Biochimica et Biophysica Acta, 589 (1980) 30—45

BBA 47783

SUPRAMOLECULAR ORGANIZATION OF CHLOROSOMES (CHLOROBIUM VESICLES) AND OF THEIR MEMBRANE ATTACHMENT SITES IN *CHLOROBIUM LIMICOLA*

L. ANDREW STAEHELIN *, JOCHEN R. GOLECKI and GERHART DREWS

Biologisches Institut II, Lehrstuhl für Mikrobiologie, Albert-Ludwigs-Universtität, Schaenzlestrasse 1, D-78 Freiburg (F.R.G.)

(Received May 7th, 1979)

Key words: Chlorosome; Chlorobium vesicle; Photosynthetic bacteria; Binding site; (Freeze fracture)

Summary

The photosynthetic green bacterium *Chlorobium limicola* 6230 has been examined by freeze-fracture electron microscopy to investigate the size, form, distribution and supramolecular architecture of its chlorosomes (chlorobium vesicles) as well as the chlorosome attachment sites on the cytoplasmic membrane. The oblong chlorosomes that underlie the cytoplasmic membrane show a considerable variation in size from about 40 × 70 nm to 100 × 260 nm and exhibit no particular orientation. The chlorosome core, which appears to be hydrophobic in nature, contains between 10 and 30 rod-shaped elements (approx. 10 nm in diameter) surrounded by an unetchable matrix. The rod elements are closely packed and extend the full length of the chlorosome. Separating the chlorosome core from the cytoplasm is a approx. 3 nm thick lipid-like envelope layer, which exhibits no substructure. A 5—6 nm thick, crystalline baseplate connects the chlorosome to the cytoplasmic membrane. The ridges of the baseplate lattice make an angle of between 40° and 60° with the longitudinal axis of the chlorosome and have a repeating distance of approx. 6 nm. In addition, each ridge exhibits a granular substructure with a periodicity of approx. 3.3 nm. The cytoplasmic membrane regions adjacent to the baseplates are enriched in large (greater than 9 nm) intramembrane particles, most of which belong to approx. 10 nm and approx. 12.5 nm particle size categories. Each chlorosome attachment site contains between 20 and 30 very large (greater than 12.0 nm diameter) intramembrane particles.

The following interpretive model of a chlorosome is discussed in terms of

* Permanent address: Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309, U.S.A.

Abbreviation: BChl, bacteriochlorophyll.

biophysical, biochemical and structural information reported by others: it is proposed that the bacteriochlorophyll *c* (BChl *c*; chlorobium chlorophyll) is located in the rod elements of the core and that it is complexed with specific proteins. The cytoplasm-associated envelope layer is depicted as consisting of a monolayer of galactosyl diacylglycerol molecules. BChl *a*-protein complexes in a planar lattice configuration most likely make up the crystalline baseplate. The greater than 12-nm particles in the chlorosome attachment sites of the cytoplasmic membrane, finally, may correspond to complexes containing a reaction center and non-crystalline light-harvesting BChl *a*. The crystalline nature of the baseplate is consistent with the notion that it serves two functions: besides transferring excitation energy to the reaction centers it could also function as a distributor of this energy amongst the reaction centers.

Introduction

The green bacteria (Chlorobiineae) constitute a small group of photosynthetic bacteria that can be readily distinguished from other photosynthetic procaryotes (purple bacteria, cyanobacteria, and prochlorophyta) by the different organization of their cytoplasm [23,27]. In 1964, Cohen-Bazire et al. [7] reported that several strains of *Chlorobium* cells contain a cortical layer of electron-lucent, oblong vesicles, immediately underlying the cytoplasmic membrane. These structures, which they named 'chlorobium vesicles', were 30—40 nm wide and 100—150 nm long, and each was surrounded by a 3—5 nm thick, single-layered 'membrane'. On very rare occasions the authors were able to reveal 1.2—2 nm thick internal fibrils oriented parallel to the long axis to some of the vesicles. While Cruden and Stanier [8] were unable to detect any filamentous substructure in micrographs of isolated and negatively stained chlorobium vesicles, they did note that a fair number of their vesicles contained between 10 and 25 ring-like structures with a diameter of approx. 10 nm and a central hole of approx. 3 nm. However, no particular organization of these rings could be detected.

Chlorobium vesicles, which serve as light-harvesting 'super antennae', have also been found in *Chloropseudomonas* [14] and in *Pelodictyon* [25]. Holt et al. [14] demonstrated further that after mechanical disruption of *Chloropseudomonas* cells many chlorobium vesicles still adhered tightly to the cytoplasmic membrane. More recently Pierson and Castenholz [26] and Madigan and Brock [16] established that cells of the thermophilic, filamentous, gliding bacterium *Chloroflexus aurantiacus*, which belongs to the second physiological group of green bacteria, the Chloroflexaceae [23], also contain 'chlorobium-type' vesicles. Since the term 'chlorobium vesicle' seemed inappropriate for describing these structures in the Chloroflexaceae, Staehelin et al. [32] introduced the term 'chlorosome' (greek for green body) for these structures*.

* The name 'chlorosome' was coined at the Gordon Conference on The Biochemical Aspects of Photosynthesis-Electron Transport, Photophosphorylation and Membrane Structure in July, 1978, by an informal group of people interested in procaryotic photosynthetic membranes consisting of R.C. Fuller, E. Gantt, S. Kaplan, J.M. Olson and with the encouragement and input of many others. At the conference the term 'chlorobium vesicle' came under severe criticism because recent data have shown that it is a double misnomer: the structure is neither a classical vesicle bounded by a unit membrane, nor is it restricted to the genus *Chlorobium*.

We propose that the name chlorosome also be used for the chlorobium vesicles of the Chlorobiaceae, both because it reflects a unifying concept and because the term suggests a functional analogy with the phycobilisomes of cyanobacteria and red algae [13].

Chlorobium cells contain two distinct antenna chlorophylls: bacteriochlorophyll *c* (BChl *c*; chlorobium chlorophyll 660) and BChl *a*. The ratio of these antenna BChls to the reaction center BChl a_p (*P*-840) is approximately 1000—1500:80:1, i.e. an order of magnitude greater than in purple bacteria [12,27]. Biochemical analyses of isolated and purified chlorosomes [4,7,8,12,14] and of purified fractions of cytoplasmic membranes [4,5,8,12] have provided information on the location of the different chlorophylls within these cells. Thus, while BChl *c* appears to be located exclusively in the chlorosomes and the reaction center BChl in the cytoplasmic membrane, the cellular distribution of BChl *a* is not as clear. After French press disruption of cells a membrane fraction ('complex I') can be isolated that contains besides reaction centers significant amounts of bound antenna BChl *a* [12,19]. Most of the BChl *a* seems to be very tightly bound to the reaction centers since even after dissociation of complex I with 2 M guanidine HCl to produce 'complex II' there are still approx. 40 BChl *a* molecules per reaction center [19]. On the other hand, certain subfractions of chlorosomes may also contain some tightly bound BChl *a*. Thus biochemical fractionation studies provide supportive evidence for the hypothesis that BChl *a* occupies an intermediary position between BChl *c* and the reaction center.

