Arch Microbiol (1981) 129:181–189

Archives of
Microbiology

The Structure of *Gloeobacter violaceus* and its Phycobilisomes

Gérard Guglielmi*, Germaine Cohen-Bazire, and Donald A. Bryant[1]

Unité de Physiologie Microbienne – C.N.R.S. E.R.A. 398 – Département de Biochimie et Génétique Moléculaire, Institut Pasteur 28, rue du Docteur Roux – 75015 Paris, France

Abstract. The fine structure of the atypical cyanobacterium *Gloeobacter violaceus* has been studied on frozen-etched replicas and compared to that of a typical unicellular strain: *Synechocystis* 6701. The complementary fracture faces of *G. violaceus* cytoplasmic membrane contain particles less numerous and more heterogenous in size than either the cytoplasmic membrane or the thylakoid membranes of *Synechocystis*. The most frequently observed particles of the exoplasmic fracture (EF) face of the *G. violaceus* cytoplasmic membrane are 11 nm in diameter and occasionally form short alignments. This particle class is similar in appearance to the numerous, aligned EF particles of *Synechocystis* thylakoid membranes. In replicas of cross-fractured *G. violaceus*, a layer 50 – 70 nm thick, composed of rod-like elements, underlies the inner surface of the cytoplasmic membrane. The rods, 12–14 nm in diameter, are oriented perpendicularly to the cytoplasmic membrane and show a 6 nm repeat along their length.

Isolated phycobilisomes of *G. violaceus* appear, after fixation and negative staining, as bundles of 6 parallel rod-shaped elements connected to an ill-defined basal structure. The bundles are 40–45 nm wide and 75–90 nm long. The rods are 10–12 nm in width; their length varies between 50 and 70 nm. These rods are morphologically similar to those observed at the periphery of hemidiscoidal phycobilisomes of other cyanobacteria, with a strong repeat at 6 nm intervals and a weaker one at 3 nm intervals along their length.

The calculated molar ratio of phycobiliproteins in isolated *G. violaceus* phycobilisomes corresponds to 1:3.9:2.9 for allophycocyanin, phycocyanin and phycoerythrin respectively. When excited at 500 nm, isolated phycobilisomes exhibit a major fluorescence emission band centered at 663 nm.

Key words: Cyanobacteria – *Gloeobacter violaceus* – *Synechocystis* – Freeze-etching – Membranes – Phycobilisomes

Gloeobacter violaceus is a small unicellular cyanobacterium of unique structure (Rippka et al. 1974). Visualized by transmission electron microscopy of thin sections, the rod-shaped cells do not contain the extensive system of photosynthetic membranes (thylakoids) characteristic of all other cyanobacteria so far examined (Stanier and Cohen-Bazire 1977). Besides the outer wall layer, the sole unit membrane system of *G. violaceus* is the cytoplasmic membrane.

Although the phycobiliproteins (PBP) of *G. violaceus* represent a large fraction of the total cellular protein, equivalent to that of other cyanobacteria (Rippka et al. 1974), the chlorophyll a content is unusually low. This is consistent with the hypothesis that chlorophyll a is confined to the cytoplasmic membrane. This membrane must therefore play a dual role: that of a typical cytoplasmic membrane (its function in cyanobacteria being largely unknown); and that of a photosynthetic membrane, site of photochemical reactions with their associated pigments (chlorophyll a and carotenoids) and electron transport machinery.

Another peculiarity of *G. violaceus* when examined in thin sections is the presence of a layer, some 50–80 nm thick, which consists of electron opaque rods oriented perpendicularly to the cytoplasmic membrane and in close proximity to its inner surface. This layer has been assumed to represent the location of PBPs in this organism (Rippka et al. 1974). The PBP complement of *G. violaceus* consists of spectroscopically typical cyanobacterial PC and AP accompanied by a PE unusal for a cyanobacterium. This PE, with 2 absorption maxima at 501 and 564 nm, bears some similarity to the PE(s) found in many red algae (Vaughan 1964) and the marine cyanobacterium *Trichodesmium thiebautii* (Fujita and Simura 1974) (see also following paper).

The cytoplasmic membrane of *G. violaceus* cannot be distinguished in thin sections form that of other cyanobacteria. Electron microscopic examination of frozen-etched replicas provides observations complementary to those of thin sections, especially when applied to membranes (Branton et al. 1975). Using this technique we have examined the structure of the cytoplasmic membrane of *G. violaceus* and compared it with the structure of the membranes (cytoplasmic and thylakoidal) of *Synechocystis* sp. 6701 (Rippka et al. 1979), a cyanobacterium that contains typical thylakoids and hemidiscoidal phycobilisomes (Bryant et al. 1979; Glazer et al. 1979; Williams et al. 1980). Furthermore, we describe the structure and some of the properties of PBP-containing elements which we assume represent the phycobilisomes of *G. violaceus*.

Materials and Methods

Biological Specimens. Axenic cultures of *Gloeobacter violaceus* and *Synechocystis* came from the Pasteur culture collection where their

Abbreviations: PBS: phycobilisome(s); PBP: phycobiliprotein(s); AP: allophycocyanin; PC: phycocyanin; PE: phycoerythrin – K-PO_4 buffer: KH_2PO_4 titrated with KOH to a given pH.

1 Present address: Section of Plant Biology, Plant Science Bldg., Cornell University – Ithaca, N. Y. 14853, USA

* *Offprint requests to* G. Guglielmi

0302-8933/81/0129/0181/$01.80

respective code numbers are 7421 and 6701 (ATCC 29082 and 27170) (Rippka et al. 1979).

Growth conditions and media have been previously described (Stanier et al. 1971; Rippka et al. 1974).

Techniques for Electron Microscopy. Freeze etching was performed using the spray freezing technique of Bachmann and collaborators (Bachmann and Schmitt 1971; Bachmann and Schmitt-Fumian 1973). The application of this technique to biological materials has been described and discussed by Plattner et al. (1973). Cells suspended in their culture medium without addition of cryoprotective agents, were sprayed into liquid propane, at −190°C. The propane was allowed to evaporate and the frozen droplets, agglomerated with butyl benzene at −85°C, were placed on a Balzer's specimen holder, precooled and maintained at −85°C. A Balzers union spray-freezing glove box and its accessories were used throughout the procedure. The samples were transferred into liquid nitrogen, placed in a BAF301 freeze-etching chamber and fractured at −110°C under a vacuum of 10^{-6} mm Hg. Carbon-platinum replicas (unidirectional shadowing) were cast after 2,5 min of etching.

