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Selective solubilization of chlorosome proteins in *Chloroflexus aurantiacus*

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Proteins were solubilized selectively from chlorosomes of *Chloroflexus aurantiacus* by electrophoretic gel filtration according to Griebenow et al. Whereas the 11 kDa and 18 kDa proteins were extracted almost completely, the remaining modified chlorosomes contained high amounts of pigment and c-protein. It was concluded that the c-protein in contradiction to the publication by Griebenow et al. is indeed localized in the interior of *Chloroflexus* chlorosomes.

Chlorosome; c-Protein; BChlc-binding polypeptide; *Chloroflexus aurantiacus*

1. INTRODUCTION

The thermophilic photosynthetic bacterium *Chloroflexus aurantiacus* contains as its primary light-harvesting antenna the B740 complex. This antenna is located in chlorosomes, vesicular oblong bodies of complex composition, which are rich in bacteriochlorophyll *c* (BChlc). Chlorosomes are situated on the inner surface of the cytoplasmic membrane; their number and size vary considerably with growth rate [1] and oxygen concentration [2].

Freeze-fracture electron miscroscopy revealed a regular substructure in the chlorosome interior [3]. By photolabeling and enzymatic cleavage experiments, Feick and Fuller [4] were able to show that the main chlorosome protein is not localized on the surface of the vesicles. Upon chemical cross-linking, dimers of this protein, subsequently called the BChlc-binding protein or 'c-protein', were found to be associated with 10–16 molecules of BChlc. Wechsler et al. [5] determined the primary structure of this polypeptide and proposed a model which was based on the sequence-data and other available information on the structure of the B740 complex [3,4]. In this model 6 dimers of c-protein were associated with 14 BChlc each. From a structural point of view, the complex thereby exhibited a similarity with other light-harvesting antennae composed of polypeptide-pigment protein complexes.

Observations of the high tendency of BChlc and related pigments for self-aggregation in vitro [6] led to a new discussion about the more ancient hypothesis of protein-free antenna complexes. Similarities were found between the spectroscopic data of chlorosomes and of in vitro aggregates [7]. Recently Holzwarth and Griebenow [8,9] described the existence of functionally unimpaired protein-depleted chromosomes obtained by a so-called electrophoretic gel filtration method [10]. They claimed that the B740 complex was the first biological antenna so far detected based exclusively on pigments.

By using the electrophoretic gel filtration method, we however were able to obtain further evidence for a structural and functional role of the c-protein in the B740-complex.

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Abbreviations: BChlc, bacteriochlorophyll *c*

2. MATERIALS AND METHODS

Cells of *Chloroflexus aurantiacus* strain J-10-fl, were grown according to Feick and Fuller [4] at low incident light intensities (500 lux) in 2 l glass bottles.

Chlorosomes were prepared by a modification of the procedure described in [4]. The whole membrane fraction was adjusted to an optical density at 740 nm of about 150, and Miranol S2M-SF (39% solution) was added to a final concentration of 8% (v/v). The suspension was stirred for 30 min at room temperature and washed twice in 10 mM Tris-HCl pH 8.0 in an ultracentrifuge (90 min, 4°C, 153 000 × *g*). Sedimented chlorosomes were resuspended in the same buffer volume as before and subjected to a second incubation with 4% (v/v) Miranol and subsequent washing. This method, useful for the preparation of large amounts of chlorosomes, permitted a purification comparable to that found in [4].

Purified chlorosomes and whole membrane fraction were submitted to the electrophoretic gel filtration system described in [10]. After incubation in 0.1% LDS, 35% sucrose for 5 min at 56°C samples were loaded on a 0.1% LDS-minigel on a BioRad Mini-Protean 11 Dual Slab Gel System. Gels were run for 1h at 150V and 4°C. Modified chlorosomes were collected by shaking the stacking gels in 10 mM Tris-HCl pH 8.0 overnight.

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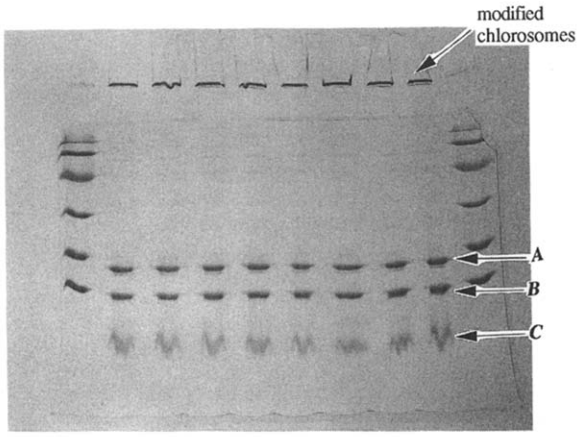

Fig. 1. Separating gel after electrophoretic gel filtration with chlorosomes. Lanes 1 and 10 show some marker proteins with molecular weights of 97.4, 66.2, 42.7, 21.5 and 14.4 kDa. The double band of the 18 kDa protein is indicated by *A*, the band of the 11 kDa protein by *B*. At the front of the gel, green pigments and traces of *c*-protein are banding as *C*.