In 1962 Olson and Romano [22] reported the isolation of a water-soluble BChl *a*-protein complex from cells of green bacteria. Subsequent fluorescence studies, [33,34] indicated that it functioned as an intermediary in the transfer of excitation energy from the main light-harvesting BChl *c* to the photochemical reaction center BChl a_p. The flow of excitation was therefore proposed to be: BChl *c* → antenna BChl *a* → reaction center BChl a_p. Since the transfer of the absorbed excitation energy to the reaction centers presumably requires a structural link between the chlorosomes and the cytoplasmic membrane, the intermediate position of the antenna BChl *a* in the fractionation experiments may reflect its physical location in the cell [21]. In this context, it is interesting to note that the major antenna BChl *a* is complexed with a protein, the BChl *a*-protein, which upon isolation can be readily crystallized from solution by the addition of ammonium sulfate [18].

This article demonstrates that freeze-fracture electron microscopy can be used to obtain a wealth of new information on the supramolecular organization of *Chlorobium* chlorosomes and of their membrane-attachment sites. The new structural observations confirm and extend our earlier findings on the chlorosomes of *C. aurantiacus* [32], and provide a much improved basis for structure-function correlations in this interesting photosynthetic system.

Materials and Methods

Cultures of *Chlorobium limicola* 6230 (German Collection of Microorganisms, Göttingen, DSM 249) were kindly provided by Professor Norbert Pfennig, Göttingen. The cells were grown anaerobically in screw cap bottles

in a medium described by Pfennig and Biebl [24] and Biebl and Pfennig [3] at a light intensity of approximately 150 lux provided by incandescence bulbs at 30° C. The cells were harvested in the exponential growth phase.

Electron microscopy. For thin sectioning the cells were fixed in the culture medium by adding 25% glutaraldehyde to a final concentration of 1%. After 60 min the cells were rinsed in a cacodylate buffer (0.05 M, pH 7.2) and post-fixed in 1% OsO_4 for 90 min before they were embedded by standard methods. Thin sections were mounted on Formvar-coated grids and stained with uranyl acetate [35] followed by lead citrate [28].

Cells to be used for freeze-fractured experiments were fixed by adding room temperature 25% glutaraldehyde to the cell cultures to a final concentration of 1%. After 30 min the cells were glycerinated by slowly (over a 1 h period) adding to an aliquot of the fixed culture an equivalent volume of a 60% glycerol/water solution. The glycerinated culture was allowed to stand for another hour before the cells were harvested by centrifugation and frozen on copper discs in liquid propane. To obtain replicas of unfixed cells an aliquot of the fresh culture was glycerinated and frozen identically to the fixed materials. Freeze-fracture replicas were prepared at −108° C in a Balzers apparatus using standard procedures. The replicas were cleaned on commercial bleach and a 40% CrO_3 solution. The membrane fracture faces are labeled according to the nomenclature of Branton et al. [6]. Biological membranes consist of a protoplasmic (P) leaflet and an exoplasmic (E) leaflet. The process of freeze-fracture splits membranes along their hydrophobic interior revealing two complementary fracture faces designated PF for P-face and EF for E-face. Each fracture face has a population of particles which represent intramembrane proteins or protein complexes. Particle size and density determinations were made as described by Staehelin [31].

Results

Our thin-section electron micrographs of *Chb. limicola* (Fig. 1a and b) depict the chlorosomes as oblong, lightly staining structures, delineated by a 3—5 nm thick envelope, and appressed to the cytoplasmic membrane, as first described by Cohen-Bazire et al. [7]. At higher magnification one can also detect a 5—6 nm thick layer interposed between the cytoplasmic membrane and the individual chlorosomes (Fig. 1b). This layer presumably corresponds to the baseplate of the chlorosome, a structure that can be seen more clearly in freeze-fracture micrographs (see below). Cross-fractured *Chlorobium* cells appear in many respects very similar to their thin-sectioned counterparts. In such cells the cross-fractured chlorosomes resemble small elongated lipid droplets that are insensitive to etching (Fig. 2). But like the thin-section images, the micrographs of cross-fractured *Chlorobium* cells provide relatively few significant insights into the architecture of the chlorosomes and of the associated areas of the cytoplasmic membrane. However, when the fracture plane passes along the cytoplasmic membrane from where it can be deflected into the attached chlorosomes, a wealth of new observations on the supra-molecular organization of chlorosomes and of their membrane attachment sites can be made (Figs. 3—13b).

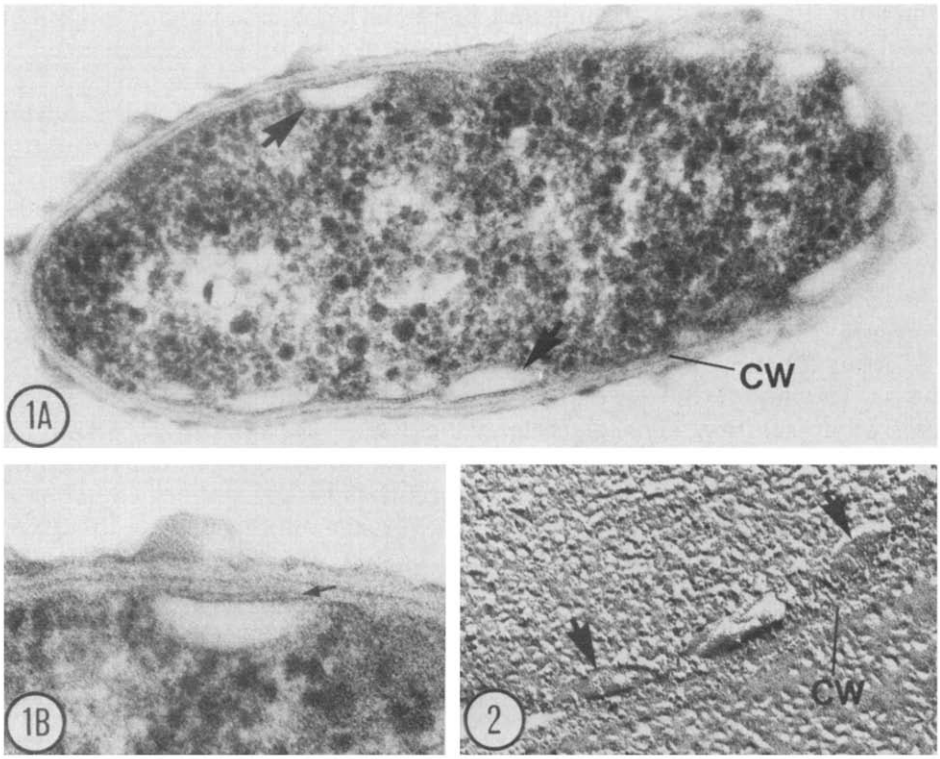


Fig. 1. (a and b) Thin section through a *Chb. limicola* cell showing several chlorosomes (chlorobium vesicles; large arrows) appressed to the cytoplasmic membrane. Inserts (b) and (c) depict selected chlorosomes and their membrane-attachment sites at higher magnification. Note the 5—6 nm thick layer (small arrow) between the chlorosome and the cytoplasmic membrane. Cell wall (CW) with extensions. Magnifications: (a) ×90 000; (b) ×180 000.

