A Photosynthetic Antenna System which Contains a Protein-Free Chromophore Aggregate

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The interior of chlorosomes, the main antenna system of the photosynthesizing bacterium *Chloroflexus aurantiacus*, is shown to contain no proteins in a fixed ratio to BChl *c* and in amounts that could be significant of direct chromophore-protein complexes. This excludes non-covalent chromophore-protein complexing – that has so far been found in all other antennae – as the main organizational principle of the interior architecture for chlorosomes of chlorophyll *C. aurantiacus*. Rather, these antennae constitute the first case of a chromophore-chromophore aggregate functioning as a photosynthetic light harvesting system.

Introduction

Photosynthetic organisms contain chromophores organized in so-called antenna complexes which harvest the light energy and transfer it to the photosynthetic reaction centers. For quite some time it has been common belief in photosynthesis research that direct binding of the chromophores to protein – either covalently as in phycobiliproteins [1] or non-covalently as with chlorophylls [2] – is *the* organizational principle used by nature in all photosynthetic antennae. This principle seemingly has been found to be abided by all antenna complexes studied so far [3, 4]. The notion of chromophore-protein binding replaced an earlier hypothesis that antenna complexes are formed by direct chromophore-chromophore aggregation without requiring a protein matrix [5, 6].

The proteins in photosynthetic antennae serve several functions. One of the most important is the optimum positioning of the chromophores to ensure both efficient energy transfer and sufficient separation to preclude excited-state self-quenching.

Chlorosomes, the main light-harvesting antennae of the green bacteria Chlorobiaceae and Chloroflexaceae [7], are extra-membranous vesicles assembling as their chromophores up to 16,000 BChl *c* along with smaller amounts of carotenoids and BChl *a* molecules [8]. It has generally been assumed that chlorosomes represent just another class of chromophore-protein complexes. And indeed, Feick *et al.* [9] identified a protein – later shown by sequence analysis to have a molecular weight of 5.6 kDa [10] – which was assigned the role of the BChl *c*-binding protein. Wechsler *et al.* [10] suggested a model of supramolecular chromophore-protein organization to form the rod-like elements detected in electron micrographs [11] of chlorosomes, assuming 5–8 BChl *c* molecules per protein monomer.

We have recently found the protein content to vary considerably with the chlorosome preparations [12]. We report now on an investigation aimed at characterizing the protein composition and to determine the chromophore-protein ratio in intact chlorosomes isolated from *Chloroflexus aurantiacus* by various procedures and at various stages of purification. An important facet is the comparison of BChl *a*-free and BChl *a*-containing chlorosomes.

Abbreviations: BChl, bacteriochlorophyll; GEF, gel-electrophoretic filtration; LDS, lithium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; SDGC, sucrose density gradient centrifugation.

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Results and Discussion

BChl *a*-free chlorosomes were prepared by GEF with 0.1–5% LDS [13]. The BChl *a*-containing chlorosomes were isolated by SDGC after incubation with one of various detergents, miranol, deriphat-160, lauryldimethylamine N-oxide, or dode-

cyl β-D-maltoside. The membranes were incubated with the detergent at 4 °C in the dark under slow stirring and layered on a 10–40% (w/v) sucrose gradient. The chlorosome band was subjected to a second SDGC using a 20–40% gradient [12]. The isolated entities were characterized by PAGE combined with an improved and highly sensitive method [12] of silver staining.

Recoveries typically of 10–30% of the total BChl *c* were estimated for both isolation procedures. This may seem a relatively small percentage, but it is in the range also for other reported procedures of chlorosome isolation. The excellent agreement in the properties of all our preparations (see below) assures that the isolated fractions are representative of the standard chlorosomes rather than merely of some non-standard sub-population.

Fig. 1 shows a typical PAGE result, comparing chlorosomes with and without BChl *a* from different purification stages. All chlorosome samples were adjusted to the same BChl *c* content. The putative BChl *c*-binding protein(s) therefore should appear on all gel traces with the same or at least very similar intensities, which in fact is clearly not the case. Four main proteins (A–D, Fig. 1) are discernible in the low-molecular weight region of some samples but not in all. None of the proteins appears with constant intensity throughout the sequence of purification steps. Rather, the content of these proteins is drastically reduced with increasing purity. This is true also for the 5.6-kDa component (protein D). In the most highly purified GEF chlorosomes the 5.6-kDa protein was not detected at all by the staining method, the minimal sensitivity of which had been calibrated with authentic 5.6-kDa protein [10] to be better than 1 ng protein per band. This exclusion of specific proteins (A–D) is especially significant with regard to the question whether proteins are contained in the interior of the chlorosomes.