The nomenclature of Branton et al. (1975) has been used to designate the complementary fracture faces of membranes. The density and size of intramembrane particles were estimated from micrographs taken at 30,000 to 40,000× and enlarged to 150,000× to 200,000×.

Specimens for thin sections were processed following the procedure described by Rippka et al. (1974).

For negative staining of PBS and PBS fragments, samples were prepared by the method described by Bryant et al. (1979). Adequately diluted samples in 0.75 M K-PO_4 buffer (pH 7) containing 0.5 M sucrose were prefixed for 10 min with 0.3% (w/v) glutaraldehyde. Collodion covered 200 mesh grids were floated on drops of the prefixed sample. The samples were further fixed by floating the grids on sucrose-phosphate buffer containing 1% (w/v) glutaraldehyde. The grids were thoroughly washed with distilled water and stained with 1% (w/v) uranyl acetate.

All electron microscopic observations were performed with a Siemens Elmiskop 101 operated at 80 KV.

Extraction and Isolation of Phycobilisomes. The procedure generally used was identical to that described by Bryant et al. (1979) for PE-rich PBS. All buffers contained 0.002 M sodium azide and all operations were performed at room temperature. One month old cultures were washed once with 0.65 M K-PO_4 buffer at pH 7 containing 0.5 M sucrose and suspended in the same buffer to a concentration of 0.1−0.2 mg (wet weight) per ml. The cells, disrupted once in a French pressure cell (Aminco) operated at 20,000 p. s. i., were treated with Triton X-100 (2% v/v final concentration) for 10−20 min with occasional mixing and centrifuged for 10 min at 27,000 g.

The supernatant was layered (0.5 ml per tube) on sucrose step gradients consisting of 0.5, 2.0, 1.5, 0.5 ml of 0.5 M, 0.75 M, 1.0 M and 2.0 M sucrose solutions in 0.75 M K-PO_4 buffer at pH 7. The gradients were centrifuged at 340,000 g for 3 h at 20°C in a swinging-bucket rotor (MSE, Cat. N° 59587).

In other experiments, the cells, disrupted in 0.75 M K-PO_4 buffer at pH 7, were treated with the zwitterionic detergent Deriphat-160 (1% w/v final concentration) (Glazer et al. 1979). Thereafter, all suspending media contained 0.1% of the detergent. PBS and their fragments were used for spectrofluorometric and electron microscopic studies as soon as possible after their recovery from the gradients.

Spectroscopic Determinations. Absorption spectra were recorded with a Cary 17 and fluorescence emission spectra with a Perkin-Elmer MPF4 spectrofluorometer equipped with a differential corrected spectral unit, using a slit width of 8 nm. Samples for fluorescence spectra were diluted with 0.75 M KPO_4 buffer at pH 7 containing 0.5 M sucrose, to an absorbance of 0.1−0.2 at 565 nm. After dialysis of samples against 50 mM K-PO_4 buffer, individual PBP concentrations were estimated spectrophotometrically using the extinction coefficients and the set of simultaneous equations published by Bryant et al. (1979) assuming a molar extinction coefficient of 456,000 M^{-1} cm^{-1} for the protomer ($\alpha\beta$) of *G. violaceus* PE (see following paper).

Results

Freeze-Etching Studies. Various planes of fracture through the cell wall and of cytoplasmic membrane of *G. violaceus* are presented in Figs. 1, 2 and 4. The two complementary fracture faces of the outer membrane of the cell wall are seen in Fig. 1 (EF-W) and in Fig. 2 (PF-W). They are similar in structure to the CW_2 layer, described by Golecki and Drews (1974) and Golecki (1977, 1979) for *Anabaena variabilis*, *Anacystis nidulans* and several other cyanobacteria.

