Plant & Cell Physiol. 23(8): 1347–1355 (1982)

Cylindrical Phycobilisomes from a Blue-Green Alga, *Anabaena variabilis*

Takahiro Isono and Tetzuya Katoh

Department of Botany, Faculty of Science, Kyoto University, Kita-shirakawa, Kyoto 606, Japan

Cylindrical 52.5-nm-long phycobilisomes were observed in *Anabaena variabilis*, differing from the generally accepted hemidiscoidal morphology. The central part of such a phycobilisome has a network-like fine structure of slightly greater diameter (16 nm) than the connected end parts of stacked-disc structure (12 nm in diameter). On the basis of this morphology, the molecular mass of this phycobilisome was calculated to be 3.27×10^6, about 60% of which is accounted for by phycocyanin with the rest being due to allophycocyanin. Separately prepared 23 *S* allophycocyanin particles with a molecular mass of 1.13×10^6 have the dimensions (16×23 nm) and network-like fine structure similar to the central part of phycobilisomes, while an aggregate form of phycocyanin (18 *S*) has a fine structure of stacked discs similar to the connecting end part of phycobilisomes, suggesting that the central part constitutes the core at which these phycobilisomes attach to the thylakoid membranes.

Key words: Blue-green alga (*Anabaena variabilis*) — Electron micrograph — Cylindrical phycobilisomes — Phycobilisomes — Protein assembly.

Blue-green algae contain phycobiliproteins as major light-harvesting pigments. C-PC (λ_{max}: 620 nm), APC (λ_{max}: 653 nm) and LWEAP [APC I (λ_{max}: 656 nm; Zilinskas et al. 1977) or APC B (λ_{max}: 671 nm; Glazer and Bryant 1975)] are common components of all cyanophycean species, while some algae also contain C-phycoerythrin (λ_{max}: 565 nm). These phycobiliproteins are confined in granules called PBS, which are attached to the outer surface of the thylakoids (Gantt and Conti 1969). Electron microscopic studies revealed that PBS isolated from blue-green algae are generally hemidiscoidal in shape with a number of stacked disc rods which fan out from a morphologically distinct core. PBS of similar morphology were found in a red alga, *Rhodella violacea* (Mörschel et al. 1977), though many PBS of red algae are hemispherical-prolate in shape (Lichtlé 1978, Gantt 1980). In hemidiscoidal PBS, the central core consisting of two or three round units of APC is assumed to convey the energy absorbed in PC or phycoerythrin in the stacked rods to the chlorophyll in the thylakoids.

Previous studies on PBS dissociation showed that the first cleavage of *Anabaena* PBS occurs between PC and APC to yield two PC subparticles and one APC-APC I complex (Kume and Katoh 1982), and that these resultant subparticles, if mixed and subjected to high phosphate concentration, can reconstitute the whole PBS (Kume et al. 1982). This evidence yields a PBS structure somewhat different from the hemidiscoidal model in which an APC core is surrounded by a number of PC rods, and thus the present work studied the morphology of PBS with special reference to the fine structures of aggregate forms of PC and APC by electron microscopy.

Abbreviations: PBS, phycobilisome(s); (C-)PC, (cyanophycean) phycocyanin; APC, allophycocyanin, LWEAP, longer wavelength emitting allophycocyanin.

Materials and Methods

Cells of *Anabaena variabilis* (strain M3), obtained from the Institute of Applied Microbiology, University of Tokyo, were grown in the C medium of Kratz and Myers (1955) with continuous flushing of CO_2 (5%)-containing air at 30°C. Cultures were illuminated with a bank of four red light-enriched fluorescent lamps (FL40-PK, Matsushita) at an incident intensity of 3.5 $mW \cdot cm^{-2}$, although the physical and morphological characteristics of the PBS of this alga are independent of the quality of the illuminating light.

PBS were isolated according to the procedure of Gantt et al. (1979) with some modifications. The entire isolation was carried out in 0.75 M K-phosphate (K_2HPO_4 : KH_2PO_4=3 : 1, pH 7.2) at 20°C. The linear sucrose gradient from 0.3 M (top) to 1.5 M (bottom) in 0.75 M K-phosphate was spun at 22,000 rpm for 18 hr at 20°C in a swinging bucket rotor (RPS 25-2) in a Hitachi ultracentrifuge. When the Triton-extract of the cells in 0.75 M K-phosphate was loaded and spun, most of the phycobiliproteins (more than 90%) were recovered in a blue band at the 1.0 to 1.2 M sucrose layer. This band (PBS) was collected with a syringe and precipitated by adding an equal volume of a mixture of 0.8 M $Na(NH_4)HPO_4$ and 1.8 M $(NH_4)_2SO_4$ to remove sucrose. The pellet obtained was suspended in 0.75 M K-phosphate and, after centrifugation at 12,000×g for 60 min to remove any contaminating membrane fragments, was used for electron microscopic studies.

