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Optical and structural properties of chlorosomes of the photosynthetic green sulfur bacterium *Chlorobium limicola*

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Isolated chlorosomes of the photosynthetic green sulfur bacterium *Chlorobium limicola* upon cooling to 4 K showed, in addition to the near-infrared absorption band at 753 nm due to bacteriochlorophyll *c*, a weak band near 800 nm that could be attributed to bacteriochlorophyll *a*. The emission spectrum showed bands of bacteriochlorophyll *c* and *a* at 788 and 828 nm, respectively. The fluorescence excitation spectrum indicated a high efficiency of energy transfer from bacteriochlorophyll *c* to bacteriochlorophyll *a*. When all bacteriochlorophyll *c* absorption had been lost upon storage, no appreciable change in the optical properties of the bacteriochlorophyll *a* contained in these 'depleted chlorosomes' was observed. The fluorescence and absorption spectra of the chlorosomal bacteriochlorophyll *a* were clearly different from those of the soluble bacteriochlorophyll *a* protein present in these bacteria. The results provide strong evidence that bacteriochlorophyll *a*, although present in a small amount, is an integral constituent of the chlorosome. It presumably functions in the transfer of energy from the chlorosome to the photosynthetic membrane; its spectral properties and the orientation of its near-infrared optical transitions as determined by linear dichroism are such as to favor this energy transfer.

Introduction

Chlorosomes of the green sulfur bacterium *Chlorobium limicola* have been found to contain small amounts of BChl *a* in addition to the main light harvesting pigment, BChl *c* [1]. At first it was not clear if BChl *a* was an intrinsic component of the chlorosome, or whether it represented a contamination derived from the soluble BChl *a* protein [2] present in this bacterium. However, it has been shown in the preceding paper [3] that a BChl *a* protein absorbing at 794 nm is associated with the chlorosomes of *C. limicola*. Under appropriate conditions a fraction with an absorption maximum at 794 nm could be isolated from broken cells that showed the characteristics of intact chlorosomes with respect to their protein content. Such 'depleted chlorosomes' contained BChl *a*, BPh *c*, and carotenoid but no or very little BChl *c* as judged from their absorption spectrum and from the absorption spectrum of the extracted pigments.

This communication reports a study of the optical properties of both intact and depleted chlorosomes by means of low-temperature absorption, fluorescence and fluorescence polarization and linear dichroism. It will be shown that the BChl *a* in both preparations is optically very similar, but different from the soluble BChl *a* protein. A high

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; LD, linear dichroism.

efficiency of energy transfer from BChl *c* to BChl *a* was observed, and both pigments showed a clear orientation with respect to the chlorosome axes. The results provide strong evidence that BChl *a* is a functional component which serves as an intermediary in the transfer of excitation energy from BChl *c* to the BChl *a* present in the membrane of Chlorobiaceae.

Materials and Methods

'Medium density' chlorosomes and 'depleted' chlorosomes (the so-called 794-band) were prepared from *Chlorobium limicola* f. *thiosulfatophilum* according to the procedure described in the preceding paper [3]. They were suspended in 10 mM Tris buffer (pH 7.8), to which 0.5 M sucrose and 50% (v/v) glycerol were added in order to obtain clear samples upon cooling. For reasons to be described below, 10 mM dithionite was normally also present.

Low-temperature absorption, fluorescence and fluorescence polarization spectroscopy were performed using a single-beam spectrophotometer as described by Rijgersberg et al. [4]. The optical pathlength in all measurements was 2.5 mm. Linear dichroism spectra were recorded on an apparatus described in Ref. 5. In these experiments sucrose and glycerol were omitted, and the chlorosomes were suspended in a disc-shaped polyacrylamide gel of 6 mm thickness. The gel was brought in between two prisms and orientation was obtained by squeezing the two prisms such as to reduce the thickness of the gel by a factor of 2. $A_{\parallel}$ was defined as the absorbance of the measuring light polarized parallel to the plane of orientation, i.e., perpendicular to the direction of squeezing. The measuring beam made an angle of 45° with this plane.