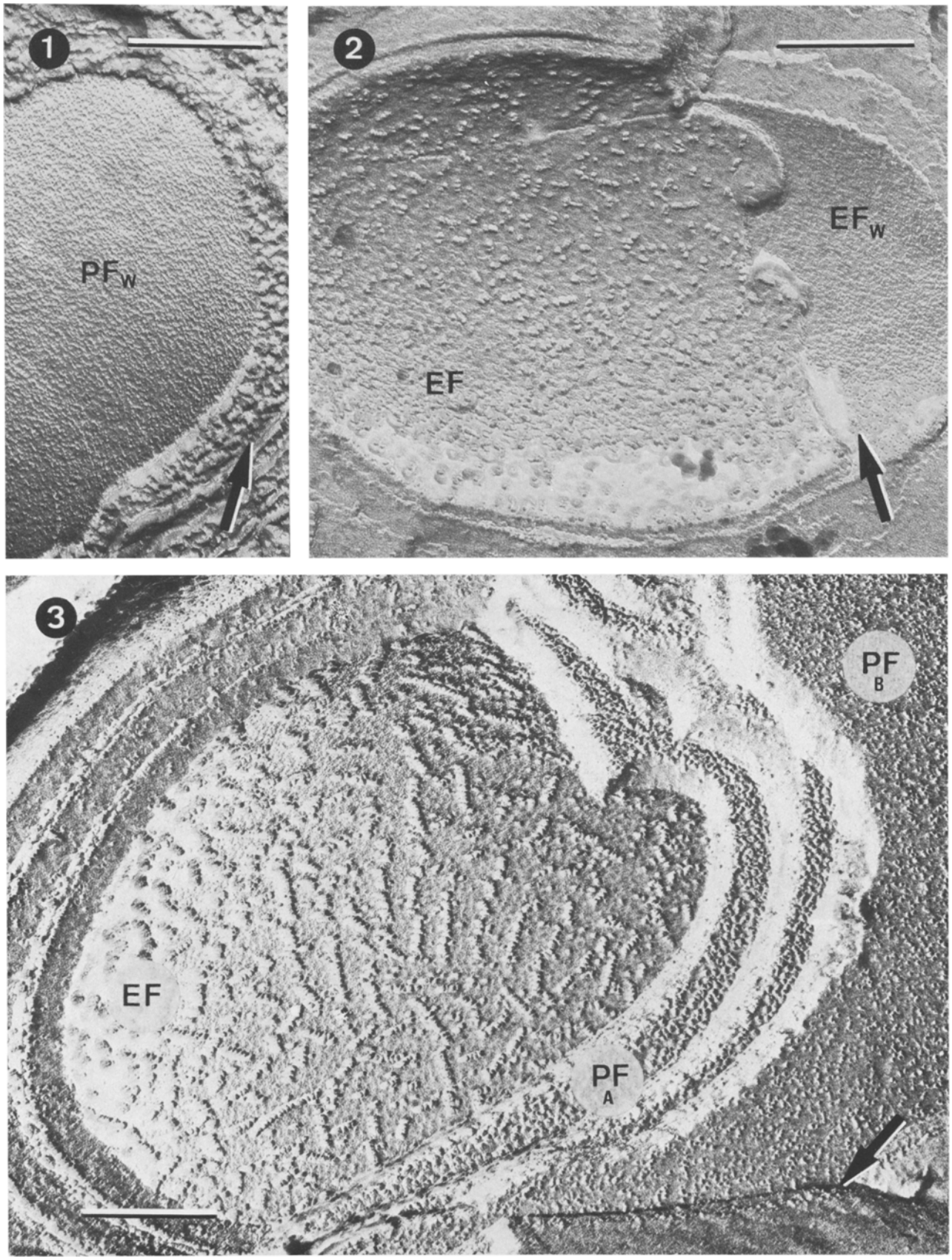
The complementary fracture faces of the cytoplasmic membrane of *G. violaceus* are shown in Figs. 2 and 4 (EF face in Fig. 2, PF face in Fig. 4). For comparison, the complementary fracture faces of the thylakoids and cytoplasmic membranes of *Synechocystis* sp. 6701 are shown in figs. 3, 5 and 6 (cytoplasmic membrane: PF face: Fig. 3; EF face: Fig. 5; thylakoid membrane: EF face, Fig. 3; PF face: Fig. 6).

The EF and PF faces of *G. violaceus* cytoplasmic membrane can be distinguished by their relative particle densities and the size distribution of their associated particles (Fig. 7). The EF face (Fig. 2) shows a particle density of approximately 1200/μm^2 with a predominance of 11 nm particles, some of them forming short alignments. The particles of the PF face (Fig. 4) are more numerous, with a density of approximately 3000/μm^2. The size distribution of particles on this face is clearly bimodal with particles 9−10 nm in diameter outnumbering the larger 17−18 nm particles. The larger particles, also observed on the EF face exhibit a complex structure upon close examination.

The density and size distribution of particles observed on the complementary fracture faces of both the cytoplasmic membrane (Fig. 3: PF face; Fig. 5: EF face) and thylakoid membranes (Fig. 3: EF face; Fig. 6: PF face) of *Synechocystis* 6701 can be compared to those of *G. violaceus* in the histograms of Fig. 9. The fractured-thylakoids of *Synechocystis* reveal rows variable in length, of 8−10 nm particles, scattered on the EF face (Fig. 3); corresponding furrows are apparent among the numerous particles of the complementary PF face (Fig. 6).

Deep etching is easily obtained in spray-frozen preparations when no cryoprotective agent is used. This had allowed the observation, in replicas of cross-fractured *G. violaceus* cells, of details in the area immediately adjacent to the inner surface of the cytoplasmic membrane, i.e. the region presumably occupied by the PBP. In Fig. 4 and in Fig. 8, this region appears composed of a series of small cylinders 50−70 nm in length and 12−14 nm in diameter, oriented perpendicularly to the cytoplasmic membrane; in favorable areas (Fig. 8), a 6 nm repeat can be resolved across their length: each cylinder appears to be formed by the stacking of 8 to 10 elements 6 nm thick.

The Phycobilisomes of G. violaceus. Ultracentrifugation on sucrose step gradients of extracts prepared in 0.65 M K-PO_4 buffer pH 7 in the presence of Triton X-100 (or in 0.75 M K-PO_4 buffer in the presence of Deriphat-160) yielded two main fractions containing varying proportions of PBP: a deep purple fraction, presumed to contain the PBS, showing little fluorescence to the naked eye, located in the lower portion of the 1.0 M sucrose layer and a lighter fraction with a marked red fluorescence, located in the lower portion of the 0.5 M sucrose layer. The relative proportions of PBP in these two fractions varied from experiment to experiment: the heavier fraction containing between ~60 and 73% and the lighter



Figs. 1 and 2. Freeze-fracture micrographs of *Gloeobacter violaceus*. **Fig. 1.** The fracture uncovers the PF face of the outer cell wall layer. In all freeze-fracture micrographs, the large arrow indicates the direction of shadowing and the bar represents 300 nm. Magnification: × 80,000. **Fig. 2.** The EF-faces of *G. violaceus* cytoplasmic membrane and outer wall layer (EF_W); few particles 11 nm in diameter are aligned on the *EF* face of the cytoplasmic membrane

Fig. 3. Freeze-fracture micrograph of *Synechocystis* 6701 exposing both fracture-faces of thylakoid membranes (*EF* and *PFA*) and the EF-face of the plasma membrane (*PFB*). The majority of particles visible on the EF-face of the thylakoid membrane are aligned in rows of diverse lengths and orientations