For electron microscopy, PBS were fixed with either i) 0.1% glutaraldehyde in 0.75 M K-phosphate for 5 min before being applied to carbon-coated grids or ii) 1% glutaraldehyde in 0.75 M K-phosphate for 2 min after application to carbon-coated grids. Excess fluid containing glutaraldehyde and phosphate was removed by floating the grids on 3 drops of deionized water with rapid transfers before placing them on a drop of 1% uranyl acetate for negative staining, with the excess being removed by filter paper. To observe the dissociation intermediates of PBS, the fixation by glutaraldehyde was conducted at a phosphate concentration in which PBS were dissociated. Stained samples were examined with a Hitachi HU-11D electron microscope operated at 75 kV, except for the observations of tilted objects made with a JEOL 200-CX operated at 80 kV. The dimensions of PBS and their derived particles were measured on the photographs enlarged to ×150,000 and the standard deviations were calculated on the basis of 200 to 500 samples.

Photosynthetic vesicles with bound PBS were isolated and observed by electron microscopy as described in an earlier paper (Katoh and Gantt 1979).

The sedimentation coefficients of PBS and the derived subparticles were determined by sucrose-density gradient centrifugation as described by Martin and Ames (1961). The linear gradients used were 12.5 ml of 0.3–0.8 M sucrose in 0.75 M K-phosphate, and 0.2 ml of sample in 0.75 M K-phosphate was loaded on the top of the gradient. After centrifugation at 36,000 rpm for 6 hr in a Hitachi RPS-40T rotor, the distance of migration of the blue band was estimated.

Calcium phosphate gel for the preparation of APC- and PC-subparticles was prepared according to Siegelman et al. (1965).

Results

Purified PBS preparation shows an absorption peak at 620 nm and a shoulder at 650 nm with the ratio of A_{650}/A_{620} nearly constant at 0.65, corresponding to the APC/PC ratio of 0.68 in terms of protein weight when calculated according to Bennett and Bogorad (1971). On excitation with the light absorbed in PC (605 nm), the PBS in 0.75 M K-phosphate emit fluorescence with a peak at 666 nm and a shoulder at 685 nm at room temperature, and a single sharp peak at 686 nm at liquid nitrogen temperature (−196°C) (solid lines in Fig. 1). Of these, the

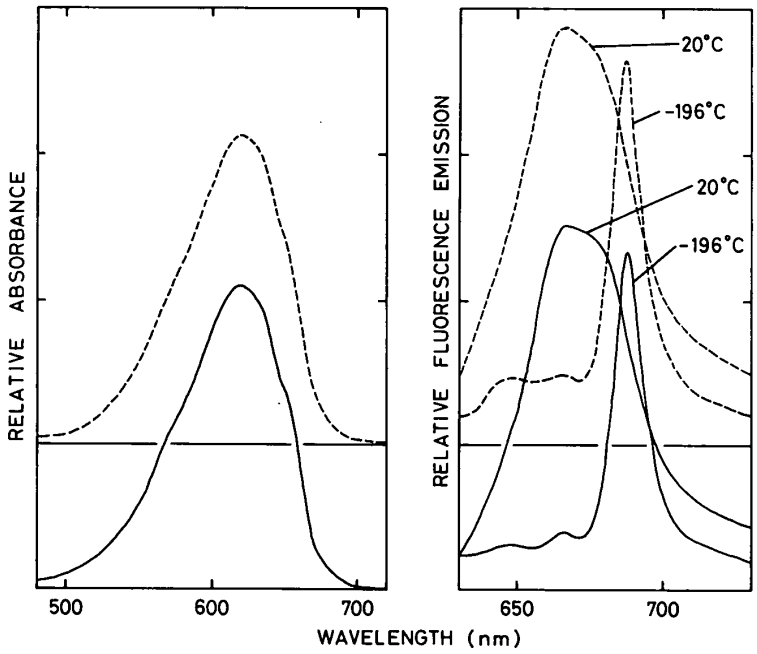

Fig. 1 Absorption (left) and fluorescence emission (right) spectra of unfixed and glutaraldehyde-fixed PBS. Measurements were made with unfixed PBS (solid lines) in 0.75 M K-phosphate, while those with fixed PBS (dashed lines) were done 10 min after PBS, first fixed with 1% glutaraldehyde in 0.75 M K-phosphate for 2 min, were diluted with 9 volumes of distilled water. Fluorescence at 20°C was measured in a 10×10 nm four-sided transparent cuvette at a PBS concentration of $A_{620}=0.1$, and that at -196°C was measured in a copper cell with a sample room of 1.2-mm optical path, fitted for setting in a Dewar bottle filled with liquid nitrogen. The excitation wavelength was 605 nm and the excitation and detection bandpasses were 10.0 and 3.0 nm, respectively.