Results and Interpretation

The absorption spectra of isolated chlorosomes of *C. limicola*, measured at 4 K and at 295 K are shown in Fig. 1A. The most prominent bands in the absorption spectrum are those of BChl *c*, but in addition the 4 K spectrum shows a weak, but clear shoulder near 800 nm, that can be attributed to BChl *a*. The amplitude of the BChl *a* band relative to that of BChl *c* cannot be determined with precision, but it is at least in qualitative agreement with the ratio of about 90 for BChl *c*/BChl *a* given in the preceding paper [3]. The band at 514 nm and the shoulder near 480 nm can be attributed to carotenoid (probably chlorobactene, see Ref. 1). The Q_y band of BChl *c* showed a red shift from 749 to 753 nm upon cooling.

Fig. 1B shows the low-temperature absorption spectrum of BChl *c*-depleted chlorosomes. The Q_y band of BChl *a* in this preparation is located at 802 nm, the Q_x band at 609 nm. As judged from the intensities of the bands at 516, 478 and 451 nm, the chlorosomes appear to have retained most of their carotenoid. The bands at 670, 558 and 423 nm are probably due to BPh *c* [6], which is presumably an artefact derived from BChl *c* originally present.

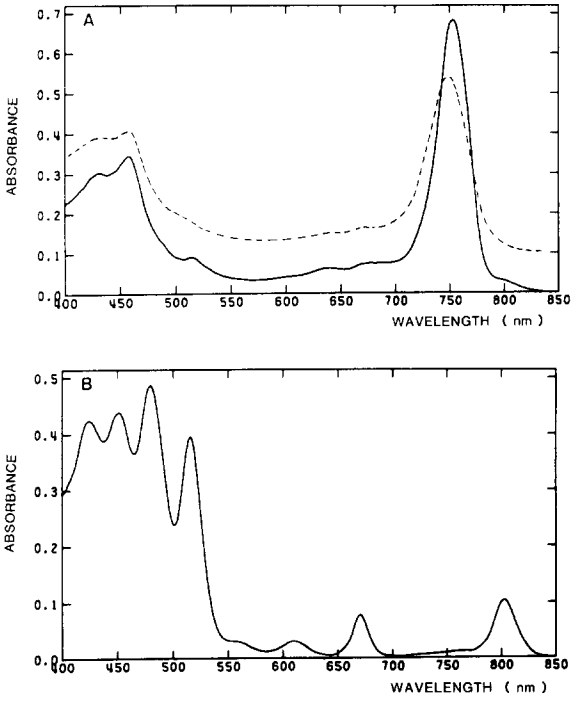

Fig. 1. Absorption spectra of (A) intact 'medium density' chlorosomes and (B) BChl *c*-depleted chlorosomes of *Chlorobium limicola* measured at 4 K. The spectrum of intact chlorosomes at 295 K is also shown (- - - - - -), shifted by 0.1 absorbance unit to enhance clarity. These spectra and those shown in the other figures were obtained with preparations that had been incubated with dithionite (see text).

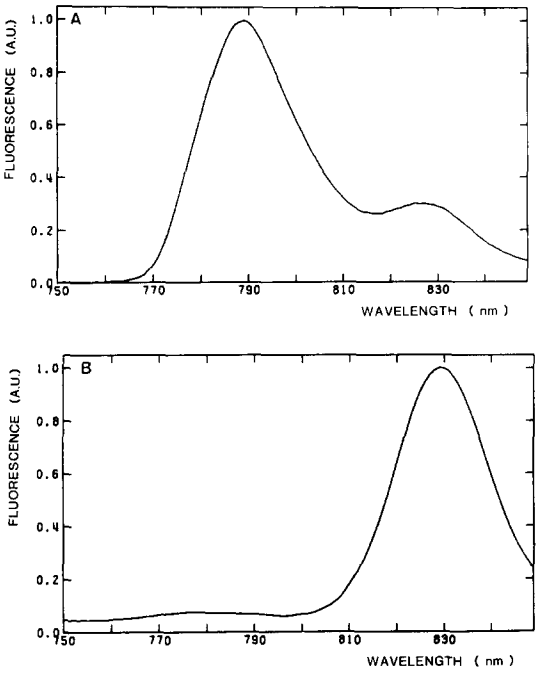

Fig. 2. Fluorescence spectra at 4 K of (A) chlorosomes and (B) BChl *c*-depleted chlorosomes. Excitation at 605 nm. The absorbance of the samples, measured at room temperature, was 0.4 at 750 nm for chlorosomes and 0.1 at 802 nm for depleted chlorosomes. The same emission spectra were obtained upon excitation at 460 nm.

