Biochimica et Biophysica Acta, 1099 (1992) 271–274

BBABIO 40259

Rapid Report

High degree of organization of bacteriochlorophyll *c* in chlorosome-like aggregates spontaneously assembled in aqueous solution

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(Received 16 January 1992)

Key words: Chlorosome; Green photosynthetic bacterium; Light-harvesting antenna; Bacteriochlorophyll *c*; Linear dichroism; Pigment aggregate

Pigment-lipid aggregates were formed in aqueous solution by diluting a chloroform/methanol extract of chlorosomes of the green photosynthetic bacterium, *Chlorobium limicola*. The aggregates showed absorption and fluorescence spectra very similar to those of intact chlorosomes. No proteins were detected in the aggregates. Electron micrographs showed that the pigment-lipid aggregates were ellipsoidal bodies with average size of 130 nm along the long axis and 86 nm along the short axis. The linear dichroism spectrum of bacteriochlorophyll *c* in the pigment-lipid aggregates oriented in a stretched polyacrylamide gel was as strong as that in chlorosomes. These results suggest that spontaneous assembly of the protein-free pigments and lipids extracted from chlorosomes restores not only direct chromophore-chromophore interactions of bacteriochlorophyll *c* molecules but also the chlorosome-like higher-order structures.

Chlorosomes are light-harvesting antenna bodies attached to the inside of the cytoplasmic membrane in green photosynthetic bacteria. They are approx. 100 nm long, 30 nm wide, 12 nm thick in *Chloroflexus aurantiacus* and are somewhat larger in Chlorobiaceae [1–3]. A large amount of bacteriochlorophyll (BChl) *c* (or *d* or *e*) is contained in the chlorosomes, together with proteins, lipids and small amounts of BChl *a* and carotenoids [4]. The function of the chlorosome is to trap light energy and to transfer this energy to the reaction centers in the cytoplasmic membrane.

The organization of BChl *c* in chlorosomes has recently been suggested to be based on chromophore–chromophore interactions among BChl *c* molecules without any participation of proteins [5–10]. BChl *c* oligomers obtained in some non-polar solvents possess spectroscopic properties similar to those of chlorosomes, and are considered to be a good model for BChl *c* in chlorosomes [5–8]. Griebenow and Holtzwarth [9,10] reported that whole proteins can be extracted from *Chloroflexus* chlorosomes with SDS without changing the spectral properties of the BChl *c*. The reversible conversion of the aggregated spectral form of BChl *c* to the monomeric form by hexanol in intact chlorosomes further supported the absence of specific BChl-*c*-binding proteins [11]. Protein localization in chlorosomes also gave no evidence for BChl *c*–protein interactions [12].

Reconstitution of chlorosomes from their components has not yet been reported, but is obviously important for elucidation of the details of structure and function of chlorosomes. In this paper we report the spontaneous formation of chlorosome-like aggregates in aqueous solution from protein-free extracts of chlorosomes from *Chlorobium limicola*. These aggregates resemble intact chlorosomes in terms of microscopic appearance, absorption and fluorescence spectra and well-ordered orientation of excitation transition moment of BChl *c*.

C. limicola was grown as described in Ref. 9. Chlorosomes were prepared by using 2 M sodium thiocyanate according to Gerola and Olson [13]. Lipids and pigments of chlorosomes were extracted with chloroform/methanol/water (1 : 1 : 2), and the chloroform

Abbreviation: BChl, bacteriochlorophyll.