Fig. 2. Portion of a freeze-fractured *Chlorobium* cell exhibiting similar features as seen in the thin section image of Figure 1. The chlorosomes (arrows) appear as oblong, amorphous bodies underlying the cytoplasmic membrane and the cell wall (CW). Magnification ×125 000.

Fig. 3 depicts a *Chlorobium* cell that was fixed with glutaraldehyde before glycerination and freezing. A dense population of intramembrane particles covers the exposed P-face of the cytoplasmic membrane. In many places the fracture plane has passed from the P-face into the underlying chlorosomes to reveal either a smooth surface image of these structures or internal, rod-shaped elements in a longitudinal orientation (Fig. 3, arrows). In Fig. 4 most of the cytoplasm of the cell has been cleaved away exposing an E-face view of the plasma membrane as well as attached fragments of chlorosomes. The E-face carries relatively few particles as well as numerous small pits. The most striking feature of Fig. 4, however, are the numerous ellipsoidal, plate-like structures exhibiting a crystalline substructure: the baseplates of the chlorosomes. Of possible developmental and functional significance is the great variation in size both of the crystalline baseplates (35 nm × 70 nm—80 nm × 250 nm; Fig. 4) and of the oblong cores of the chlorosomes (40 nm × 75 nm—100 nm × 260

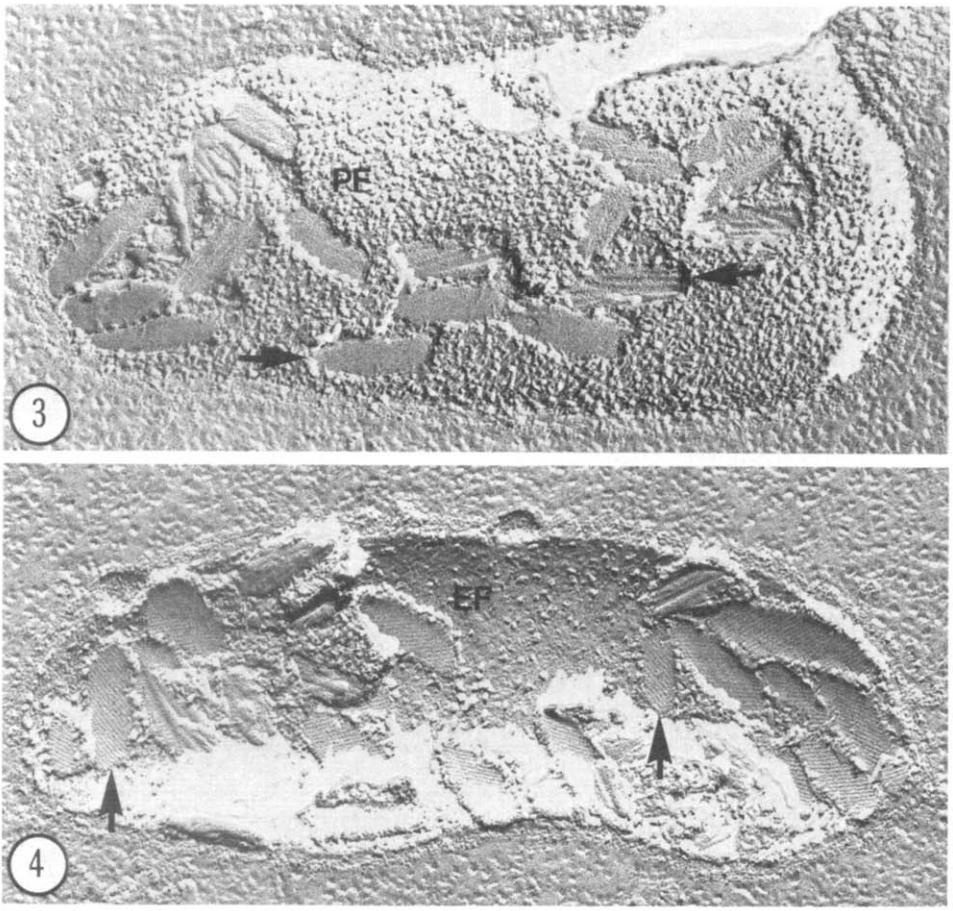


Fig. 3. Freeze-fractured *Chlorobium* cell fixed with glutaraldehyde and infiltrated with glycerol before freezing. The fracturing process has exposed both a P-face (PF) view of the cytoplasmic membrane, which is covered by numerous intramembrane particles, and underlying chlorosomes (arrows). The exposed surface of the core material of these elongated bodies appears smooth, while closely packed rod-shaped elements can be detected in their interior (see also Figs. 5 and 8). Magnification ×100 000.

Fig. 4. Freeze-fractured *Chlorobium* cell showing an E-face (EF) view of the cytoplasmic membrane and baseplates (arrows) of associated chlorosomes. The baseplates exhibit more or less distinct striations due to their crystalline substructure. Note the close packing of the baseplates on the right, and the great variation in their size. Magnification ×90 000.

nm; Fig. 5). No preferred orientation of the chlorosomes in the cells is observed.

Figs. 5—9 illustrate fine structural details of chlorosomes at higher magnification. In Fig. 5 chlorosomes are shown whose crystalline baseplates have been broken away during the fracturing process to reveal the adjacent, extremely smooth surface of the core material. The arrow in Fig. 5 points to the tip of a chlorosome that has been pushed against the side of another and thereby has produced a dent in the latter. Figs. 6 and 7 depict fracture faces of chlorosome baseplates that are complimentary in nature to the smooth surface fractures of the chlorosome cores shown in Fig. 5. The packing geometry of the closely