The spectra of Fig. 1 were obtained with preparations that had been incubated for 2–3 h at 0°C with 10 mM sodium dithionite. Without such incubation the amount of BPh *c* in the preparations was larger. This was particularly clear for the depleted chlorosomes, which without incubation showed strong bands at 670, 554 and 419 nm. Treatment with dithionite reduced the band at 670 nm by a factor of 3, while the band at 802 nm was not affected.

The fluorescence emission spectrum of the chlorosomes, when measured at room temperature, showed a broad band at 780 nm, due to BChl *c*. Emission by BChl *a* could not be discerned at room temperature, but upon cooling a long-wave band developed, and at 4 K two separate emission bands from BChl *c* and BChl *a* were clearly visible, located at 788 and 828 nm, respectively (Fig. 2A). Depleted chlorosomes showed mainly BChl *a* emission (Fig. 2B), together with weak bands near 680 nm (not shown) and 780 nm, which may be due to BPh *c* and BChl *c*, respectively. When the dithionite incubation was omitted, the preparation showed in addition strong emission bands near 670, 720 and 740 nm, which were probably due to artefacts, whereas a weak band at 680 nm appeared in the emission spectrum of chlorosomes (not shown).

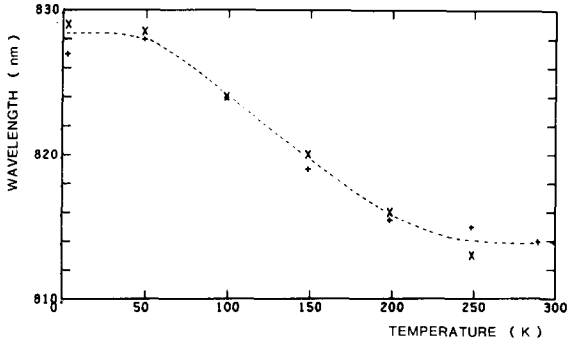

Fig. 3. Location of the maxima of the BChl *a* emission bands of chlorosomes (x) and of BChl *c*-depleted chlorosomes (+) as a function of temperature. When necessary the wavelengths of the maxima were determined from the second derivatives of the spectra. Excitation wavelength was 460 nm.

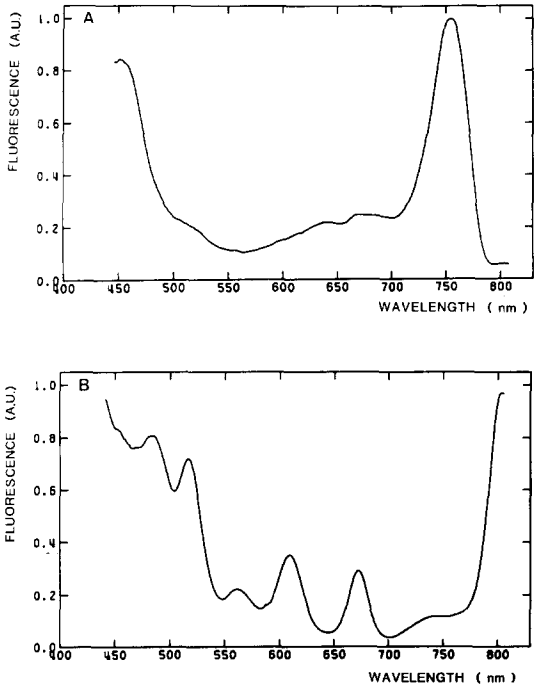

Fig. 4. Excitation spectra of BChl *a* fluorescence at 830 nm of (A) chlorosomes ($A_{750} = 0.7$) and (B) BChl *c*-depleted chlorosomes ($A_{820} = 0.1$), measured at 4 K.

The long-wave emission bands of chlorosomes and of depleted chlorosomes showed the same red shifts upon cooling (Fig. 3). For both preparations the BChl *a* band shifted from 814 nm at 250 K to 828 nm at 4 K. At each temperature only a single BChl *a* emission band was observed, unlike the situation with the water-soluble BChl *a* protein from green sulfur bacteria, where a band at 817 nm is gradually replaced by one at 828 nm upon cooling [7,8].

Excitation spectra are shown in Fig. 4. The spectrum of the depleted chlorosomes (Fig. 4B) showed relatively strong Q_y and Q_x bands of BChl *a*, confirming that the emission at 828 nm indeed is due to this pigment. Comparison with the absorption (1 − T) spectrum showed that light energy absorbed by BPh *c* was transferred to BChl *a* with an efficiency of 35%, whereas the efficiency of energy transfer from carotenoid to BChl *a* appears to be about 20%.

