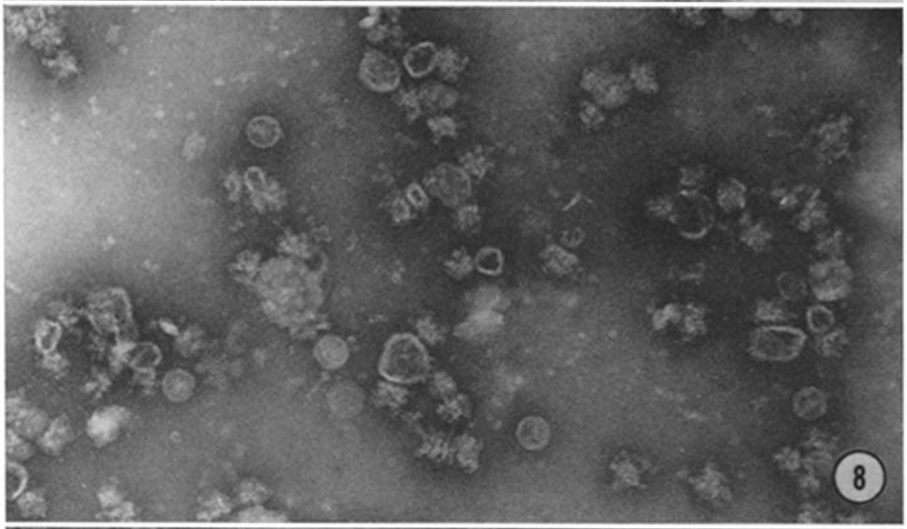
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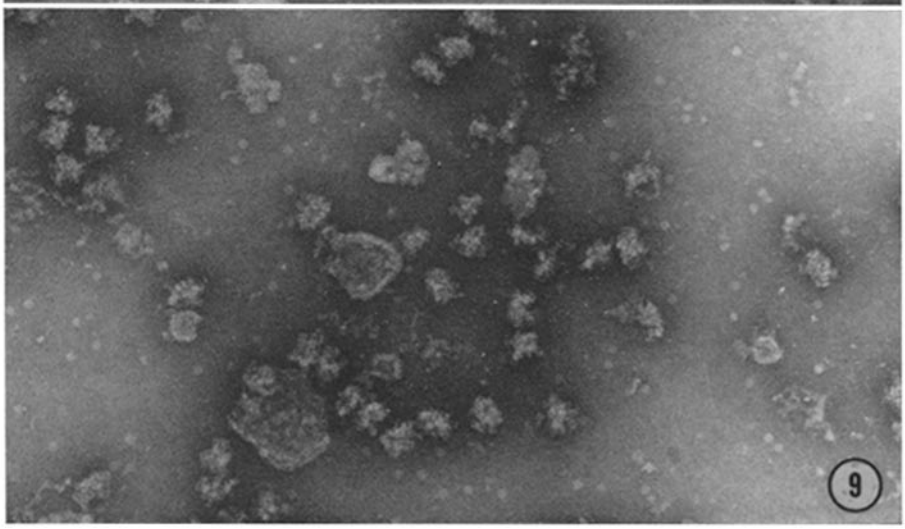
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determinations and examination by electron microscopy (Figs. 4, 7, 8, 9).

Phycobilisome recovery varied with the detergent type and concentration used. In the control, where no detergent was present, the phycobilisomes remained attached to small membrane vesicles (Fig. 7). The control fraction also contained a large amount of chlorophyll relative to phycoerythrin. With deoxycholate (0.5 and 1.0%) the recovery of phycoerythrin in the 1 M sucrose band was lower than in the control. Furthermore, there were no identifiable phycobilisomes present with this detergent at the conclusion of the isolation (150 min), which suggests that deoxycholate destroyed unfixed phycobilisome structure. Both Tween 80 and Triton X-100 caused release of phycobilisomes from the photosynthetic membranes and resulted in the recovery of intact phycobilisomes. At low Triton concentrations (0.1%) some phycobilisomes were released, but most remained attached to the membranes (Fig. 8). The membrane contamination was high, as was the chlorophyll content (Fig. 6). Invariably, high chlorophyll content was paralleled by high vesicle contamination.

Most phycobilisomes were freed with Tween 80 (Fig. 9) but the contaminating membranes were not so readily removed by centrifugation on the sucrose step gradient as with Triton X-100. The cleanest preparations were obtained with Triton X-100 at a concentration of 0.5% and above. Chlorophyll absorption at 675 nm was not observable as a peak or shoulder at higher concentrations; therefore, the absorption at that point was indicated as dotted lines in the bar graph in Fig. 6. Because of these results, a 1% Triton concentration was chosen for the standard isolation procedure.

Other Factors Relevant to Phycobilisome Isolation

Since the chloroplast accounts for most of the cell volume, chloroplast membrane vesicles are the expected contaminants of phycobilisome fractions. Therefore, a comparison was made between the chlorophyll content of the whole cell homogenate and that of the phycobilisome fraction. The whole cell homogenate contained 120 μg of chlorophyll for every 1 mg of phycoerythrin, whereas the phycobilisome preparations had as little as 0.7 μg of chlorophyll per 1 mg phycoerythrin. Although there is a positive correlation between the chlorophyll content and vesicle contamination as shown by the detergent treatments (Figs. 6–9), it is not certain that chlorophyll is only in the vesicles. Perhaps a small fraction of chlorophyll is intimately involved in the normal structure of phycobilisomes.