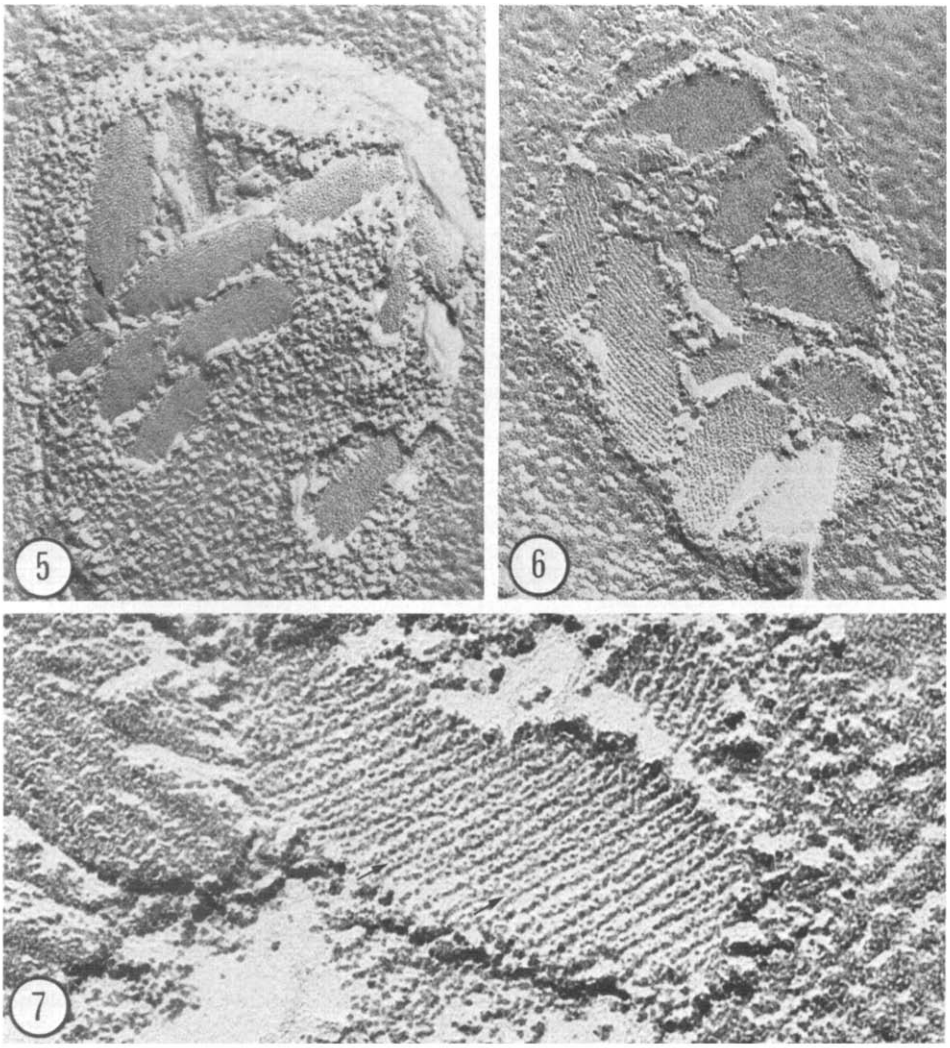


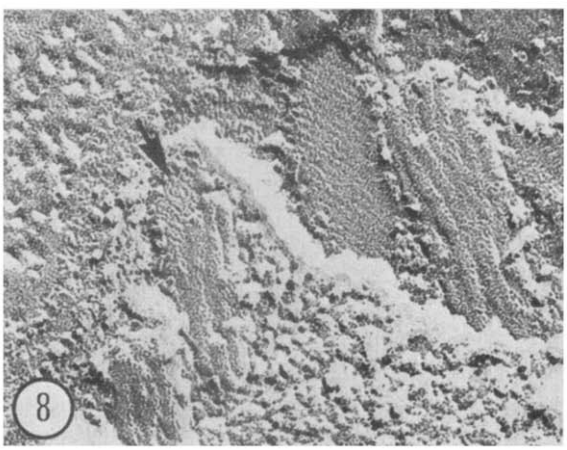
Fig. 5. P-face view of the cytoplasmic membrane and of underlying chlorosomes of a *Chlorobium* cell. The smooth fracture faces of chlorosomes as seen in this micrograph are produced when the crystalline baseplate is split away from the chlorosome core. This figure and Fig. 6 exhibit complementary-type fracture faces of chlorosomes. Variations in size and form of the chlorosomes are quite evident. The dent in the chlorosome marked by an arrow, and caused by the abutting tip of an adjacent one, suggests that the chlorosome core is deformable and not a rigid structure. Magnification X120 000.

Fig. 6. Chlorosome baseplates of a freeze-fractured *Chlorobium* cell. In living cells these thin, striated, plate-like structures are sandwiched between the chlorosome cores and the cytoplasmic membrane, presumably forming a functional link between the two structures. The fracture faces of the baseplates shown in this and the following figure are complementary in nature to the smooth faces of the core material seen in Fig. 5. Magnification X145 000.

Fig. 7. Micrograph of a highly magnified baseplate of a chlorosome in a freeze-fractured *Chlorobium* cell. The baseplate exhibits a crystalline substructure, the distinct ridges of which made an angle of between 40° and 60° with the longitudinal axis of the baseplate and which have a repeat distance of approx. 6 nm. Closer examination reveals further that each ridge possesses a regular, globular substructure with a periodicity of approx. 3.3 nm (small arrows). Magnification X360 000.

spaced baseplates as well as the variations in the orientation of their lattices is clearly seen in Fig. 6. The very low profile of the lattice components that give rise to the baseplate striations can be deduced from the fact that the visualization of these striations is highly sensitive to the shadowing angle. Thus, even the slight inherent curvature of the baseplates causes significant variations in the clarity with which the lattice structures can be recognized (Figs. 4,6 and 7). Fig. 7 depicts a very highly enlarged baseplate of a freeze-fractured *Chlorobium* cell in which the supramolecular organization of the lattice is demonstrated. Careful examination of the lattice image reveals that the ridges (periodicity approx. 6 nm) possess a distinct granular substructure with a periodicity of approx. 3.3 nm. Due to the inherent curvature of most of the baseplates we have not been able to use simple optical diffraction and reconstitution techniques to further enhance the resolution of the lattice structure. However, since fairly flat baseplates are occasionally found this type of analysis might become feasible in future studies.

A very clear image of the internal rod elements of a *Chlorobium* chlorosome and their relationship to a baseplate is presented in Fig. 8 (arrow). In this micrograph and in Fig. 3 and 4 the approximately 10-nm-thick rod elements appear closely packed and oriented parallel to the long axis of the chlorosomes. (The curved appearance of some of the rod elements seen in Fig. 8 can be traced to plastic deformation associated with the fracturing process.) Simple calculations indicate that, depending on its size, a chlorosome may contain between 10 and 30 rod elements. The fact that no rod elements can be detected at the interface between the core and baseplate (Fig. 5) and between the core and the envelope layer (Fig. 9) of chlorosomes, as well as the finding that etching does not change the appearance of cross-fractured chlorosomes,



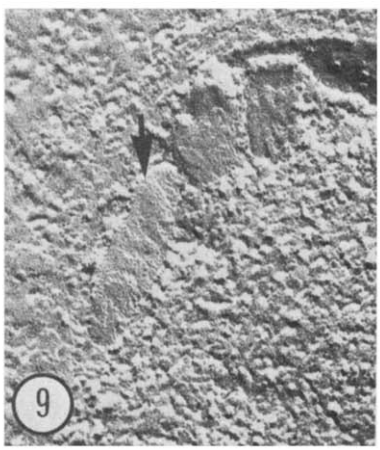
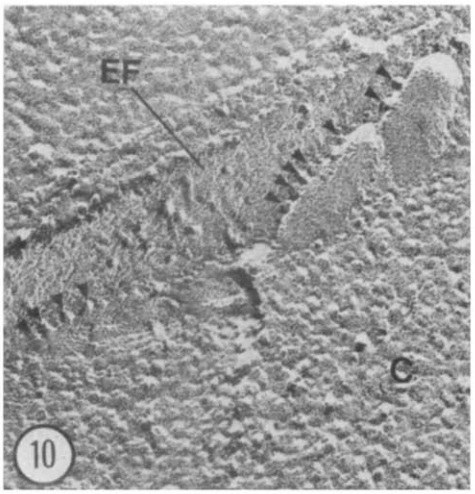


Fig. 8. Freeze-fractured chlorosomes illustrating the closely packed, parallel rod elements (diameter approx. 10 nm) and their relationship to the crystalline baseplate (arrow). The curved appearance of the rod elements of the chlorosome on the right is due to plastic deformation during the fracturing process. Magnification ×190 000.