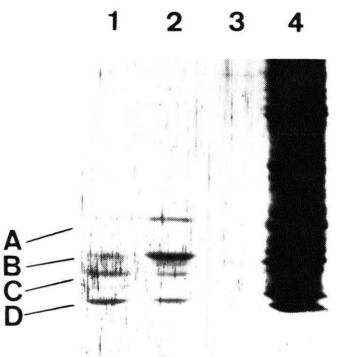

Fig. 1. PAGE (conventional 1 mm gel, 15% acrylamide) of BChl *a*-containing chlorosomes, of BChl *a*-free GEF chlorosomes, and of cytoplasmic membrane with attached chlorosomes. Lane 1: BChl *a*-containing chlorosomes after purification with dodecyl β-D-maltoside; lane 2: BChl *a*-containing chlorosomes after purification with miranol; lane 3: GEF preparation; lane 4: membranes. The samples in lanes 1–3 were adjusted to 25 nmol BChl *c*/ml, the membrane preparation to 20 nmol BChl *c*/ml. Samples of 50 µl were used for each lane. Note that the 5.6-kDa protein (D) is overloaded in the membrane preparation at this concentration (lane 4) and is readily detectable together with a large number of other proteins. Lanes 1 and 2 show samples where proteins (A, 17 kDa; B, 13 kDa; C, 12.5 kDa; D, 4–5 kDa) could be detected by PAGE and silver staining; in the GEF samples other than that in lane 3 often no proteins at all were found. Protein patterns and molecular weights of the individual protein bands in lanes 1 and 2 are compatible with the patterns and values reported previously (*e.g.*, Feick and Fuller [9]).

Very conservative estimates for our samples give BChl *c*/protein ratios at least five times higher than previously assumed*, which in terms of constructing complex chromophore-protein association models seriously aggravates the difficulties already encountered with 5–8 BChls/5.6-kDa protein [9].

We are thus forced to conclude that none of the proteins still present – albeit in very small amounts in most preparations – are functional in BChl *c* binding with the chlorosomes. In particular the 5.6-kDa protein must be excluded as a BChl *c*-binding protein. The small but varying amounts of protein still detectable in isolated chlorosomes either are contaminations from the cytoplasmic membrane or serve in the monolayer membrane enveloping the chlorosome some organizational

* The estimate is based on the sensitivity of silver staining in Pharmacia PhastSystem PAGE analyses of GEF chlorosomes [12, 13] as follows: assuming a 5–8:1 ratio of BChl *c* to 5.6-kDa protein [9], a sample containing 5–8 nmol/ml BChl *c* should contain 1 nmol/ml (= 5.6 µg/ml) of the protein. Since 1 µl of such a sample solution is used per lane, the maximum concentration of BChl *c* per lane needed for detection of the 5.6-kDa protein, would be 1–2 nmol/ml, given the assumed ratio. However, although the staining sensitivity is 1 ng protein per individual protein band, the 5.6-kDa or any other protein has normally not been detected in the GEF samples even at higher concentrations, *viz.* 10 nmol/ml BChl *c* (hence a lower limit for the BChl *c*:5.6-kDa-protein ratio of 25–40:1).

function other than direct BChl *c* binding in the interior. If the latter were true, partial removal of such proteins by the detergent would explain the variation found in the chromophore-protein ratio. Nevertheless, it might well leave the interior chlorosome structure intact.

Indeed, both the BChl *a*-free and BChl *a*-containing chlorosome preparations fulfilled all criteria which have been accepted in the literature to establish the specific structural and functional integrity of intact chlorosomes. In particular we ruled out the possibility that the chlorosomes had accidentally been disintegrated and bacteriochlorophyll-containing micelles had been formed instead: (i) As described previously, the 740-nm absorption band of BChl *c* in functional chlorosomes was fully retained [13]. (ii) Stationary fluorescence confirmed that energy transfer from the carotenoids to BChl *c* was still operative [12, 13]. (iii) Time-resolved fluorescence of BChl *a*-free and BChl *a*-containing preparations indicated that both the lifetimes of BChl *c* and the energy transfer processes between different BChl *c* pools remained essentially intact [14, 15]. Two additional observations have further confirmed the intact chlorosomal nature of our preparations: both types of preparations were characterized (iv) in their electron micrographs (negative staining) by unaltered dimensions typical of intact chlorosomes (*ca.* 30 × 100 nm) [16], and (v) in their linear dichroism by identical orientations of the chromophores [17].

It is appropriate to note here that Olson and collaborators [18] recently concluded that on treatment with LDS the chlorosomes remained intact as they obviously also do under our conditions of isolation.

Our biochemical evidence shows for the first time that chromophore-protein complexes as the exclusive building blocks of antenna pigments can no longer be upheld as a dogma. Rather, the organizational principle of chlorosomes – at least in the case of the functional antenna from *C. aurantiacus* – appears to be direct chromophore-chromophore interaction, presumably leading to large units of BChl *c* aggregates. It is well documented [6, 19, 20] that BChl *c* in non-polar solvents forms aggregates with absorption spectra very similar to those of BChl *c* in chlorosomes. Furthermore, Raman [21] and FTIR spectra [22] of BChl *c* indicate similar interactions in *in vitro* aggregates and in chlorosomes.

The structure of the BChl *c* aggregates in the chlorosomes of *C. aurantiacus* is unclear at present. It is of particular interest in this regard that Worcester *et al.* [6] have observed that artificial BChl *c* aggregates form tubular structures of the same diameter as found also for the rod-like structures in the interior of the chlorosomes [11]. Our finding may thus well be a link between the BChl *c* organization *in vivo* within the chlorosomes and the assemblies of the pure pigment *in vitro* in artificial media. The finding also bears on the feasibility of artificial antennae to be used for light harvesting in synthetic charge separation systems [6], in particular with respect to the properties that allow BChl *c* aggregates to function as efficient light-harvesting antennae without adverse self-quenching.

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