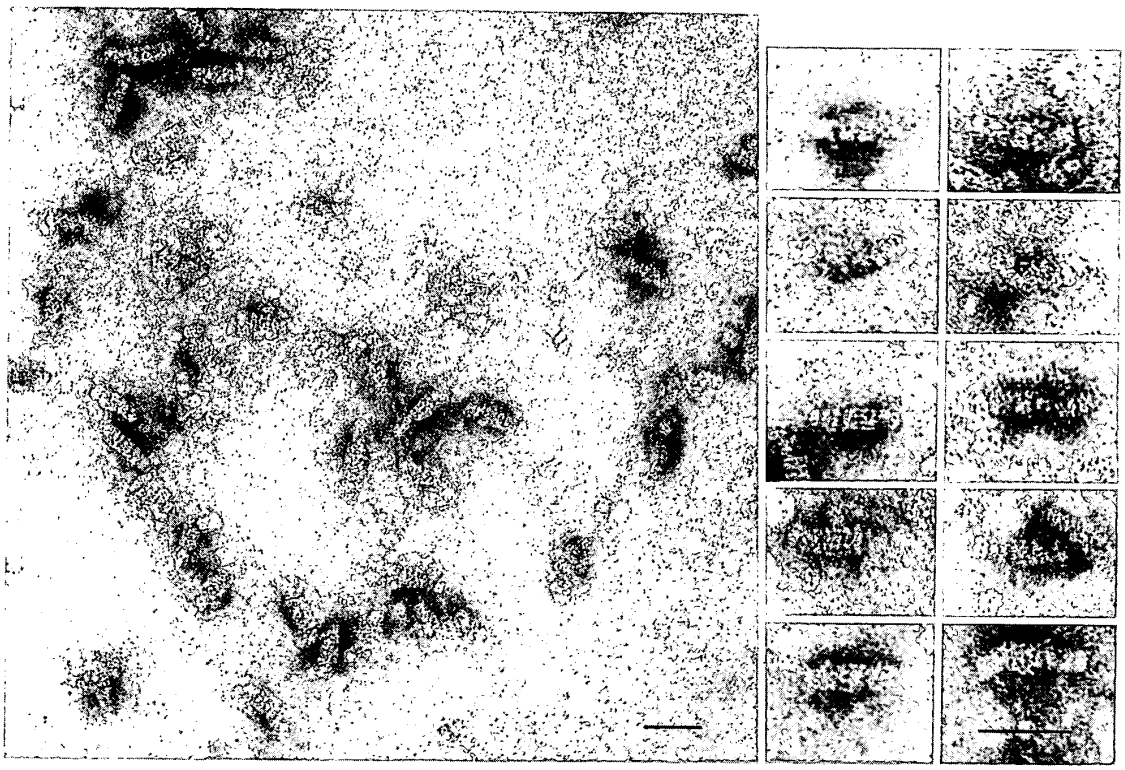

Fig. 2 Electron micrographs of intact PBS, fixed with 1% glutaraldehyde in 0.75 M K-phosphate. Negatively stained with 1% uranyl acetate. Bars represent 50 nm.