Fig. 9. Chlorosome (arrow) fractured along its cytoplasm-associated envelope layer. This layer exhibits no discernible substructure and also lacks imprints of the underlying rod elements. Magnification ×150 000.



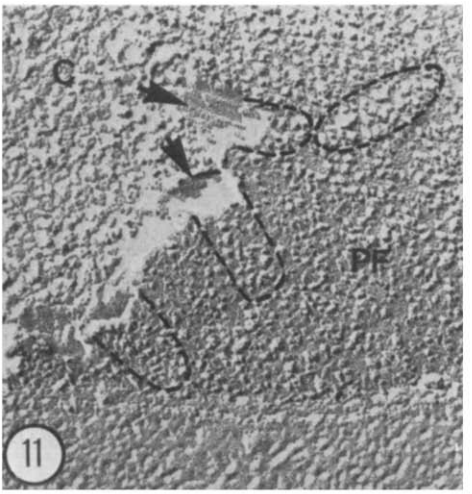


Fig. 10. Freeze-fractured chlorosomes and adjacent cytoplasmic membrane (EF) of a *Chlorobium* cell. Note the intramembrane particles (arrowheads) to which the chlorosomes appear to be attached. Cytoplasm (C). Magnification X160 000.

Fig. 11. Micrographs revealing a P-face (PF) view of the cytoplasmic membrane as well as cross-fractured cytoplasm (c) with membrane-associated chlorosomes (arrows). Where the chlorosomes underlie the cytoplasmic membrane a slightly different population of intramembrane particles (mostly larger than average) may be discerned. These differentiated membrane regions have been outlined. Magnification X115 000.

suggests that the rod elements in the core are surrounded by a matrix.

Our documentary evidence pertaining to the attachment of the chlorosomes to the cytoplasmic membrane is presented in Figs. 10—13b. Edge on views of chlorosomes and their membrane adhesion sites reveal large intramembrane particles in the contact zone (Fig. 10). Figs. 11—13b show that distinct elongated patches of P-face particles may be recognized on the fracture faces of the cytoplasmic membrane at the presumptive attachment sites of the chlorosomes. A particularly clear example of this type of membrane differentiation is pointed out by the arrow in Fig. 12a. The number of particles per site may vary from 20 to 45, the average being 32. Sites with many very large particles (13—16 nm in diameter) usually contain less than 30. Particle size measurements confirm the visual impression that these attachment sites contain a significantly higher percentage of large (greater than 9.5 nm in diameter) particles than the cytoplasmic membrane as a whole (compare Fig. 14a—c). As seen in Fig. 14b 62% of the particles in the chlorosome attachment regions are greater than 9.5 nm, while only 31% of the particles are greater than 9.5 nm in the surrounding areas (Fig. 14c). Since no significant differences in the size of the P- and E-face particles of unfixed and glutaraldehyde-prefixed *Chlorobium* cells were found, the measurements have been pooled in the histograms (Fig. 14a, b and d). As seen in Fig. 12a four distinct size categories of particles with peaks at approx. 6.5 nm, approx. 8 nm, approx. 10 nm and at approx. 12.5 nm may be recognized on the P-face of the cytoplasmic membrane. Of these the 12.5 nm size category seems to be characteristic for the chlorosome attachment sites, while most of the 6.5-nm and 8-nm particles appear to be located in the surrounding areas (compare Fig. 14b and c). The

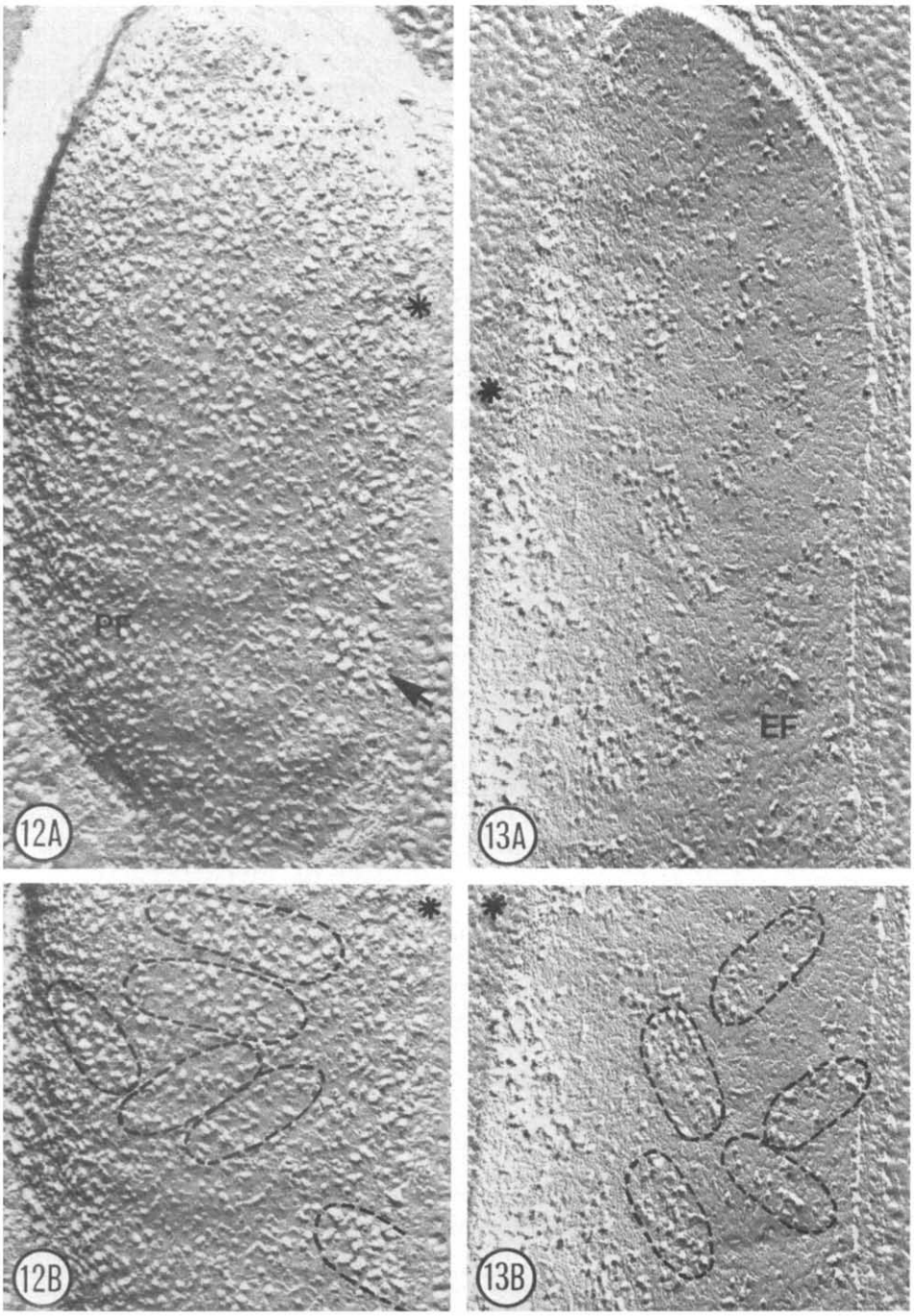


Fig. 12. (a and b) P-face (PF) of the cytoplasmic membrane of a glutaraldehyde-fixed and glycerinated *Chlorobium* cell. The membrane fracture face reveals elongated patches of mostly larger particles (arrow) corresponding to the attachment sites of the underlying chlorosomes. Some of these patches have been outlined in (b). The asterisks mark the same point of reference in both micrographs. Magnification X135 000.