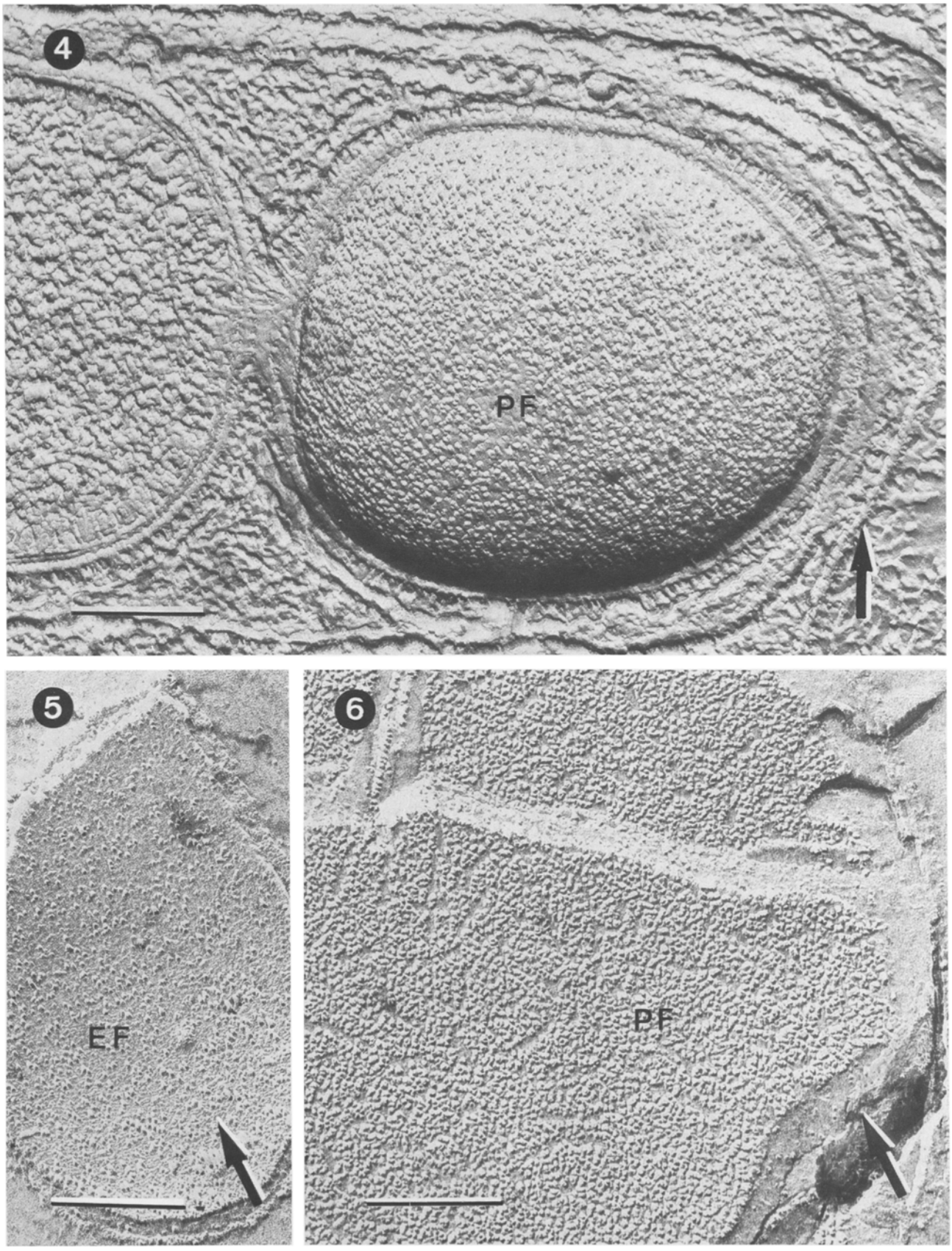


Fig. 4. PF-face of *G. violaceus* plasma membrane. The distribution and size of particles closely resembles those observed on the PF-face of *Synechocystis* thylakoid membrane (see Fig. 6)

Fig. 5. EF-face of *Synechocystis* cytoplasmic membrane

Fig. 6. Large fracture areas showing the structure of the PF-face of *Synechocystis* thylakoid membranes. Note the particle-free grooves among the numerous particles on this fracture face

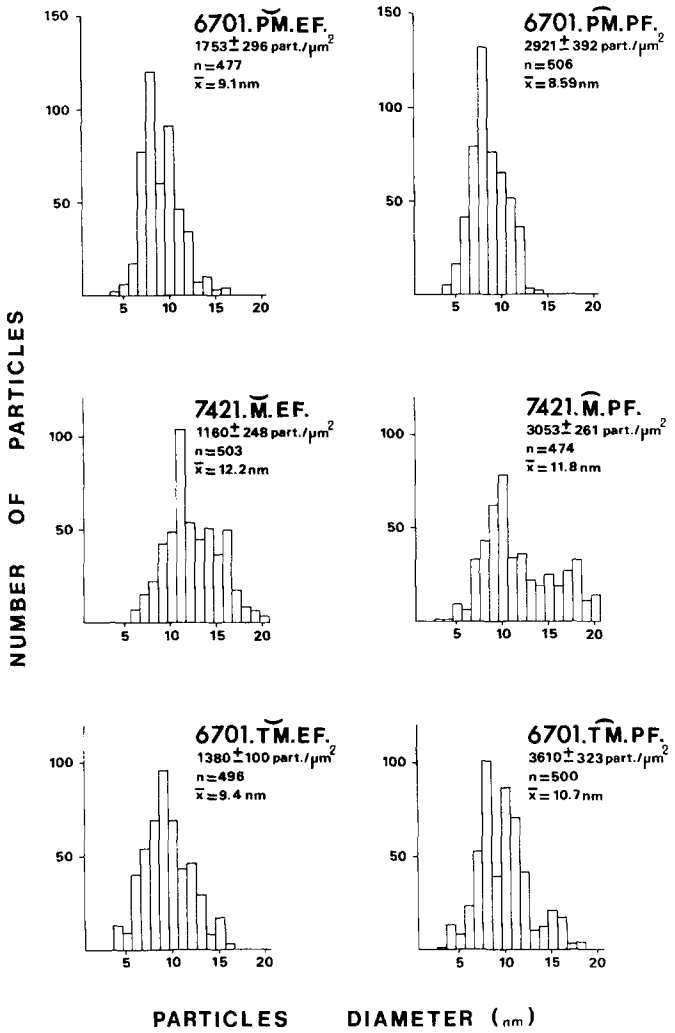


Fig. 7. Particle size histograms of PF and EF faces of *Gloeobacter violaceus* plasma membrane (*M*) and, of *Synechocystis* plasma membrane (*PM*) and thylakoid membranes (*TM*). The density of particles is indicated on each bar-graph. *n*: number of particles measured; $\bar{x}$: mean diameter