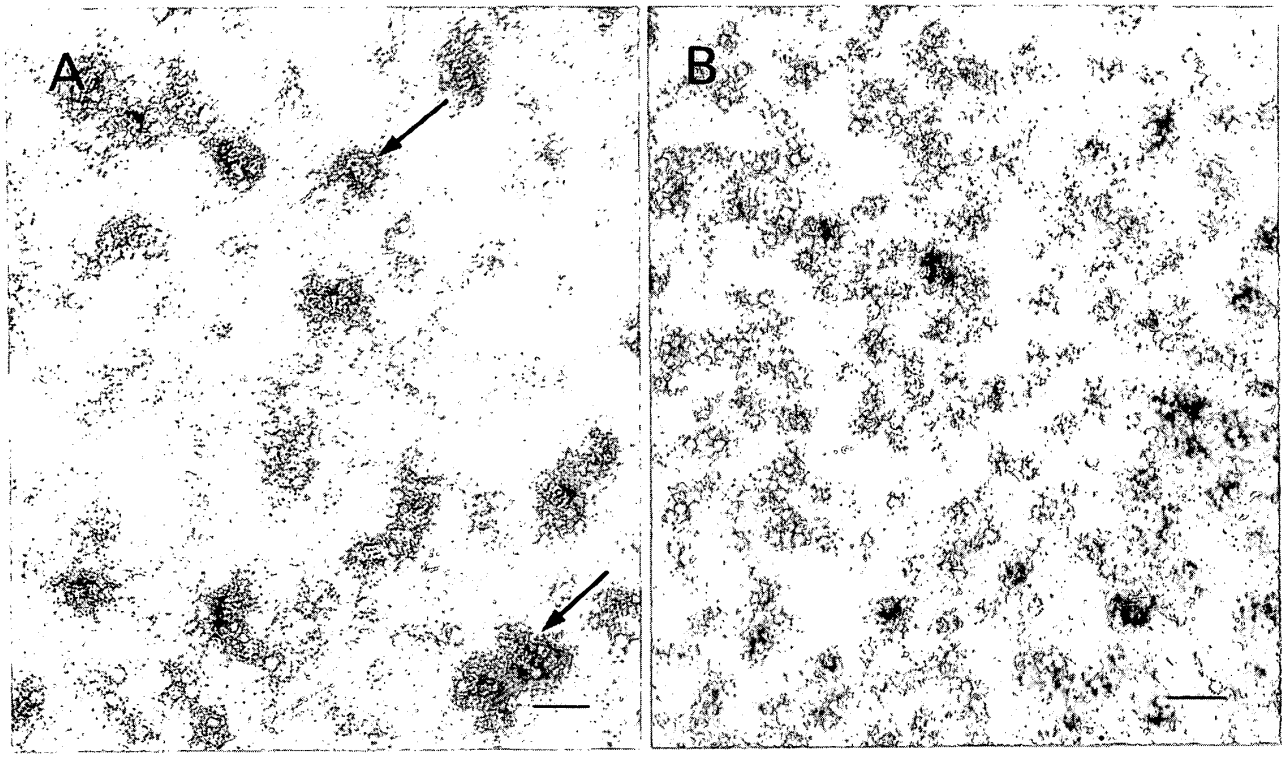

Fig. 3 Electron micrographs of dissociating PBS. A. 4 hr after transfer into 0.25 M K-phosphate. Arrows indicate squarish bodies with dimensions close those of to the central part of PBS. B. 24 hr after transfer into 0.1 M K-phosphate. Bars represent 50 nm.

666 nm fluorescence originates from APC and the rest from LWEAP, reflecting an efficient exciton transfer between these pigments.

The structure of intact PBS isolated was examined after fixation by glutaraldehyde in 0.75 M

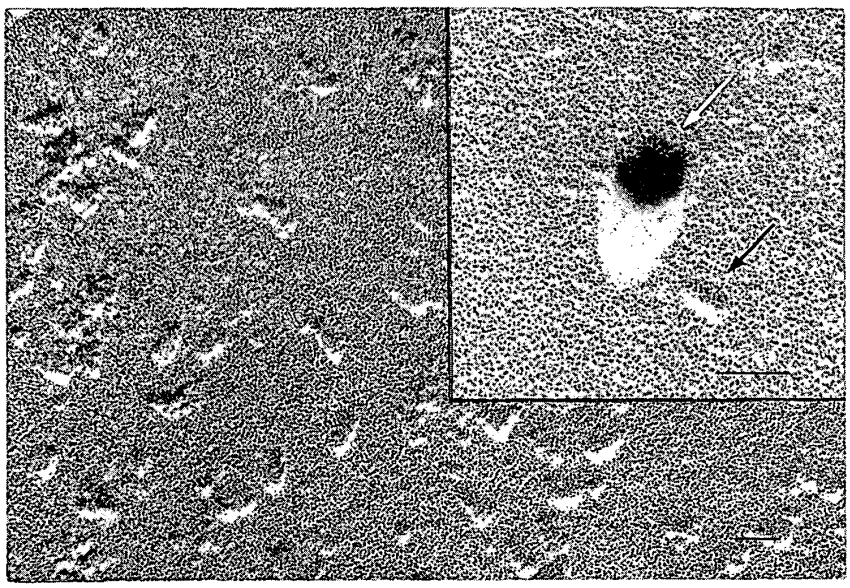

Fig. 4 Intact PBS observed after platinum-palladium shadowing from an angle of 27° against the grid plane, which gives a shadow twice as long as the thickness of the object. White arrow indicates 50-nm bacteriophage T3 as the control against the intact PBS (black arrow). Bars represent 50 nm.

K-phosphate. When sufficiently fixed, PBS retained their excitation coupling even in the absence of high phosphate concentration (dashed lines in Fig. 1). The fixed PBS were found to have a cylindrical structure 52.2±6.5 nm long and 15.7±2.2 nm in diameter at the fat middle part (Fig. 2). The cylinder is slimmer at both ends (11.5±1.2 nm in diameter), which appear to be composed of stacked discs. The fatter middle part has a defined, network-looking substructure.

The disc-shaped subunits were more distinctly visible when PBS were dissociated under moderate conditions. Four-hour incubation in 0.25 M K-phosphate resulted in the dissociation of PBS into many separate discs of 11.9±1.2 nm in diameter, sometimes with an accumulation of stain at the center, suggesting that the discs have a ring structure (Fig. 3A). A few squarish subparticles (approximately 15.4 nm in diameter and 24.5 nm long) with a fine structure similar to the middle part of PBS were also detected. According to the spectral resolution of fluorescence in the course of PBS dissociation, the energy migration between PC and APC was almost absent at this stage, although the coupling between APC and LWEAP remained mostly intact (Kume and Katoh 1982). Further dissociation led to uncoupling of the exciton migration, with accompanying change of all the subparticles into discoidal form (Fig. 3B).