Fig. 13. (a and b) E-face (EF) of the cytoplasmic membrane of a glycerinated but not chemically fixed *Chlorobium* cell. Several elongated patches enriched in particles and/or pits, some of which are outlined in (b), may be recognized against the relatively smooth background. *, reference points. Magnification X 115 000.

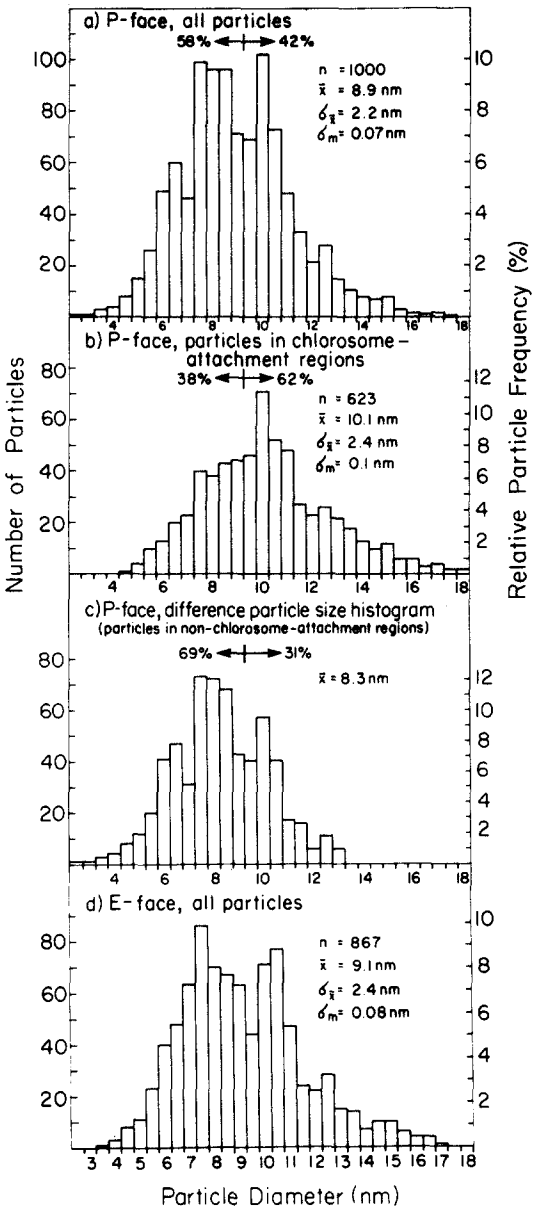


Fig.14. Particle size histograms of P- and E-faces of the cytoplasmic membrane of *Chb. limicola*. Measurements made on micrographs of both glutaraldehyde-prefixed and of unfixed cells have been pooled because no significant difference in particle sizes were observed between these two types of samples. For preparing the histogram (b) the measures were limited to very clearly delineated chlorosome attachment sites as marked in Fig. 12b. The difference particle size histogram (c) was calculated from histograms (a) and (b) assuming that the chlorosome attachment sites constitute 40% of the total plasma membrane surface area.

average density of P-face particles is 4229 ± 154/μm^2.

E-face images of the cytoplasmic membrane also reveal several interesting features (Figs. 13a, b and 14d). In unfixed cells (Fig. 13a) the E-faces tend to carry more particles (average 1586 ± 604/μm^2) than E-faces of glutaraldehyde-

prefixed cells (average 1240 ± 171/μm^2; not shown). The histogram Fig. 14d of E-face particle sizes is remarkable for its similarity with the histogram Fig. 14a of P-face particles. Besides having very similar mean values (8.9 nm vs. 9.1 nm), both histograms show peaks of comparable size at approx. 8 nm, approx. 10 nm and at approx. 12.5 nm.

Discussion

The wealth of new structural information on chlorosomes and their membrane attachment sites that can be derived from freeze-fracture studies of *Chlorobium* cells is quite remarkable considering the rather limited observations that can be made on thin-sectioned cells, and on isolated chlorosomes and fragments of cytoplasmic membranes examined after negative staining. It should also be noted that the structures we describe in this paper are consistently seen in all appropriately fractured samples.

Envelope layer of chlorosomes

The envelope layer of the chlorosomes adjacent to the cytoplasm is still very poorly defined. In thin sections it appears as a single dense line whose width may vary from 2 nm to 5 nm. Freeze-fracture micrographs depict the envelope layer as being very thin, lipid-like and devoid of a distinctive substructure. Olson et al. [21] suggest that all the chlorosome proteins are located in the envelope layer in the form of globular subunits with a diameter of about 3 nm. They also propose that the chlorosome interior is filled with a pool of approximately 10 000 BChl *c* molecules. In our model (Fig. 15) the cytoplasm-associated envelope layer is suggested to consist of a monolayer of monogalactosyl diacylglycerol molecules, which make up approximately 16% of the dry weight of a chlorosome [8]. This assignment is not only consistent with the freeze-fracture appearance of the envelope layer but also with the recent finding of Boyce [4] that when isolated chlorosome are exposed to the non-membrane permeating chemical label pyridoxal 5′-phosphate in the presence of borotritride, virtually no labeling of the chlorosomes occurs. This suggests that chlorosomes have no free amino groups accessible to the label on their surface. If the envelope layer consisted of proteins one would expect a significant amount of labeling to occur. The notion of having a monolayer of polar lipid molecules forming a boundary layer between the largely hydrophobic chlorosome core and the surrounding cytoplasm has an analogy in eucaryotic cells. Schwarzenbach [29] has found in developing seeds of *Ricinus* that during the differentiation of spherosomes into lipid droplets the triacylglycerol molecules accumulate between the inner and the outer layer of the unit membranes. This growth mechanism not only forces the two leaflets of the bilayer apart, but also insures that the surface of the lipid droplet is covered by a monolayer of phospholipid molecules.

Chlorosome core

The core of the chlorosome can be shown to contain between 10 and 30 rod-shaped elements with a diameter of approx. 10 nm. The closely spaced and longitudinally oriented rods are embedded in an unetchable matrix.

Whether the 10-nm rod elements are related to the 10 nm in diameter ring-like structures described by Cruden and Stanier [8] is unknown. However, the fact that the number of rings and rods per chlorosome as well as the diameter of the two structures coincides, suggests that there may be more than a casual relationship between the two. Could the rings represent initiating centers for the assembly of the rod elements? It is now well documented that both the assembly of bacterial flagella (reviewed by Ref. 15) and of microtubules (reviewed by Ref. 9) depends on such initiating structures.