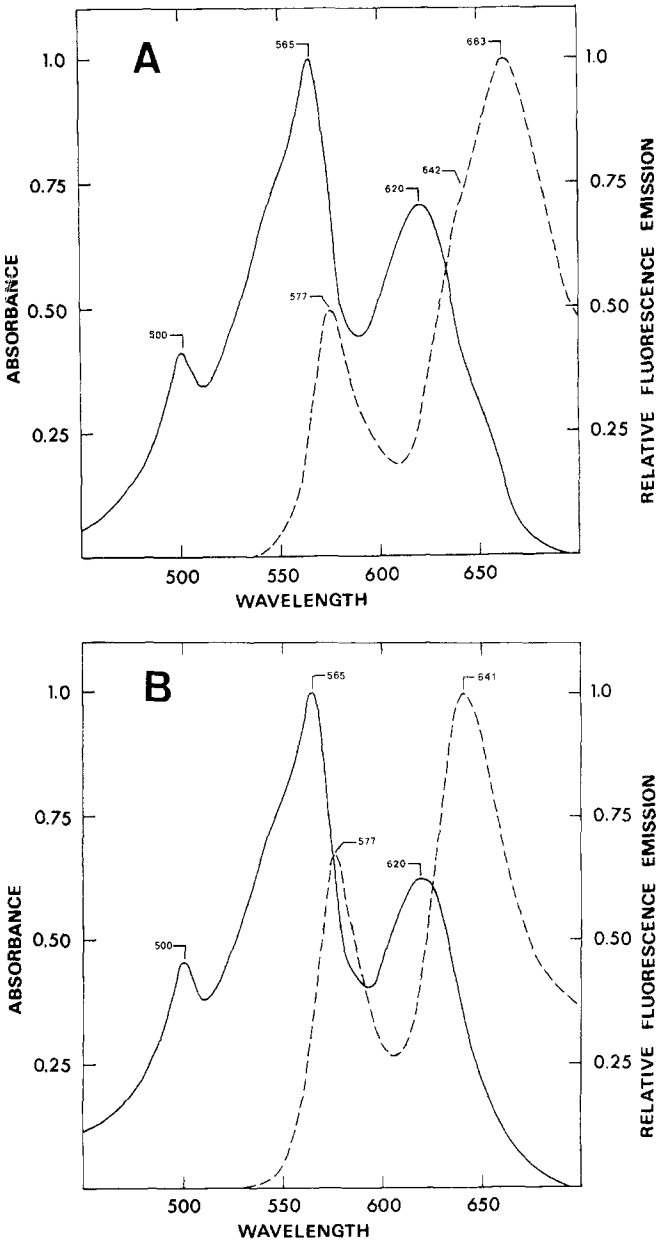


Fig. 12A and B. Absorption and fluorescence emission spectra of the two main biliprotein containing fractions obtained after sucrose gradient centrifugation of a Triton X-100 extract of *G. violaceus*. **A** Heavy fraction containing the phycobilisomes shown in Fig. 9. **B** Lighter PE and PC rich fraction containing predominantly the rod-shaped elements shown in Fig. 10

fractions between ~7 and 15% of the total PBP layered on the gradients.

The elements contained in the heavier purple fraction are shown, negatively stained, in Fig. 9. They consist of bundles of rods, packed almost parallel to each other and attached to an ill-defined basal structure. In overall dimensions, the bundles are 40–45 nm in width and 75–90 nm in length. The individual rods are 10–12 nm wide and their length varies between 50 and 70 nm. The average number of rods in the bundles appears to be 6, although the counting is difficult, due to their superpositioning. Strong repeats at 6 nm intermals and weaker ones at 3 nm intervals are visible perpendicular to the long axis of the individual rods. Each 6 nm repeat delimits a double disc similar to those forming the peripheral rods of hemidiscoidal phycobilisomes (Bryant et al. 1979; Williams et al. 1980). Each rod consists of a long coaxial stack of 8 to 10 of such disc-shaped subunits. At their distal end, the rods terminate with a 6 nm thick disc; their proximal extremities end in an ill-defined lobed structure some 20–30 nm thick. This structure is visible in Fig. 11a, b, c which show partially flattened and disrupted bundles occasionally observed in the lighter biliprotein containing fraction. The elements contained in this fraction are shown, negatively stained in Fig. 10. They consist mainly of isolated rods, some of them broken, with the same organization as those present in the bundles: they vary in length between 30 and 50 nm and consist of coaxial stacks of 6 nm thick double discs.

The molar ratios of biliproteins (AP:PC:PE) in the two fractions were approximately 1:3.9:2.9 for the PBS fraction and 1:6:5.3 for the lighter rod fraction. Their absorption and fluorescence emission spectra are shown in Fig. 12A and B. The two absorption spectra are closely similar except for a pronounced shoulder around 650 nm, attributable to AP, present in the absorption spectrum of the PBS fraction and not noticeable in the rod fraction; the maxima at 500 and