To elucidate the three-dimensional structure of intact PBS, examinations by shadowing as well as by grid-tilting were attempted. With a platinum-palladium beam from the angle of 27°, 50-nm T3 phages gave shadows about 100 nm long, whereas the shadows of PBS were about 25 nm, indicating that they were about as thick as their width (Fig. 4). This was confirmed by the pictures taken with tilted specimens (Fig. 5). On tilting the grids by 30° either to the left or right, PBS remained in essentially the same rod shape, indicating no extension in the direction vertical to the grid plane.

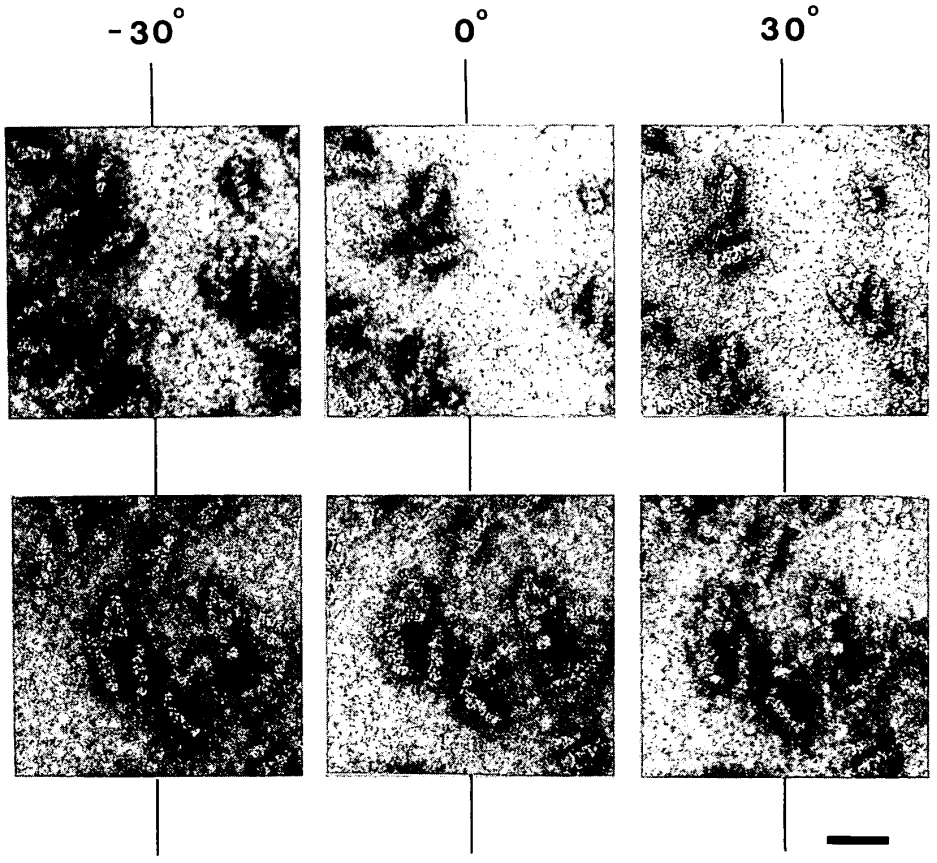

Fig. 5 Upright and tilted views of intact PBS. Negatively stained PBS were observed from right above (center) and at a tilted angle of 30° to the right (left) or the left (right) along the indicated line. Bar represents 50 nm.