Low molecular substances introduced by the electrophoretic gel filtration procedure, like sucrose and LDS, were removed from the modified chlorosomes by repeated washing in the ultracentrifuge (90 min, 4°C, 150000 × *g*). After addition of formic acid to a final concentration of 50% (v/v) and sonification, gel filtrations were run on a BioGel P-10 column in 50% formic acid. Previously dried samples could be loaded on a SDS-minigel-system containing 7.5 M urea.

Amino acid sequences were determined by the automated Edman degradation procedure on an Applied Biosystems 470A protein sequencer. The PTH amino acids were identified as described in [11].

For amino acid analysis polypeptide samples were hydrolyzed in constant boiling 6 N HCl for 24 h at 110°C in vacuo and analyzed on a Biotronic LC 6000 E analyzer.

BChlc contents were determined as described in [12]. Absorption spectra were taken on a Perkin Elmer Lambda 5 UV/VIS spectrophotometer.

3. RESULTS AND DISCUSSION

Absorption spectra of the B740 complex were unaltered in chlorosomes treated by the method of electrophoretic gel filtration. Baseplate absorption however was lacking in modified chlorosomes as described in [10]. Modified chlorosomes showed a slight tendency for aggregation, so that the absorption characteristics could be affected by scattering.

In a 10% to 45% sucrose gradient, where native chlorosomes banded at 30% sucrose, chlorosomes accumulated after incubation with 0.1% LDS at about 20%. This effect may be due to a swelling of the chlorosomes caused by penetration of LDS or to a binding of detergent to the surface of the vesicles. Nevertheless modified chlorosomes could still be sedimented at 150000 g.

The separating gel of electrophoretic gel filtration showed two major bands at about 11 kDa and 18 kDa (Fig. 1). The 18 kDa protein always appeared as a double-band. Minor fractions of higher molecular weight and some pigment at the front of the gel were also detected.

The separating gel of electrophoretic gel filtration further shows that very small amounts of c-protein were eluted electrophoretically from the chlorosomes on the top of the gel (Fig. 1). In chlorosomes previously subjected to electrophoretic gel filtration however, a high content of c-protein and pigment was observed by SDS-minigels with 7.5 M urea. The 11 kDa and 18 kDa proteins formed small fractions when compared to native chlorosomes (Fig. 2).

We assume that by using 7.5 M urea we were able to solubilize the c-protein in modified chlorosomes. It may

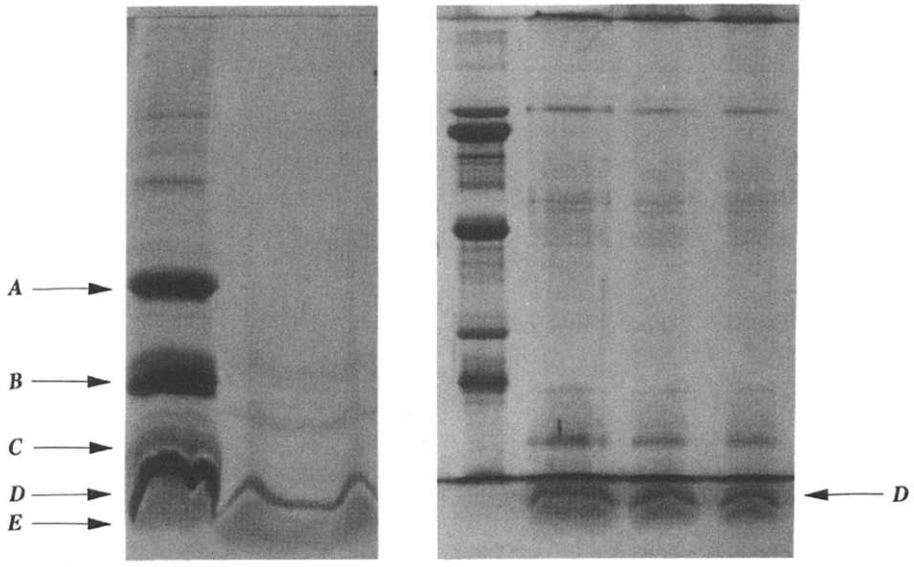

Fig. 2. SDS-minigel with 7.5 M urea. Lane 1 shows native chlorosomes and lane 2 modified chlorosomes. Lane 3 in a second gel contains some marker proteins with molecular weights of 97.4, 66.2, 31, 21.5 and 14.4 kDa. Pigments are again banding at the front of the gel (*E*). The BChlc-binding protein is indicated as *D*, the baseplate protein as *C*. The 18 kDa- and 11 kDa-proteins are marked with *A* and *B*. Lanes 4–6 show a second preparation of modified chromosomes, the marker Bromophenol blue banding above the *c*-protein (*D*).