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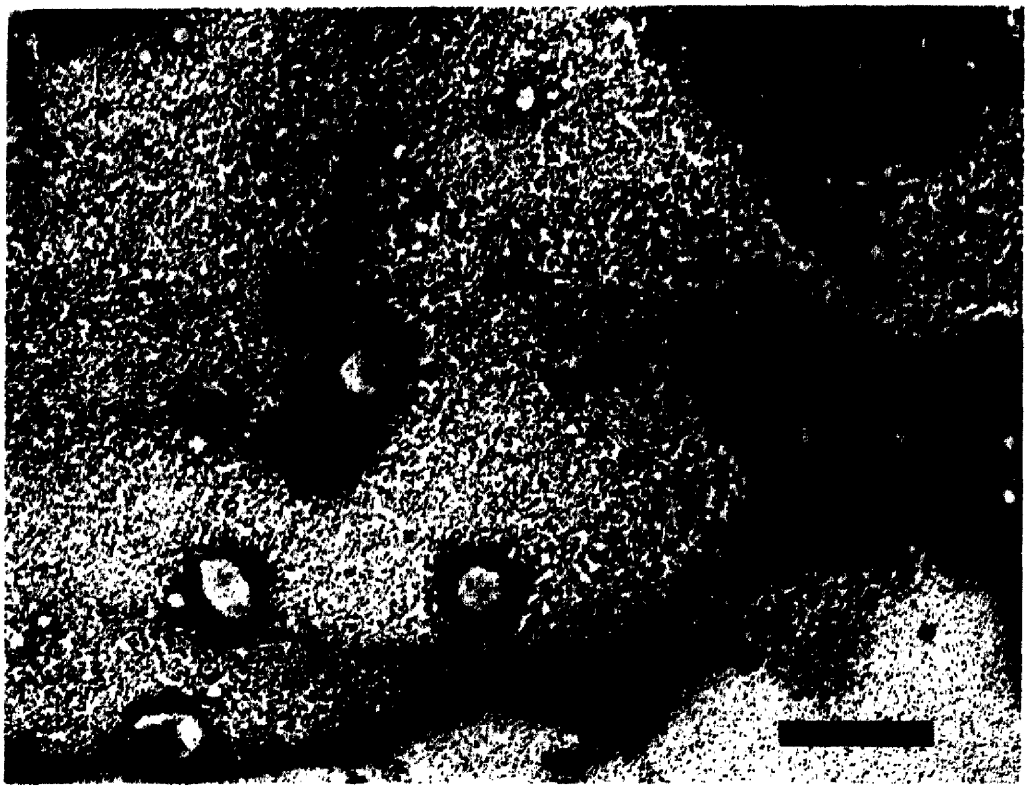

Fig. 1. The electron micrograph of pigment-lipid aggregates stained negatively. The pigment-lipid aggregates formed as described in the text was collected by ultracentrifugation (200000 × g, 20 min) and suspended in a small volume of buffer. The aggregate were negatively stained on the collodion membrane grid (150-B mesh, Ohken) with 4% phosphotungstic acid (pH 7.0). A Hitachi H-300 electron microscope was used. The bar represents 500 nm.

phase after centrifugation was dried and dissolved in a small amount of methanol. This fraction contained all of BChl *c*, BChl *a* and carotenoids in chlorosomes judging from the absorption spectrum. It is also likely that all of monogalactosyl diacylglycerol molecules which were reported to be present at 18% of the dry weight of chlorosomes of *C. limicola* [14] are contained in this extract. 10 μl of the pigment-lipid mixture in methanol was rapidly mixed in 3 ml of 10 mM potassium phosphate buffer (pH 7.2) at room temperature. The particles formed can be sedimented by ultracentrifugation (200000 × g, 20 min).

Fig. 1 shows an electron micrograph of the pigment-lipid aggregates negatively stained by phosphotungstic acid. Round and elliptical particles of various sizes ranging from 40 nm to 400 nm were observed. Examination of 360 particles in several micrographs revealed the particles to have average dimensions of 150 nm by 86 nm. Their shape and the size seem to correspond to those of intact chlorosomes, despite their rather globular shape and wide size distribution.

The absorption spectrum of the aqueous aggregates is remarkably similar to that of intact chlorosomes (Fig. 2). The absorption maximum of BChl *c* in intact chlorosomes was 750 nm, and that of the pigment-lipid aggregates was at 740 nm. The small peak at 670 nm may be due to 'monomeric' form of BChl *c* in the aqueous aggregates. The shoulder at 510 nm belongs to carotenoid.