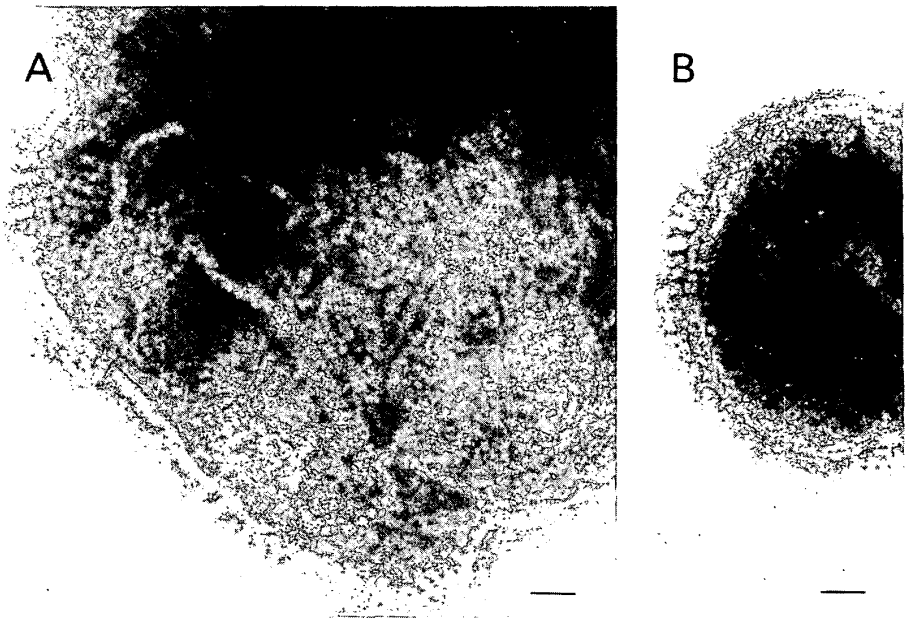

Fig. 6A and 6B Photosynthetic vesicles with bound phycobilisomes. Isolated vesicles were fixed with 1% glutaraldehyde in a mixture of 0.5 M sucrose, 0.5 M K-phosphate and 0.3 M Na_3-citrate. Negatively stained with 1% uranyl acetate. Bars represent 50 nm.

When photosynthetic vesicles with bound PBS were prepared in a medium containing phosphate, citrate and sucrose (Katoh and Gantt 1979), the membranes were found to be profusely covered with cylindrical bodies of dimension (57.5 ± 4.8 nm long) and morphology close to those of isolated PBS. At the periphery of the thylakoids, however, the attached rods looked shorter (25 to 30 nm long) than isolated PBS, and pairing of rods could be frequently seen (Fig. 6).

To identify the localization of APC and PC in PBS, APC- and PC-subparticles were prepared

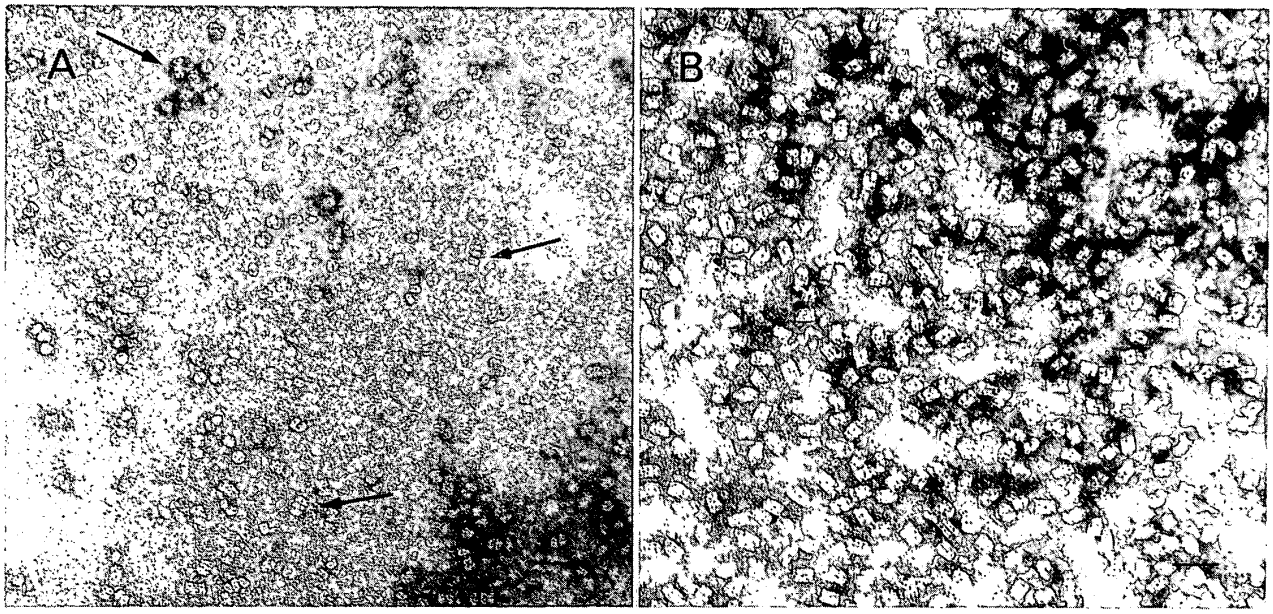

Fig. 7 Particles obtained from dissociated PBS. A. 18.3 *S* particles consisted exclusively of PC. Arrows indicate three stacked discs. B. 23 *S* particles consisted of APC and LWEAP. For details, see text. Bars represent 50 nm.

from partially dissociated PBS and their fine structures were examined by electron microscopy. When PBS, after dissociation in 10 mM K-phosphate at the PBS concentration of $A_{620} \cong 100$, were applied to calcium phosphate gel, the APC-subparticle species were adsorbed on the gel and separated from the PC-subparticle species which escaped from the gel unadsorbed. The former was eluted with 0.75 M K-phosphate, while the latter was brought to 0.75 M phosphate with the addition of 2.0 M K-phosphate, and both were allowed to stand for 24 hr at 20°C. When these suspensions were analyzed for their subparticle distribution in sucrose density-gradient centrifugation in 0.75 M K-phosphate, both were found to contain subparticle bands which migrate faster than their trimetric forms (7 *S*). The PC suspension, incubated in 0.75 M K-phosphate, yielded, in addition to 7 *S* (trimer), 10 *S* (hexamer) and 15.2 *S* (dodecamer), a larger 18.3 *S* band which was assumed to be an octadecamer of PC from its fine structure consisting of three stacked discs (Fig. 7A). On the other hand, the largest APC-subparticles with the sedimentation rate of 23 *S* had the dimensions of 22.6 ± 2.1 nm $\times$ 15.6 ± 0.8 nm and showed a defined substructure, similar to the central part of PBS (Fig. 7B).