The excitation spectrum for BChl *a* fluorescence of chlorosomes was dominated by the bands of BChl *c* (Fig. 4A), whereas the band of BChl *a* itself was quite weak. The fluorescence was measured at 830 nm. The height of the BChl *c* bands in the excitation spectrum may be exaggerated, because some of the emission at 830 nm may still be due to BChl *c*. Nevertheless, the relative heights of the BChl *c* and BChl *a* bands indicate a high efficiency of energy transfer from BChl *c* to BChl *a*, which may well approach 100%. The excitation spectrum for BChl *c* fluorescence at 795 nm (not shown) was similar to that of BChl *a*, as was to be expected.

When dithionite was omitted from the chlorosome preparation, an about 10-fold lower emission intensity was observed for both BChl *c* and BChl *a* upon excitation at 750 nm. A similar, but weaker quenching has been reported for the BChl *a* protein and for membrane protein complexes from *Prosthecochloris aestuarii* [9]. The excitation spectrum for BChl *a* fluorescence now showed a strongly reduced contribution of BChl *c* at 750 nm and in the Soret region, corresponding to an efficiency of energy transfer from BChl *c* to BChl *a* of only about 30%. This shows that a strong quenching mainly occurred in BChl *c*. Apparently dithionite removes non-fluorescing quenching centers [9] which effectively compete with the energy transfer from BChl *c* to BChl *a*.

In order to obtain information about the orientation of the pigments in the chlorosomes we also measured linear dichroism spectra. The spectrum of the chlorosomes (Fig. 5A) showed a strong positive dichroic signal in the Q_y band of BChl *c*, which means that the orientation of the Q_y transitions is predominantly parallel to the plane of

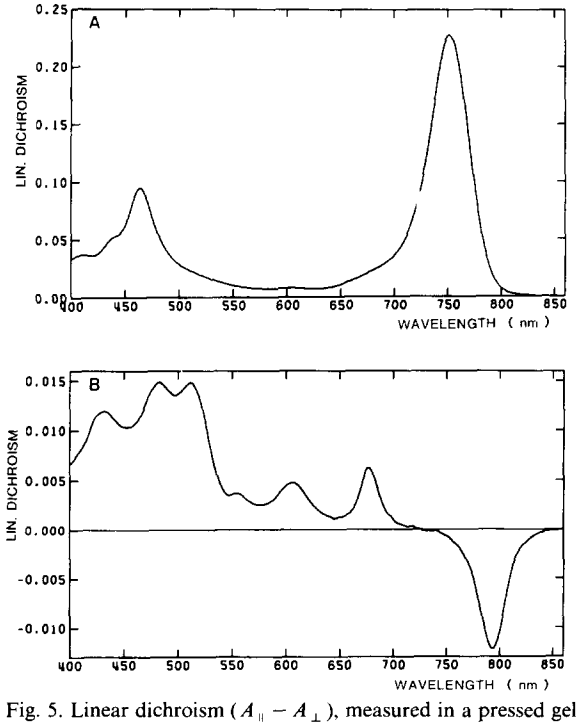

Fig. 5. Linear dichroism ($A_{\parallel} - A_{\perp}$), measured in a pressed gel at 295 K of (A) chlorosomes ($A_{750} = 0.90$) and (B) BChl *c*-depleted chlorosomes ($A_{794} = 0.12$).

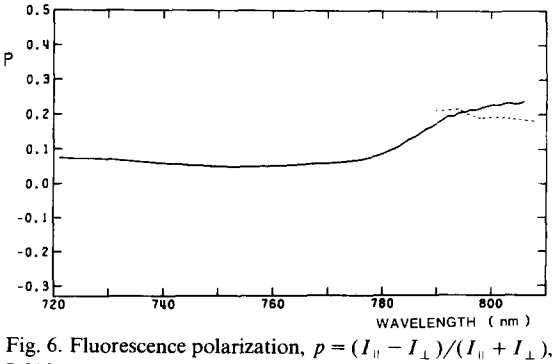

Fig. 6. Fluorescence polarization, $p = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$, of BChl *a* at 830 nm, measured at 4 K, as a function of the excitation wavelength for chlorosomes (———) and for BChl *c*-depleted chlorosomes (- - - - -).

orientation (see Materials and Methods). If we assume that the chlorosomes are oriented with their long axes preferentially parallel to the plane of orientation, this means that the Q_y transitions are predominantly parallel to the plane of these chlorosome axes. In the Soret region the band at about 460 nm shows a stronger orientation than the one at 430 nm. Bands of carotenoid and of BChl *a* are not observed in the LD spectrum.