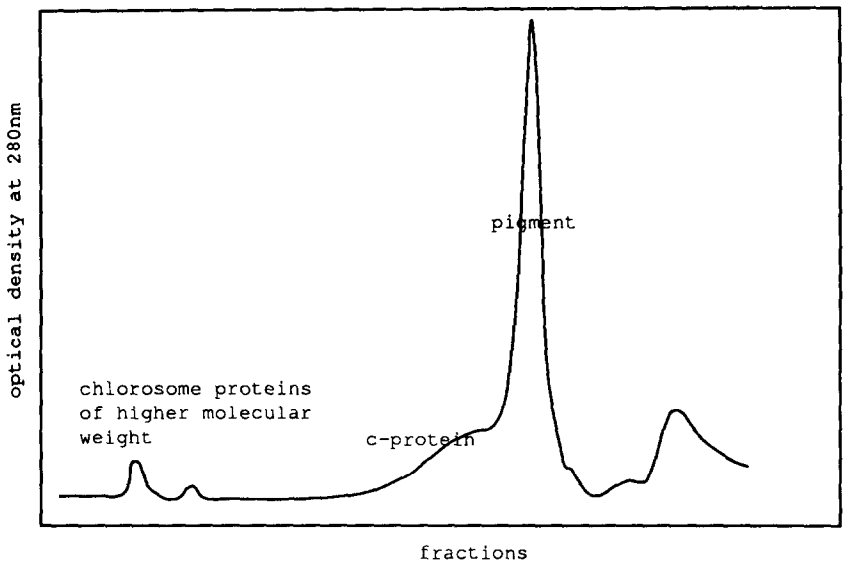

Fig. 3. Elution profile of modified chlorosomes on BioGel P-10 in 50% formic acid.

be assumed that Holzwarth et al. [8,9] on SDS-gels failed to detect this protein because of unsuitable solubilization procedures. Chlorosomes in general and especially modified chlorosomes which obviously have an altered internal structure often resist solubilization. This effect might be due to aggregation of proteins particularly in modified chlorosomes.

Gel filtration of modified chlorosomes on BioGel P-10 likewise revealed two minor polypeptides of higher molecular weight, probably small amounts of 11 kDa and 18 kDa protein (Fig. 3). In the void volume fraction 18 kDa protein could hardly be detected by its N-terminal sequence of ANETTNERDGLFEMAAGVF because amounts of protein were too small. As the 11 kDa protein of *Chloroflexus aurantiacus* is blocked at its N-terminus, identification was not possible in this case either. The main fraction accumulating near to a peak of pigments consisted of c-protein ATRGWF-SESSAQVAQIGDIM... [5].

The pigment/protein ratio in modified chlorosomes amounted to 20–25/1 compared to values of about 15/1 in native chlorosomes. The ratio determined by us for native chlorosomes is thus roughly twice as high as that published by Feick and Fuller [4]. Protein concentrations were determined by amino acid analysis. Measurements were complicated as in chlorosomes c-protein always coexists with the other chlorosome proteins of higher molecular weight. As a basis for our calculations we assumed, that all chlorosome protein was composed of one single 6 kDa polypeptide, or that characteristic amino acids originated mainly from the c-protein. The pigment/protein ratio is expected therefore to be even somewhat higher than the indicated value. For evaluation of pigment concentrations an in vitro coefficient $\epsilon = 74 mM^{-1}\ cm^{-1}$ [12] was used for BChlc in organic solvent. The in vivo coefficient $\epsilon = 97 mM^{-1}\ cm^{-1}$ was determined by our own experiments and led to identical results. It should be noticed, however, that because of changes in the composition of chlorosomes depending on growth rate and oxygen concentration, in vivo coefficients might vary with growth conditions.

Our results in accordance to [4] confirm the localization of the c-protein in the chlorosome interior, whereas the 18 kDa and 11 kDa proteins seem to be situated on the surface of the vesicles. Thus the important structural role of the c-protein in the B740 complex as the BChlc-binding protein postulated earlier in [5] is still possible. It becomes even more probable as preliminary quantitative analysis of high-light and low-light cells suggest a correlation between BChlc- and c-protein content.

The common structural principle of light-harvesting protein/pigment complexes in all antenna complexes known so far could thus be confirmed for *Chloroflexus aurantiacus* in contrast to Holzwarth et al. [8,9].

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