Discussion

The cylindrical morphology of *Anabaena* PBS revealed in the present study is markedly different from the generally accepted shape of cyanophycean PBS (Bryant et al. 1979, Gantt 1980, Williams et al. 1980, Rosinski et al. 1981). Our pictures might have been a top view of hemidiscoidal bodies which were adsorbed perpendicularly onto carbon-coated grids, but examinations with shadowing as well as plate-tilting techniques revealed no evidence for upright-standing rods.

The question arising with this shape of PBS is how they are attached to the thylakoid membranes. The photosynthetic vesicles isolated from this alga are profusely covered with PBS which look as though they are attached upright at one end (Fig. 6; see also Katoh and Gantt 1979). Rod-shaped PBS have been shown to fill the intra-thylakoidal space in *Anabaena* and *Nostoc*, although the detailed morphology has not been discussed (Wildman and Bowen 1974). However, LWEAP in combination with APC is assumed to form the core of PBS and to be localized at the attachment site to the thylakoid (Gantt et al. 1976, Bryant et al. 1979). If either of the two ends of cylindrical PBS is the attachment site, then the other end should consist of the PC moiety with a fine structure that would more or less differ from the attached end. However, our result (Fig. 2) shows essentially identical stacked structures for both ends. Recently, Yu et al. (1982) showed that the aggregate forms of PC build up stacked discs. Consistent with this, the subparticles comprising of PC form stacked discs, while the APC subparticles have a network-like fine structure, like the central part of PBS (Fig. 7B). Thus, the central part of PBS probably consist of the APC species and has PC rods with the stacked-disc structures attached on two opposite sides.

From the morphological data presented here and from other physicochemical information, the molecular mass of the PBS can be calculated. On the basis of a sedimentation velocity, $S_{20,w}$, of 43.0 for the intact PBS after corrections for the density and viscosity of the suspending medium and assuming this PBS to be a cylindrical body 52.5 nm long and 12 nm in diameter which gives a friction coefficient of 3.41×10^{-7} g·s^{-1} (Broersma 1955), calculation gave a molecular mass of 3.27×10^6. Likewise, the molecular mass of 23 *S* APC subparticles is estimated as 1.13×10^6, with their Stokes radius assumed to be 9.8 nm. As the APC content of PBS is about 40%, the molecular mass of APC in one PBS should be about 1.31×10^6, which is roughly the same magnitude as 23 *S* APC subparticles. This fact, together with the similarity in their fine structures, suggests that the APC moiety of PBS can be reversibly reconstituted from the dissociated subunits of APC.

The APC content in this PBS preparation is higher than those of other algae (Lemasson et al. 1973, Tandeau de Marsac 1977, Glazer 1979). From this fact, the cylindrical morphology of this PBS can be regarded as a special case of hemidiscoidal PBS with only two stacked disc rods attached onto the central core. The possibility that some of the PC rods have been detached from otherwise hemidiscoidal PBS in the course of preparation can be excluded from a high recovery of cellular phycobiliproteins as PBS preparation (>90%). Thus, this PBS contrasts with the case of *Synechococcus sp.* 6312, in which a higher APC content (APC/PC=0.66) is reflected in shortened PC rods, with the number of rods attached to the central core being unchanged. The PBS of *Anabaena* appear closer to those of a mutant of *Synechocystis* 6701, in which fewer attached rods extend from the central core (Williams et al. 1980).

Attachment of PBS to the thylakoid membranes at the center of the cylindrical body seems somewhat awkward. A possible explanation would be that the attached PBS are folded into a V-form with the pair of PC rods extending from the same side of the APC core, while PBS detached from the membranes assume the cylindrical form. Paired rods 30 nm long seen at the periphery of the vesicle preparation (Fig. 6B) may be attached in the V-form. Further detailed studies are needed to clarify the attachment of PBS to photosynthetic membranes.

The authors wish to express their gratitude to Dr. Satoru Murakami, Department of Biology, College of General Education, University of Tokyo, Dr. Tsuneyoshi Kuroiwa, National Institute for Basic Biology and Dr. Hisako Matsuo-Kato in this Department, for their valuable advice on electron microscopic techniques. This work was supported by Grants-in-Aid of Scientific Research (00534027, 00548009, 56040054) from the Ministry of Education, Science and Culture, Japan to T. K.

References

Bennett, A. and L. Bogorad (1973) Complementary chromatic adaptation in a filamentous blue-green alga. *J. Cell Biol.* 58: 419–435.