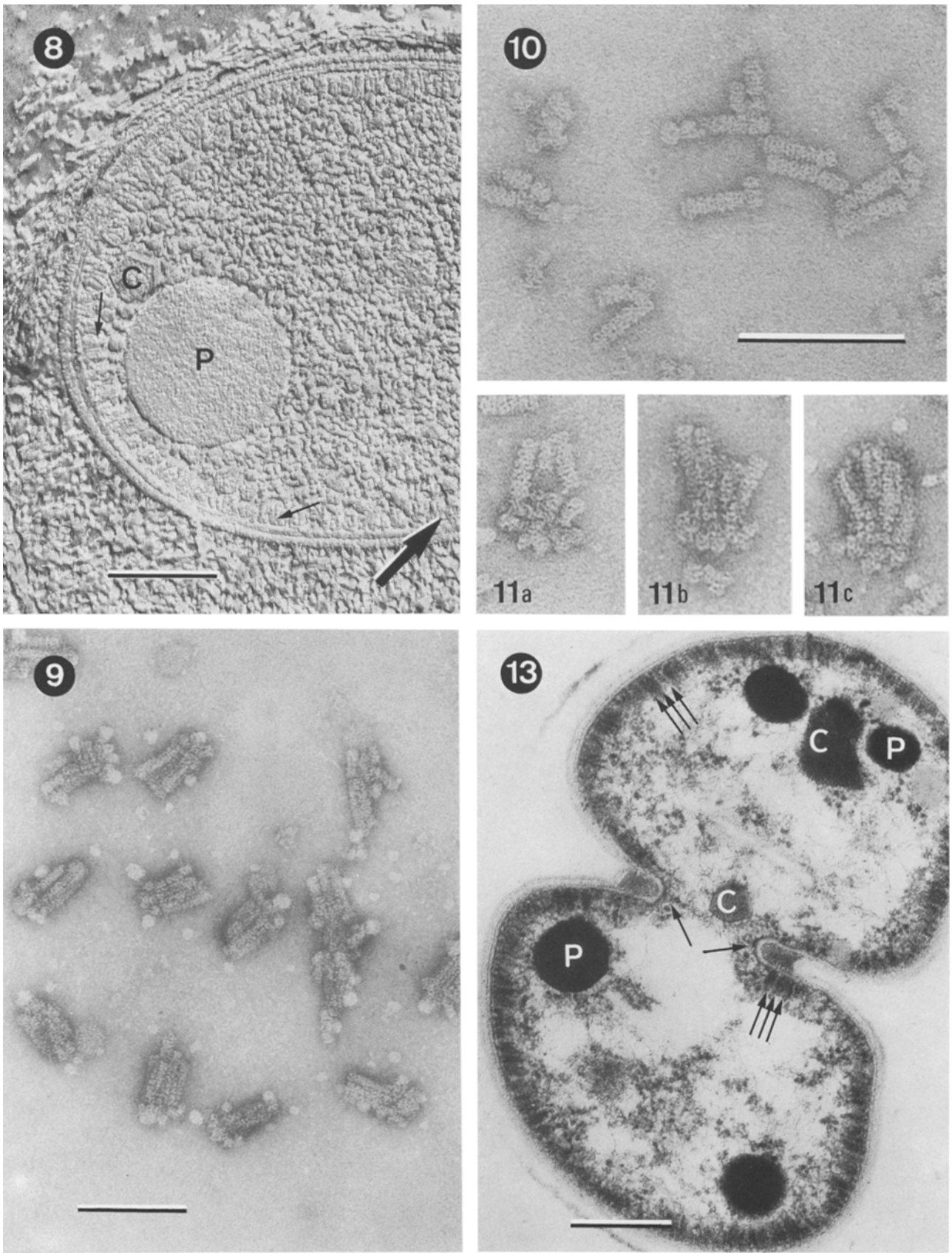


Fig. 8. Cross-fractured cell of *G. violaceus* exposing the rod shaped elements in the cortical area adjacent to the inner surface of the plasma membrane a 6 nm repeat across their long axis is visible in some areas indicated by small arrows. The cell contains a large polyphosphate granule (*P*) and a small carboxysome (C)

Fig. 9. Phycobilisomes isolated from *G. violaceus*, negatively stained with uranyl acetate. The bar represents 100 nm

Fig. 10. Negatively stained rod-shaped elements contained in the 0.5 M sucrose fraction in the gradient. The bar represents 100 nm

565 nm correspond to the absorption maxima of *G. violaceus* PE and at 620 to PC (Rippka et al. 1974). The fluorescence emission spectra of the two fractions are quite different. The heavy fraction (Fig. 12A) shows some fluorescence of PE at 577 nm and a main broad fluorescence emission band centered at 663 nm with a slight shoulder at 641 – 642 nm. The lighter fraction (Fig. 12B) shows a relatively strong emission at 577 nm and a main narrow emission band centered at 641 nm, corresponding to the fluorescence of PC.

Although the absorption spectra of Deriphat-160 and Triton X-100 extracted PBS were superimposable, the fluorescence maxima of Deriphat-160 extracted PBS were shifted to lower wavelengths, 575 nm and 656 nm respectively (spectra not shown).

Figure 13 shows a photomicrograph of a thin section of a fixed cell of *G. violaceus*. The preceding results help to interpret the structure of the cortical PBP layer which underlies the inner surface of the cytoplasmic membrane. This layer, composed of electron-opaque elements of various widths and lengths standing perpendicular to the membrane, represent the bundle-shaped PBS sectioned in various planes. It should be noted that in this photomicrograph of a cell in the process of division, PBS are absent from the region adjacent to the cytoplasmic membrane engaged in septum formation.

Discussion

Observations of freeze-etched preparations of *Gloeobacter violaceus* have provided some insights into the structure of this atypical cyanobacterium. The cell wall is of a gram-negative nature with only one plane of fracture passing through the lipopolysaccharidic outer membrane layer (Salton and Owen 1976). Both fracture faces have the same substructures, analogous to those described in gram-negative bacteria (Van Gool and Nanninga 1971; Gilleland et al. 1973) and various cyanobacteria (Golecki and Drews 1974; Golecki 1977, 1979).

As observed by Giddings and Staehelin (1978, 1979), Golecki (1979), and as is also apparent in the present freeze-fracture study of *Synechocystis* 6701, the cytoplasmic membrane and thylakoid membranes of typical cyanobacteria are structurally well differentiated. The distribution of particle sizes and the relative particle densities of both the EF and PF faces of the *G. violaceus* cytoplasmic membrane are similar to those observed for the EF and PF faces of *Synechocystis* thylakoid membranes. Thus, the architecture of the *G. violaceus* plasma membrane, the only membrane present in this organism, confirms its assumed dual function: that of a combined cytoplasmic and photosynthetic membrane.