Dry chlorosomes of *Chb. limicola* contain besides the already mentioned lipids, 28% BChl *c*, 33% protein and 15% carbohydrates [8]. Although we have at present no direct chemical information on the rod elements, the matrix and the baseplate of chlorosomes, we would like to make several proposals (summarized in the diagram Fig. 15) concerning the chemical nature of these structures based on the following observations. The chlorosome core is probably mostly hydrophobic in nature since in frozen cells it usually seems to offer less resistance to the cleaving forces generated by the freeze-fracture process than the adjacent cytoplasmic membrane. The general exclusion of

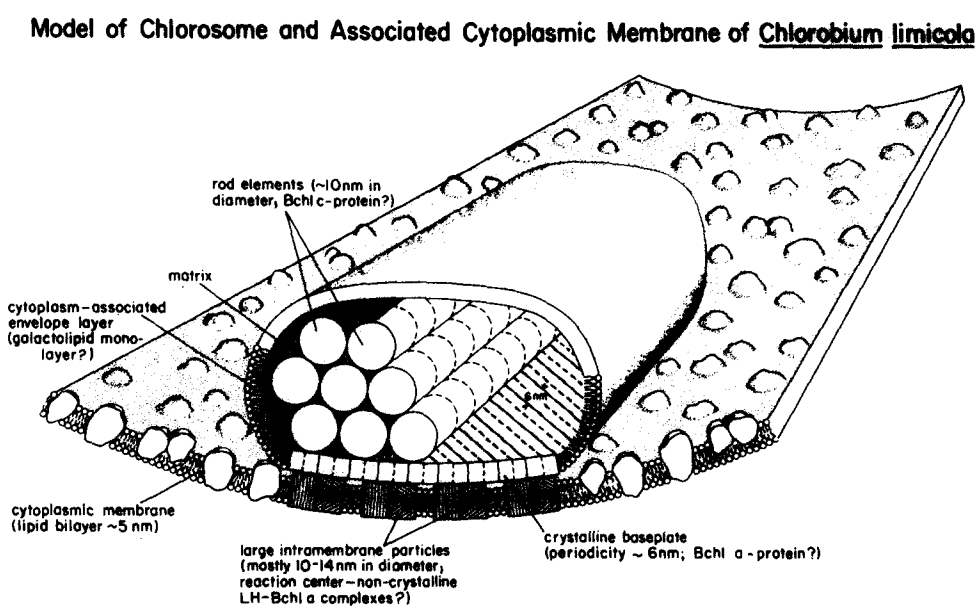


Fig. 15. Model of a chlorosome and its associated cytoplasmic membrane of *Chb. limicola* based on the freeze-fracture observations made in this study as well as on biochemical and biophysical studies of others [4,7,8,10,12,14,17,19,20,21,26]. The hydrophobic core of the chlorosome contains between 10 and 30 longitudinally oriented rod elements (approx. 10 nm in diameter) surrounded by matrix material. It is proposed that the rods contain BChl *c* complexed with specific proteins. The 2—4 nm wide envelope layer adjacent to the cytoplasm is poorly defined but may contain the monogalactolipid molecules in a monolayer configuration. A crystalline baseplate (5—6 nm thick; periodicity approx. 6 nm) provides the mechanical and presumably functional coupling between the light-harvesting components in the chlorosome core and the reaction centers in the cytoplasmic membrane. The crystalline nature of the baseplate and its intermediate location suggest that it consists of BChl *a*-protein complexes organized in the form of a lattice. The membrane attachment site of the chlorosome contains on the average about 30 large intramembrane particles that could repesent complexes consisting of a reaction center and tightly bound, non-crystalline antenna BChl *a*-proteins.

negative stains from the interior of the chlorosomes [14] also supports this notion. The fact that isolated chlorosomes can be stabilized by glutaraldehyde [8] as well as the precise shape and size of the rod elements leads us to suggest that the rod elements and probably the matrix contain proteins. Since the rod elements are the major component of the chlorosome core it seems not unreasonable to further propose that they also contain the BChl *c* molecules, probably complexed with specific proteins. No such complexes have been found to date. However, since all available evidence suggests that the core material must be rather hydrophobic in nature, the postulated core proteins would have to contain a high proportion of non-polar amino acids. That such proteins exist in nature can be seen from the fact that the dicyclohexylcarbodiimide binding protein of the ATPase of *Escherichia coli* contains approx. 85% non-polar amino acids [1,30]. We have no information that would allow us to speculate on the nature of the matrix material.

Membrane-attachment site of chlorosome

The boundary layer between the chlorosome and the cytoplasmic membrane is formed by a thin plate-like structure with a crystalline substructure, which we have termed the chlorosome baseplate (Fig. 15). Where the baseplate is attached to the cytoplasmic membrane the latter appears to have a different composition of integral membrane proteins as evidenced by the significantly higher proportion of large (greater than 9.5 nm in diameter) intramembrane particles on the P-face in these regions (compare Fig. 14a—c). It is tempting to speculate that the very large (greater than 12 nm in diameter) and distinct P-face particles located in these chlorosome attachment sites could be structural equivalents of photosynthetic units composed of a reaction center and non-crystalline light-harvesting BChl *a*. In this context it is interesting to note that in higher plant thylakoids the largest categories of intramembrane particles have been shown to consist of a Photosystem II core element and one, two or four aggregates of chlorophyll *a*/*b* light-harvesting complexes [2]. Olson et al. [21] have estimated that each chlorosome funnels energy into approximately ten reaction centers. Considering the many assumptions that had to be incorporated into the calculations this figure is remarkably close to the 20—30 very large P-face particles we find associated with each chlorosome attachment site.

Chlorosome baseplate

Both the physical location of the chlorosome baseplate between the BChl *c*-containing core and the reaction center-containing cytoplasmic membrane, and its crystalline nature are fully compatible with the notion that it consists of BChl *a*-protein complexes organized in the form of a planar lattice. As pointed out earlier, BChl *a*-protein complexes isolated without the use of detergents readily aggregate into crystals when they are precipitated from solution by the addition of ammonium sulfate [18]. Such crystals have been successfully examined by X-ray crystallographic techniques to determine the size and arrangement of the subunits and the molecular organization of the polypeptide chains and their associated BChl *a* molecules [10,11,17]. These studies have shown that the basic unit of the crystals is a trimer of molecular

weight 140 000 and that each of the identical subunits contains seven molecules of BChl *a*. The molecular dimensions estimated from the unit cell dimensions of the BChl *a*-protein crystals indicate that the space occupied by the trimer is roughly that of an oblate ellipsoid of revolution 5.7 nm along the short axid and 8.3 nm along the long axis. In this context the 6 nm periodicity of the crystalline baseplates of the chlorosomes appears remarkably similar to the 5.7 nm dimension of the trimer molecules along their three-fold axis. Future studies, however, will have to prove whether the two parameters are indeed related and whether the BChl *a*-proteins are organized into trimers in the baseplate. Since the approx. 6 nm thick baseplate is a two-dimensional and not a three-dimensional crystal, it is conceivable that the BChl *a*-protein molecules are arranged in a different configuration. On the other hand, Matthews et al. [17] have recently pointed out that the cyclic symmetry C_3 is the expected symmetry for close-packed, planar arrays, and that a second crystal form of the BChl *a*-protein has been found which contains close-packed layers.