Broersma, S. (1960) Viscous force constant for a closed cylinder. *J. Chem. Phys.* 32: 1632–1635.

Bryant, D. A., G. Guglielmi, N. Tandeau de Marsac, A.-M. Castets and G. Cohen-Bazire (1979) Structure of cyanobacterial phycobilisomes: A model. *Arch. Microbiol.* 123: 113–127.

Gantt, E. (1980) Structure and function of phycobilisomes: Light harvesting pigment complexes in red and blue-green algae. *Internatl. Rev. Cytol.* 66: 45–80.

Gantt, E. (1981) Phycobilisomes. *Annu. Rev. Plant Physiol.* 32: 327–347.

Gantt, E. and S. F. Conti (1969) Ultrastructure of blue-green algae. *J. Bacteriol.* 97: 1486–1493.

Gantt, E., C. A. Lipschultz, J. Grabowski and B. K. Zimmerman (1979) Phycobilisomes from blue-green and red algae. Isolation criteria and dissociation characteristics. *Plant Physiol.* 63: 615–620.

Gantt, E., C. A. Lipschultz and B. A. Zilinskas (1976) Phycobilisomes in relation to the thylakoid membranes. *Brookhaven Symp. Biol.* 28: 347–357.

Glazer, A. N. and D. A. Bryant (1975) Allophycocyanin B (λ_{max} 671, 618 nm). A new cyanobacterial phycobiliprotein. *Arch. Microbiol.* 104: 15–22.

Glazer, A. N., R. C. Williams, G. Yamanaka and H. K. Schachman (1979) Characterization of cyanobacterial phycobilisomes in zwitterionic detergents. *Proc. Natl. Acad. Sci. USA* 76: 6162–6166.

Katoh, T. and E. Gantt (1979) Photosynthetic vesicles with bound phycobilisomes from *Anabaena variabilis*. *Biochim. Biophys. Acta* 546: 383–393.

Kratz, W. A. and J. Myers (1955) Nutrition and growth of several blue-green algae. *Amer. J. Bot.* 42: 282–287.

Kume, N., T. Isono and T. Katoh (1982) Stability of cyanobacterial phycobilisomes in reference to their concentration. *Photobiochem. Photobiophys.* 4: 25–37.

Kume, N. and T. Katoh (1982) Dissociation kinetics of *Anabaena* phycobilisomes. *Plant & Cell Physiol.* 23: 803–812.

Lemasson, C., N. Tandeau de Marsac and G. Cohen-Bazire (1973) Role of allophycocyanin as a light harvesting pigment in cyanobacteria. *Proc. Natl. Acad. Sci. USA* 70: 3130–3133.

Lichtlé, C. (1978) Étude experimentable d'une cryptophycée, analyse particulière de l'appareil photosynthètique; comparaison avec quelque rhodophycées. Thesis.

Martin, R. G. and B. N. Ames (1961) A method for determining the sedimentation behaviour of enzymes: Application to protein mixtures. *J. Biol. Chem.* 236: 1372–1379.

Mörschel, E., K.-P. Koller, W. Wehrmeyer and H. Schneider (1977) Biliprotein assembly in the disc-shaped phycobilisomes of *Rhodella violacea* I. Electron microscopy of phycobilisomes in situ and analysis of their architecture after isolation and negative staining. *Cytobiologie* 16: 118–129.

Rosinski, J., J. E. Hainfeld, M. Rigbi and H. W. Siegelman (1981) Phycobilisome ultrastructure and chromatic adaptation in *Fremyella diplosiphon*. *Ann. Bot.* 47: 1–12.

Siegelman, H. W., G. A. Wieczorek and B. C. Turner (1965) Preparation of calcium phosphate for protein chromatography. *Anal. Biochem.* 13: 402–404.

Tandeau de Marsac, N. and G. Cohen-Bazire (1977) Molecular composition of cyanobacterial phycobilisomes. *Proc. Natl. Acad. Sci. USA* 74: 1635–1639.

Wildman, R. B. and C. C. Bowen (1974) Phycobilisomes in blue-green algae. *J. Bacteriol.* 117: 866–881.

Williams, R. C., J. C. Gingrich and A. N. Glazer (1980) Cyanobacterial phycobilisomes. Particles from *Synechocystis* 6701 and two pigment mutants. *J. Cell Biol.* 85: 558–566.

Yu, M.-H., A. N. Glazer and R. C. Williams (1981) Cyanobacterial phycobilisomes. Phycocyanin assembly in the rod substructures of *Anabaena variabilis* phycobilisomes. *J. Biol. Chem.* 256: 13130–13136.

Zilinskas, B. A., B. K. Zimmerman and E. Gantt (1978) Allophycocyanin forms isolated from *Nostoc* sp. phycobilisomes. *Photochem. Photobiol.* 27: 587–595.

(Received June 5, 1982; Accepted September 21, 1982)