Examination by electron microscopy sometimes revealed two types of contaminants, one being the very small membrane vesicles mentioned in the previous section, and the other being small amounts of very fine fibrous material. The fibrous material had the same appearance as the sheath which surrounds whole cells of *P. cruentum*. Since it was present in small amounts and would not be expected to contain any pigments, nor to interfere with any further treatments, it was tolerated.

Numerous other variations were tested in developing the phycobilisome isolation procedure. A few which may have been critical are: the addition of sodium chloride (0.1–2.0 M) to dilute phosphate buffer, sorbitol (0.25 M), Ficoll (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) (2.5%), mercapthoethanol (1.5 mM), magnesium chloride (5 mM), and the replacement of phosphate buffer with either 0.05 M Tricine (California Biochemical Corp., Los Angeles, Calif.) (pH 7.2) containing 1.5 mM disodium ethylenediaminetetraacetate, or 0.1 M acetate buffer (pH 4.3). Although not tested exhaustively, none of these improved the final phycobilisome yield.

Phycobilisomes were not resistant to freezing. After cells were frozen in liquid nitrogen and stored for several weeks, the yield of phycobilisomes was small, but the yield of free phycobiliproteins was large. Though the phycobilisome structure was destroyed, freezing did not cause any significant alteration of the pigments themselves.

DISCUSSION

Phycobilisomes from *P. cruentum* can now be isolated in a relatively pure and intact state and used for further analysis of their components. Upon dissociation these preparations yield phycobiliproteins in a native state as judged by their absorption spectra and fluorescence. The new procedure reported here has overcome the major disadvantages of our previous procedure (16): (*a*) Fixation is not required to keep the phycobilisomes intact. (*b*) The nonionic detergent Triton X-100, used for phycobilisome release from membranes, is much more gentle than deoxycholate previously employed. (*c*) Use of the sucrose step gradient and 0.5 M phosphate buffer permitted the rapid recovery (150 min) of phycobilisomes in high yield (60%).

For the present, we are assuming that all phycobilisomes in a particular fraction are essentially the same. Negatively stained intact phycobilisomes fall within the size range of 400–500 A (long axis) by 300–320 A (short axis). These values are higher than those previously obtained from sectioned material of 350 A (15, 16). The difference is no doubt due to the two different methods of preparation, because the dehydrating reagents used in the embedding process cause a much greater shrinkage than air drying of negatively stained material.

Are all phycobiliproteins in the chloroplast contained in phycobilisomes? On the basis of phycoerythrin, which is the major phycobilisome constituent, most of it is in phycobilisomes. As seen in Table II, 60% (0.5 M phosphate) is in phycobilisomes; the 15% present as "free" phycoerythrin is probably a result of dissociation on cell breakage. The 25% in the pellet is presumed to be located in the phycobilisomes visible in the cell fragments.

Phycobilisome pigment composition is more complex than the absorption spectrum suggests (Fig. 3). The phycocyanin is of the R-type, possessing both a red and a blue chromophore (27). On gel electrophoresis there are two bands of allophycocyanin and two groups of phycoerythrin. The small phycoerythrin(s) lack a shoulder at 500 nm and show very little cross-reactivity with antiserum made against the large phycoerythrin(s).

Although the amount of chlorophyll present in the phycobilisome preparations is small, we cannot be certain whether it is only a contaminant or a constituent. Calculations show that even at 0.7 μg chlorophyll/mg phycoerythrin there would be about 10 chlorophyll molecules per phycobilisome. It would be very interesting to have chlorophyll as a constituent, because for effective energy transfer chlorophyll and phycobiliproteins must be closely associated (1, 10, 13). Direct contact between chlorophyll and phycocyanin (or perhaps allophycocyanin) would be the most efficient arrangement, but it does not necessitate the inclusion of chlorophyll into the phycobilisome complex Phycobilisomes are attached at very specific sites on the membrane (8, 15, 16). Perhaps chlorophyll is exposed at these membrane-phycobilisome sites. The density of about 1.310 of phycobilisomes (unpublished) rules out the presence of any substantial amounts of membrane fragments or chlorophyll.

While phycobilisomes are alike in size and sedimentation rate, there is still the possibility that we could have more than one pigment type. It is impossible, in terms of energy transfer, to have phycobilisomes composed only of phycoerythrin. It would be possible, however, to have phycobilisomes composed only of phycocyanin. Some of our results (unpublished) on controlled dissociation pertain to this problem. Phycobilisomes with the greatest integrity had the highest phycoerythrin/phycocyanin ratio. Upon dissociation more phycoerythrin than phycocyanin was freed initially, which resulted in the relative increase of phycocyanin over phycoerythrin in the undissociated remainder. Two explanations are possible: that the phycoerythrin surrounds the phycocyanin in the phycobilisome, or that there is more than one type of phycobilisome which dissociates at different rates. We favor the first suggestion beause a phycocyanin core covered by a phycoerythrin shell is consistent with the energy transfer mechanism, and one would expect exposed phycocyanin aggregates to be more unstable at pH 6.8 than phycoerythrin-covered aggregates (4, 25, 29). Furthermore, there is no evidence of more than one morphological type in sectioned cells or in the isolated phycobilisome preparation.

Although allophycocyanin is commonly found in red and blue-green algae (3, 28) its role in the energy transfer scheme has been generally ignored. In our phycobilisome preparations allophycocyanin is as constant a constituent as R-phycocyanin and the phycoerythrins. Therefore, one can reasonably assume that allophycocyanin, like the latter pigments, is an essential component of the phycobilisome structure, and we suspect that it may be present in the lamellae as well. It is likely that allophycocyanin and/or phycocyanin physically connect phycoerythrin and chlorophyll and thus would be the bridge between the chloroplast lamellae and the projecting phycobilisome.

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