Numerous aligned particles are observed on the EF face of *Synechocystis* thylakoids[1]. The proposal of Lefort-Tran et al. (1973), that a direct correlation exists between the EF particle alignments within the thylakoids and the PBS at their surface, was later supported by the comparative studies of Lichtlé and Thomas (1976). Since the PBS constitute the light harvesting antennae of photosystem II in cyanobacteria and red algae (Butler 1978), the hypothesis that many or all of the 10 nm intrathylakoidal particles represented the photosystem II chlorophyll complexes in these organisms seemed attractive and has been strengthened by recent observations. Giddings and Staehelin (1979), in comparing the structure of *Anabaena variabilis* thylakoid membranes from vegetative cells and heterocysts, found that the 10 nm EF particles in the thylakoids of vegetative cells were conspicuously absent from heterocyst thylakoids, in agreement with the absence of photosystem II activity in these specialized cells (Haselkorn 1978). Wollman (1979) recently observed that a mutant of the red alga *Cyanidium caldarium* lacking biliproteins contained twice as many 10 nm EF particles in its chloroplast thylakoids as the wild type, in correlation with a doubling of photosystem II units.

Approximately 20% of the EF particles of *G. violaceus* have an apparent diameter of 11 nm and short alignments of particles of this size class are occasionally observed. However, a correlation between these EF particles and PBS appears difficult to establish for *G. violaceus*. Thin sections of fixed cells provide little information on the structure, size and distribution of PBS in this organism, possibly because the PBS are not arranged in regular arrays. Freeze-etched replicas of cross-fractured cells reveal closely packed rod-shaped elements which radiate from the inner surface of the plasma membrane. Do these rods, or some larger structure composed of several such rods, form the PBS of *G. violaceus?*

The true structure of the PBS of *G. violaceus* PBS could be determined only after their isolation. Applying the technique successfully used for the extraction of PE-rich cyanobacterial PBS (Bryant et al. 1979), up to 73% of the cellular biliprotein content was recovered after sucrose gradient centrifugation as a relatively homogeneous fraction. This fraction, consisting of cylindrical bundle-shaped structures, contained all three major biliproteins in proportions similar to that of whole cell extracts. On average, each bundle is composed of six parallel, rod-shaped elements. These rods, with strong repeats at 6 nm intervals and weaker ones at 3 nm intervals (perpendicular to their long axis), appear to be formed by the coaxial stacking of 8 to 10 double discs 6 nm thick and 12 nm in diameter; they are morphologically similar to the peripheral rods of hemidiscoidal PBS (Mörschel et al. 1977; Bryant et al. 1979; Glazer et al. 1979). Whereas the peripheral rods of hemidiscoidal PBS are composed of 2 to 4 or 5 double discs 6 nm thick, the rods of *G. violaceus* PBS are considerably longer. Within the bundles each rod terminates at one extremity, their distal end, with a double disc; the proximal extremities of the rods end in an ill-defined lobed structure which assumedly makes the contact with the inner surface of the cytoplasmic membrane within the cell. It should be noted that substitution of Deriphat-160 for Triton X-100 during the PBS isolation resulted in the recovery of structurally similar particles. Deriphat-160, a zwitterionic detergent soluble in the high

1 In *Synechocystis* the majority of aligned EF thylakoid particles have an apparent mean diameter of 9 nm. Similar types of particles with an apparent diameter of 10 nm have been observed in cyanobacterial and red algal thylakoids (see the review of Staehelin et al. 1978). This size discrepancy – if such it be – may be attributed to the close packing of the particles in *Synechocystis*.

Fig. 11a–c. Partially disrupted or flattened *G. violaceus* phycobilisomes occasionally present in the 0.5 M sucrose fraction in the gradient; their base appears lobed with no clear fine structure. Same magnification as Fig. 10

Fig. 13. Thin section of a fixed *G. violaceus* cell in the process of division. The three grouped arrows show individualized phycobilisomes. No phycobilisomes are detected in the region of cross-wall formation (*small arrows*). *P*: polyphosphate granules; *C*: carboxysomes. The bar represents 300 nm

ionic strength buffers used for PBS isolation, has been reported to prevent the aggreagation of isolated PBS (Glazer et al. 1979).

As in hemidiscoidal PBS, the 6 nm thick double discs presumably represent hexamers of PC and PE. Such discs are the only visible elements forming the rods present in the PC- and PE-rich, lighter biliprotein fraction isolated on sucrose gradients. As shown in Fig. 12B, this fraction exhibits two narrow fluorescence emission bands when excited by light at 500 nm (absorbed >95% by *G. violaceus* PE). The first, centered at 577 nm corresponds to the emission of PE; the second, centered at 641 nm, corresponds to the emission of PC. The relatively strong emission at 641 nm in this fraction composed of approximately equimolar amounts of PE and PC indicates that much of the PE is energetically coupled to PC.

When the PBS fraction is excited by light at 500 nm, largely absorbed by PE, a major, broad fluorescence emission band centered at 663 nm is observed (Fig. 12A). This emission maximum, which corresponds to that of *G. violaceus* AP (see following paper), indicates a good energy coupling between the three major component biliproteins. Since the 663 nm emission band is broad and extends well beyond 680 nm, it could encompass the emission from a small amount of allophycocyanin B (Glazer and Bryant 1975; Ley et al. 1977). Allophycocyanin B was not detected, however, during the purification of *G. violaceus* biliproteins (see following paper). The fluorescence emission of PE in *G. violaceus* PBS is more pronounced than that observed in PBS from other PE-rich cyanobacteria or red algae (Bryant et al. 1979; Gantt et al. 1979) and may be a consequence of their unusual geometry.

The PBS of *G. violaceus* represent a type of PBS unique among the cyanobacteria so far examined. Nonetheless, they bear unmistakeable structural similarities to the PBS of red algae (Mörschel et al. 1977; Gantt 1980) and other cyanobacteria (Bryant et al. 1979; Glazer et al. 1979; Williams et al. 1980). All known PBS are built of rod-shaped elements formed by the coaxial stacking of hexamers of PC or of PC and PE which converge on a core of AP.

Acknowledgements. The authors are particularly grateful to Mr. S. Kouprach for the preparation of freeze-etched replicas and to the Balzers Company for lending a spray freezing apparatus. We thank Drs A. Ryter, L. G. Chevance and O. Croissant for the use of their electron microscopy facilities.