The LD spectrum of BChl *c*-depleted chlorosomes gave information on the orientation of BChl *a* and carotenoid. Fig. 5B shows that the orientation of the BChl *a* transitions is basically different from those of BChl *c*, the BChl *a* Q_y transitions being more or less perpendicular to the plane of the long axes of the chlorosome. A positive, but weaker LD is observed for carotenoid and for the BPh *c* transition dipoles at 670 and near 555 and 430 nm.

The fluorescence polarization, *p*, for BChl *a* fluorescence excited in the Q_y band was 0.18 for depleted chlorosomes (Fig. 6). A similar value of 0.22 ± 0.08 was obtained with intact chlorosomes, but here the accuracy was much lower, because of the relatively weak band of BChl *a* in the excitation spectrum. A strong depolarization of fluorescence ($p = 0.05$) was observed upon excitation in the Q_y band of BChl *c*.

Discussion

The results reported here give strong evidence that BChl *a* forms an integral component of the chlorosomes of *Chlorobium limicola*. The BChl *a*-to-BChl *c* ratio is about three times lower than has been found in chlorosomes of the gliding green bacterium *Chloroflexus aurantiacus* [1,10]. The BChl *a* is spectroscopically quite distinct from that of the soluble BChl *a* protein: it has an absorption band near 802 nm at 4 K and a single fluorescence band that shifts from 814 nm at room temperature to 828 nm at 50 K and below. A similar red shift was observed for the emission band of BChl *a* in *Chloroflexus* chlorosomes [11]. Chlorosomes can be obtained that lack the BChl *c* absorption bands, without observing an appreciable change in the optical properties of BChl *a*.

The most obvious function of the chlorosomal BChl *a* is to transfer excitation energy from BChl *c* to the soluble BChl *a* protein, and hence to BChl *a* in the membrane. This energy transfer has not been demonstrated directly, but the large overlap integral of the emission band and the main absorption band of the BChl *a* protein would make the BChl *a* of the chlorosome eminently suited for such a function. The excitation spectrum of Fig. 4A demonstrates efficient energy transfer from BChl *c* to BChl *a* in the isolated chlorosome.

The LD spectra obtained with both chlorosomes and depleted chlorosomes show that significant orientation can be obtained by the gel-press method, first described by Abdourakhmanov et al. [12] for bacterial reaction centers. A more detailed study on chlorosomes of *Chloroflexus aurantiacus* [13] shows that the method is quite efficient and may be used to obtain accurate angles of transition dipoles with respect to the orientation axes. Electron micrographs [14,15] show that the longest axes of the chlorosomes are parallel to the plane of the cytoplasmic membrane. Thus, the positive LD for the Q_y bands of BChl *c* in chlorosomes agrees with that observed earlier for whole cells of *Prosthecochloris aestuarii*, which indicated that the BChl *c* Q_y transitions are predominantly parallel to the membrane [16]. Comparison of our spectrum of depleted chlorosomes with that of intact cells of *P. aestuarii* also shows that the Q_y transitions of BChl *a* in the chlorosome have about the same orientation as the strongest Q_y transition dipole of BChl *a* in the BChl *a* protein, i.e., approximately perpendicular to the membrane. Such an orientation would favor energy transfer from BChl *a* in the chlorosome to the BChl *a* protein.

The fluorescence polarization of BChl *a* upon excitation in the Q_y band is significantly lower than for isolated, monomeric BChl *a* [17,18]. This is consistent with the circular dichroism spectrum which indicates a dimeric or oligomeric structure for BChl *a* [3]. As the polarization is higher than the value of 0.14 that applies to a circularly degenerate system, we conclude that, if BChl *a* is a dimer, the angle between the Q_y transitions of the constituent monomers must be smaller than 90°. Upon excitation in the Q_y band of BChl *c*, the BChl *a* fluorescence of chlorosomes was largely depolarized to a value of approx. 0.05, indicating that on the average the Q_y transitions of BChl *c*

and a make an angle which is slightly less than the magic angle of 55°.

Acknowledgements

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