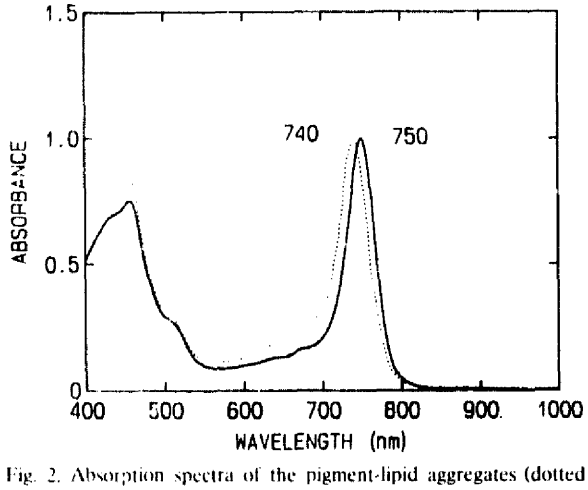

Fig. 2. Absorption spectra of the pigment-lipid aggregates (dotted line) and intact chlorosomes isolated from *C. limicola* (solid line). The aggregates and chlorosomes were suspended in 10 mM potassium phosphate buffer (pH 7.2). The two spectra have been normalized at the near infrared peaks.

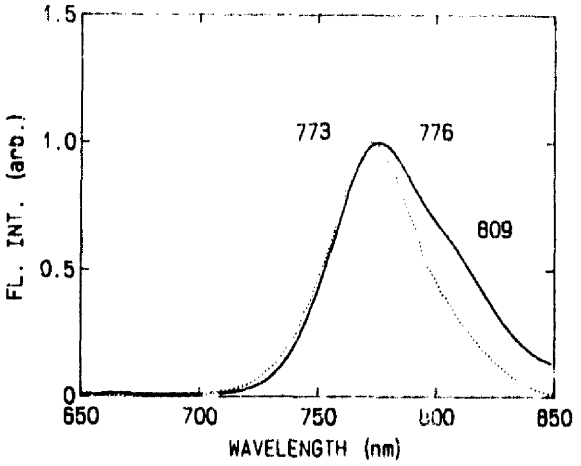

Fig. 3. Fluorescence emission spectra of the pigment-lipid aggregates (dotted line) and intact chlorosomes from *C. limicola* (solid line). The aggregates and chlorosomes were suspended in 10 mM potassium phosphate (pH 7.2) supplemented with a small amount of sodium dithionite. Fluorescence was excited in the blue wavelength region (380–540 nm) through a broad band path filter (Corning 4–96). The two spectra have been normalized at the peaks.

Fluorescence emission spectra (Fig. 3) were measured in the presence of sodium dithionite. Dithionite increased the fluorescence intensity by about 20-fold in intact chlorosomes [15] and also by about 10-fold in the pigment-lipid aggregates. The emission spectrum in the aggregates (solid line) was similar to that of the intact chlorosomes (dashed line) except for the BChl *a* shoulder around 809 nm. This shoulder was not observed in the pigment-lipid aggregates, probably because the baseplate BChl *a* was not reconstituted, and BChl *a* which was present in a small amount in the aggregate suspension is not energetically coupled to the aggregated BChl *c*. The difference in emission maxima was only 3 nm, 776 nm in chlorosomes and 773 nm in the pigment-lipid aggregates, in spite of the 10 nm difference in the absorption maxima of BChl *c*. This similarity in fluorescence suggests that the main component responsible for the emission in the aggregates is almost identical to that in chlorosomes, although there seem to be more shorter-wavelength components in the aggregates than in chlorosomes judging from the 10 nm difference in the absorption spectra (Fig. 2). This means that the excitation energy is transferred efficiently from the shorter-wavelength components to the longer-wavelength component in the pigment-lipid aggregates.

The pigment lipid aggregates did not contain any proteins as indicated by analysis after SDS polyacrylamide gel electrophoresis. No protein bands were detected in the pigment-lipid aggregates at the same BChl *c* content as that in the intact chlorosomes which gave a densely stained band at 6.3 kDa and more than five minor bands (data not shown). The absence of proteins in the aqueous aggregates suggests that BChl *c* and lipids can make chlorosome-like particles by self-aggregation without proteins. This is consistent with the finding by Griebenow and Holtzwarth [9,10] that all proteins can be extracted from chlorosomes with SDS without affecting the fundamental properties of chlorosomes.