One of us (D.A.B.) was supported by a post-doctoral fellowship awarded in an exchange program of the National Science Foundation and the Centre National de la Recherche Scientifique and gratefully acknowledges the hospitality of the Unité de Physiologie Microbienne, Institut Pasteur.

This research was supported by grants from the Centre National de la Recherche Scientifique (E. R. A. 398) and the Délégation Générale à la Recherche Scientifique et Technique (décision d'aide N° 77-7-1751).

References

Bachmann L, Schmitt WW (1971) Improved cryofixation applicable to freeze-etching. Proc Natl Acad Sci (Wash.) 68:2149–2152

Bachmann L, Schmitt-Fumian WW (1973) Spray-freezing and freeze-etching. In: Freeze-etching techniques and applications. Benedetti EL, Favard F (eds). Société Française de Microscopie Electronique Paris pp 73–79

Branton D, Bullivant S, Gilula NB, Karnovsky MJ, Moor H, Mühlethaler K, Northcote DH, Packer L, Satir B, Satir P, Speth V, Staehelin LA, Steere RL, Weinstein RS (1975) Freeze-etching nomenclature. Science 190:54–56

Bryant DA, Guglielmi G, Tandeau de Marsac N, Castets AM, Cohen-Bazire G (1979) The structure of cyanobacterial phycobilisomes. Arch Microbiol 123:113–127

Butler WL (1978) Energy distribution in the photochemical apparatus of photosynthesis. Ann Rev Plant Physiol 29:345–378

Fujita Y, Shimura S (1974) Phycoerythrin of the marine blue-green alga *Trichodesmium thiebautii*. Plant Cell Physiol 15:939–942

Gantt L (1980) Structure and function of phycobilisomes: light harvesting pigment complexes in red and blue-green algae. Int Rev Cytol 66:45–80

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

Giddings TH, Jr, Staehelin LA (1978) Plasma membrane architecture of *Anabaena cylindrica:* occurrence of microplasmodesmata and changes associated with heterocyst development and the cell cycle. Cytobiol 16:235–249

Giddings TH, Jr, Staehelin LA (1979) Changes in thylakoid structure associated with the differentiation of heterocysts in the cyanobacterium *Anabaena cylindrica*. Biochim Biophys Acta 546:373–382

Gilleland HE, Stinnet JD, Roth IL, Eagon, RG (1973) Freeze-etching study of *Pseudomonas aeruginosa:* localization within the cell wall of an ethylenediamine tetraacetate-extractable component. J Bacteriol 113:417–432

Glazer AN, Bryant DA (1975) Allophycocyanin B (λ max 671, 618 nm): a new cyanobacterial phycobiliprotein. Arch Microbiol 104:15–22

Glazer AN, Williams RC, Yamanaka G, Schachman HK (1979) Characterization of cyanobacterial phycobilisomes in zwitterionic detergents. Proc Natl Acad Sci (Wash) 76:6162–6166

Golecki JR (1977) Studies on ultrastructure and composition of cell walls of the cyanobacterium *Anacystis nidulans*. Arch Microbiol 114:35–41

Golecki JR (1979) Ultrastructure of cell wall and thylakoid membranes of the thermophilic cyanobacterium *Synechococcus lividus* under the influence of temperature shifts. Arch Microbiol 120:125–133

Golecki JR, Drews G (1974) Zur Struktur der Blaualgen-Zellwand. Gefrierätzuntersuchungen an normalen und extrahierten Zellwänden von *Anabaena variabilis*. Cytobiologie 8:213–227

Haselkorn R (1978) Heterocysts. Ann Rev Plant Physiol 29:319–344

Lefort-Tran M, Cohen-Bazire G, Pouphile M (1973) Les membranes photosynthétiques des algues à biliprotéines observées après cryodécapage. J Ultrastruct Res 44:199–209

Ley AC, Butler WL, Bryant, DA, Glazer AN (1977) Isolation and function of allophycocyanin B of *Porphyridium cruentum*. Plant Physiol 59:974–980

Lichtlé C, Thomas JC (1976) Etude ultrastructural des algues à phycobiliprotéines, comparaison des résultats obtenus par fixation classique et cryodécapage. Phycologia 15:393–404

Mörschel E, Koller KP, Wehrmeyer W, Schneider H (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

Plattner H, Schmitt-Fumian WW, Bachmann L (1973) Cryofixation of single cells by spray-freezing In: Freeze-etching Techniques and Applications (Benedetti EL, Favard F (eds). Société Francaise de Microscopie Electronique Paris pp 83–100

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1973) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J gen. Microbiol 111:1–61

Rippka R, Waterbury JB, Cohen-Bazire G (1974) A cyanobacterium which lacks thylakoids. Arch Microbiol 100:419–436

Salton MRJ, Owen P (1976) Bacterial membrane structure. Ann Rev Microbiol 30:451–482

Staehelin LA, Giddings TH, Badami P, Krzymowski WW (1978) A comparison of the supra-molecular architecture of photosynthetic

membranes of blue green, red and green algae and of higher plants. In: Deamer DW (ed) Light transducing membranes, structure, function and evolution. Academic Press New York pp 335–355

Stanier RY, Cohen-Bazire G (1977) Phototrophic prokaryotes: The cyanobacteria. Ann Rev Microbiol 31:225–274

Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol Rev 35:171–205

Van Gool AP, Nanninga N (1971) Fracture faces in the cell envelope of *Escherichia coli*. J Bacteriol 108:474–481

Vaughan MH, Jr (1964) Structure and comparative studies of the algal protein phycoerythrin. Ph. D. Thesis, Boston: Massachusetts Institute of Technology

Williams RC, Gingrich JC, Glazer AN (1980) Cyanobacterial phycobilisomes. Particles from *Synechocystis* 6701 and two pigment mutants. J Cell Biol 85:558–566

Wollman FA (1979) Ultrastructural comparison of *Cyanidium caldarium* wild type and III-C mutant lacking phycobilisomes. Plant Physiol 63:375–381

Yamanaka G, Glazer AN, Williams RC (1978) Cyanobacterial phycobilisomes. Characterization of the phycobilisomes of *Synechococcus* sp. 6301. J Biol Chem 253:8303–8310

Received October 9, 1980