As far as the functional significance of the two-dimensional lattice organization of the BChl *a*-protein complexes is concerned, we propose that this arrangement allows for optimal lateral transfer of excitation energy between the BChl *a*-protein complexes. Calculations of exciton interactions between the chlorophyll molecules of the BChl *a*-protein trimers [10] have shown that significant intersubunit interactions can occur between certain of the chromophores. Thus it seems not unreasonable to assume that such molecular interactions could also take place between adjacent BChl *a*-protein complexes in the baseplate lattice, particularly since excitation energy transfer over even greater distances must occur in the chlorosome core. This being the case, the baseplate could serve two functions: besides transferring excitation energy to the reactions centers it could also function as a distributor of this energy amongst the reaction centers.

Comparison with Chloroflexus chlorosomes

A comparison of the supramolecular architecture of the *Chlorobium* chlorosomes with those of *Chloroflexus* cells [32] reveals that in both systems the chlorosomes possess the same basic structural elements and organization, but that virtually all of the components have smaller dimensions in *Chloroflexus*. Thus, besides having a smaller overall size (on the average 106 × 33 × 12 nm, but variable among different strains [16,26]) the chlorosomes of *Chloroflexus* contain rod elements with a diameter of only 5.2 nm (vs. 10 nm for *Chlorobium*). The thickness of their envelope layer is only about 2 nm and the repeating distance of the baseplate striations 2.5—3.0 nm (vs. approx. 6 nm for *Chlorobium*). Finally, our most recent studies have revealed that the membrane-attachment sites of the *Chloroflexus* chlorosomes consist of arrays of much smaller than average intramembrane particles (approx. 5.5 nm; Staehelin, L.A., unpublished results) and not of larger than average particles as in *Chlorobium*. Future studies will have to show how these differences in size affect the functional parameters of these 'super antennae'.

Acknowledgements

Thanks are due to Dr. Clinton R. Fuller for numerous stimulating and helpful discussions on structure-function relationships in green bacteria, and to Drs. David P. Carter and Jean C. Linder for critically reading the manuscript. The technical assistance of Marcia DeWit in preparing the photographic plates is gratefully acknowledged. This work was supported by the Deutsche Forschungsgemeinschaft, an award by the Alexander von Humboldt Stiftung (to L.A.S.), and by grant GM 18639 from the National Institute of General Medical Sciences.

References

1 Altendorf, K. (1977) FEBS Lett. 73, 271—275
2 Armond, P.A., Staehelin, L.A. and Arntzen, C.J. (1978) J. Cell Biol. 73, 400—418
3 Biebl, H. and Pfennig, N. (1978) Arch. Mikrobiol. 117, 9—16
4 Boyce, C.D. (1978) Ph.D. Thesis, University of Massachusetts, Amherst, MA
5 Boyce, C.O.L., Oyewole, S.H. and Fuller, R.C. (1977) in Brookhaven Symposium on Biology No. 28 (Olson, J.M. and Hind, G., eds.), p. 365
6 Branton, D., Bullivant, S., Gilula, N.B., Karnovsky, M.J., Moor, H., Mühlethaler, K., Northcote, D.H., Packer, L., Satir, B., Satir, P., Speth, V., Staehelin, L.A., Steere, R.L. and Weinstein, R.S. (1975) Science 190, 54—56
7 Cohen-Bazire, G., Pfennig, N. and Kunisawa, R. (1964) J. Cell Biol. 22, 207—225
8 Cruden, D.L. and Stanier, R.Y. (1970) Arch. Mikrobiol. 72, 115—134
9 Dustin, P. (1978) Microtubules, Springer Verlag, Berlin
10 Fenna, R.E. and Matthews, B.W. (1977) in Brookhaven Symposium on Biology No. 28 (Olson, J.M. and Hind, G., eds.), pp. 170—182
11 Fenna, R.E., Matthews, B.W., Olson, J.M. and Shaw, E.K. (1974) J. Mol. Biol. 84, 231—240
12 Fowler, C.F., Nugent, N.A. and Fuller, R.C. (1971) Proc. Natl. Acad. Sci. U.S. 68, 2278—2282
13 Gantt, E. (1975) Bioscience 25, 781—790
14 Holt, S.C., Conti, S.F. and Fuller, R.C. (1966) J. Bacteriol. 91, 311—323
15 Iino, T. (1977) Annu. Rev. Genet. 11, 161—182
16 Madigan, M.T. and Brock, T.D. (1977) J. Gen. Mikrobiol. 102, 279—285
17 Matthews, B.W., Fenna, R.E., Bolognesi, M.C. and Schmid, M.F. (1979) J. Mol. Biol., in the press
18 Olson, J.M. (1972) Methods Enzymol. 23A, 636—644
19 Olson, J.M., Giddings, T.H. and Shaw, E.K. (1976) Biochim. Biophys. Acta 449, 197—208
20 Olson, J.M., Koenig, D.F. and Ledbetter, M.C. (1969) Arch. Biochem. Biophys. 129, 42—48
21 Olson, J.M., Prince, R.C. and Brun, D.C. (1977) in Brookhaven Symposium on Biology No. 28 (Olson, J.M. and Hind, G., eds.), pp. 238—246
22 Olson, J. M. and Romano, C.A. (1962) Biochim. Biophys. Acta 59, 726
23 Pfennig, N. (1977) Annu. Rev. Microbiol. 31, 275—290
24 Pfennig, N. and Biebl, H. (1976) Arch. Mikrobiol. 110, 3—12
25 Pfennig, N. and Cohen-Bazire, G. (1967) Arch. Mikrobiol. 59, 226—236
26 Pierson, B.K. and Castenholz, R.W. (1974) Arch. Mikrobiol. 100, 5—24
27 Pierson, B.K. and Castenholz, R.W. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 179—197, Plenum Publishing Corp
28 Reynolds, E.S. (1963) J. Cell Biol. 17, 208—212
29 Schwarzenbach, A.M. (1971) Cytobiol. 1, 145—147
30 Sebald, W. and Wachter, E. (1978) in 29th Mosbacher Colloquium on Energy Conservation in Biological Membranes (Schafer, G. and Klingenberg, M., eds.), pp. 228—236, Springer Verlag, Berlin-Heidelberg-New York
31 Staehelin, L.A. (1976) J. Cell Biol. 71, 136—158
32 Staehelin, L.A., Golecki, J.R., Fuller, R.C. and Drews, G. (1978) Arch. Mikrobiol. 119, 269—277
33 Sybesma, C. and Olson, J.M. (1963) Proc. Natl. Acad. Sci. U.S. 49, 248—253
34 Sybesma, C. and Vredenberg, W.J. (1964) Biochim. Biophys. Acta 88, 205—207
35 Watson, M.L. (1958) J. Biophys. Biochem. Cytol. 4, 475—478