It has been reported that BChl *c* alone forms aggregates in non-polar solvents which resemble chlorosomes in their absorption and fluorescence spectra [5–8]. In our preliminary experiments, aqueous aggregates from purified BChl *c* instead of the crude extracts did not give a chlorosome-like absorption band around 740–750 nm. When phospholipids were added to purified BChl *c*, however, the 740 nm absorption band appeared in aqueous aggregates (studies in progress). We suggest, therefore, that amphipathic lipids are indispensable for the chlorosome-like aggregation in aqueous solution. Since the absorption spectrum of BChl *c* aggregates in nonpolar solvents is almost the same as that of the aqueous aggregates shown in this study, the porphyrin rings of BChl *c* in the aqueous aggregates may be located in the hydrophobic environment formed by the amphipathic lipids.

In intact chlorosomes, it has been shown that BChl *c* molecules are highly ordered, i.e., the Q_y transition of BChl *c* is almost parallel to the long axis of chlorosomes [16–19]. Since the pigment-lipid aggregates have also ellipsoidal shape like intact chlorosomes (Fig. 1), linear dichroism was measured in oriented aggregates. The pigment-lipid aggregates embedded in a polyacrylamide gel were unidirectionally oriented by compression of the gel [16–18]. Absorption spectra were measured with linearly polarized light beams parallel and perpendicular to the stretched direction of the gel (Fig. 4). The absorbance at 740 nm with the parallel beam was about 5-times larger than that with the perpendicular beam. This observation indicates that the Q_y transition moment of BChl *c* is almost parallel to the long axis of the pigment-lipid aggregates, as it is in intact chlorosomes.

Miller and Olson (unpublished data) have observed that pigment-lipid aggregates can also be formed by diluting extracts of whole cells of the green filamentous bacterium, *Chloroflexus aurantiacus*. The properties of the aggregates from *Chloroflexus* are very similar to those from *Chlorobium* described in this paper.

From these results, we conclude that the spontaneous aggregates in aqueous solution from the crude pigment-lipid extracts of chlorosomes restored not only the direct chromophore–chromophore interaction of BChl *c* molecules but also higher-order structure in the ellipsoidal bodies. A possible model for the structure of an aggregate to explain the presented observations is a bundle of cylindrical micelles [20] of BChl *c* surrounded by a monolayer of lipids, in which Q_y

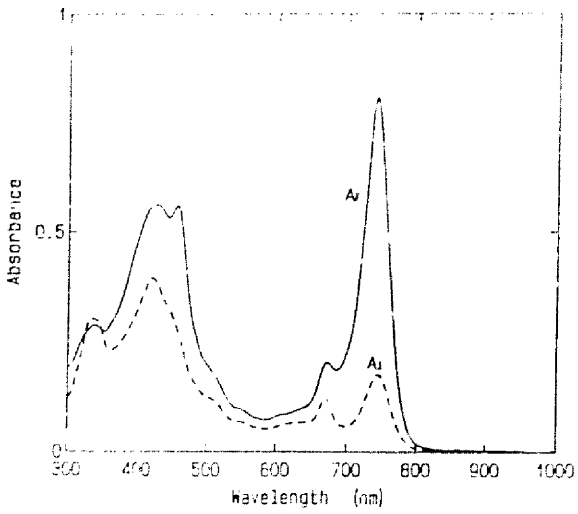

Fig. 4. Absorption spectra of the pigment lipid aggregates unidirectionally oriented in a stretched polyacrylamide gel with linearly polarized beams parallel ($A_{\parallel}$) and perpendicular ($A_{\perp}$) to the stretched direction. 12% acrylamide was polymerized in a form of a cube (10 × 10 × 10 mm) in the presence of the pigment-lipid aggregates, 50% glycerol, and 5 mM potassium phosphate (pH 7.2). The gel was compressed in a special holder from one direction allowing it to stretch in another direction until it took the form 5 × 20 × 10 mm. The spectra were measured through the third direction with the length of 10 mm.

transition of BChl *c* is almost parallel to the long axis. The highly oriented organization of BChl *c* in intact chlorosomes may result from similar spontaneous self-aggregation of BChl *c*. Further studies on the pigment-lipid aggregates in aqueous solution are in progress to clarify the details of organization of pigments and other components in chlorosomes.

We thank Dr. Isao Uemura for his help in electron microscopy, and Dr. Mamoru Mimuro for valuable suggestions and discussions. This study was supported by a grant from the Ministry of Education, Science and Culture of Japan. M.M. and J.M.O. were supported by the Danish Natural Sciences Research Council.

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