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Antenna organization and energy transfer in membranes of *Heliobacterium chlorum*

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Absorption, fluorescence emission and fluorescence excitation spectra of membranes of the recently discovered photosynthetic bacterium *Heliobacterium chlorum* (Gest, H. and Favinger, J.L. (1983) Arch. Microbiol. 136, 11–16) showed that at 4 K at least three spectroscopically different forms of bacteriochlorophyll *g* (BChl *g* 778, BChl *g* 793 and BChl *g* 808) can be discerned in the antenna system. Efficient energy transfer occurs from the short-wave-absorbing bacteriochlorophylls to BChl *g* 808. Energy transfer to bacteriochlorophyll, albeit with lower efficiency (70%), also occurred from the main carotenoid, neurosporene, and from a pigment absorbing at 670 nm. The complex structure of the antenna system is also reflected by fluorescence polarization and linear and circular dichroism spectra. Significant circular dichroism was only observed for BChl *g* 793, and different orientations were observed for the various Q_y transition dipoles, the one of BChl *g* 808 making a smaller angle with the plane of the membrane than those of the other bacteriochlorophylls.

Introduction

Heliobacterium chlorum is a recently discovered photosynthetic bacterium [1] that contains a hitherto unknown species of bacteriochlorophyll, BChl *g* [2]. Neither its unusual pigmentation, nor its ultrastructure [1] provide a clue to its taxonomic position; there is no evidence for the presence of chlorosomes, as in green bacteria, nor for invaginations of the cytoplasmic membrane, as in purple bacteria. Its mechanism of photosynthesis is largely unknown, but recent studies of Fuller and coworkers [3] have demonstrated a light-induced absorbance decrease centered at 798 nm that was ascribed to photooxidation of a primary electron donor.

In this paper we present a study of the antenna organization and energy transfer in the photosynthetic membrane of *H. chlorum*. Several spectral forms of BChl *g* were observed that transfer their excitation energy efficiently to a long-wave BChl *g* absorbing at 808 nm at 4 K. Energy transfer, albeit with lower efficiency, does also occur from carotenoid and an unidentified pigment absorbing near 670 nm, that may be similar to a BChl *c*-like pigment present in membranes of green sulfur bacteria (Braumann, T. and Vasmel, H., unpublished results). The complicated structure of the antenna system of *H. chlorum* is also reflected by fluorescence polarization and linear and circular dichroism spectra.

Materials and Methods

H. chlorum was grown anaerobically in medium 112 of the American Type Culture Collection [1]

Abbreviations: BChl, bacteriochlorophyll; CD, circular dichroism; LD, linear dichroism; *A*, absorbance.

containing 2.5 mM ascorbate. Cells were harvested by centrifugation and resuspended in a buffer of pH 8.0, comprising 10 mM Tris/10 mM ascorbate/2 mM dithiothreitol. Membrane fragments were prepared by sonication followed by centrifugation for 15 min at 27000 × *g*. Normally, the supernatant was used for the experiments, but essentially the same results were obtained after additional purification of the membrane fragments by centrifugation at 180000 × *g* for 1 h. The preparation was stored in the dark at 5°C.

Absorption, fluorescence and fluorescence polarization spectra were recorded on a single beam spectrophotometer [4]. Circular and linear dichroism spectroscopy was performed on an apparatus described in Ref. 5. For low temperature experiments 0.5 M sucrose and 50% (v/v) glycerol were added to obtain clear samples upon cooling. For measurements of linear dichroism the membranes (in buffer) were suspended in an 8% (w/v) gelatin gel. Orientation was obtained by squeezing the gel between two prisms. $A_{\parallel}$ 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.

For qualitative analysis of the pigment content, samples were extracted with acetone/methanol (7 : 2, v/v), followed by an additional extraction with pure methanol. The pigments were transferred to light petroleum ether (40–60°) and separated by thin-layer chromatography on precoated silicagel plates (Merck; Kieselgel 60, 0.25 mm) developed with light petroleum ether (40–60°)/acetone/propanol-2 (80 : 15 : 5, v/v/v).

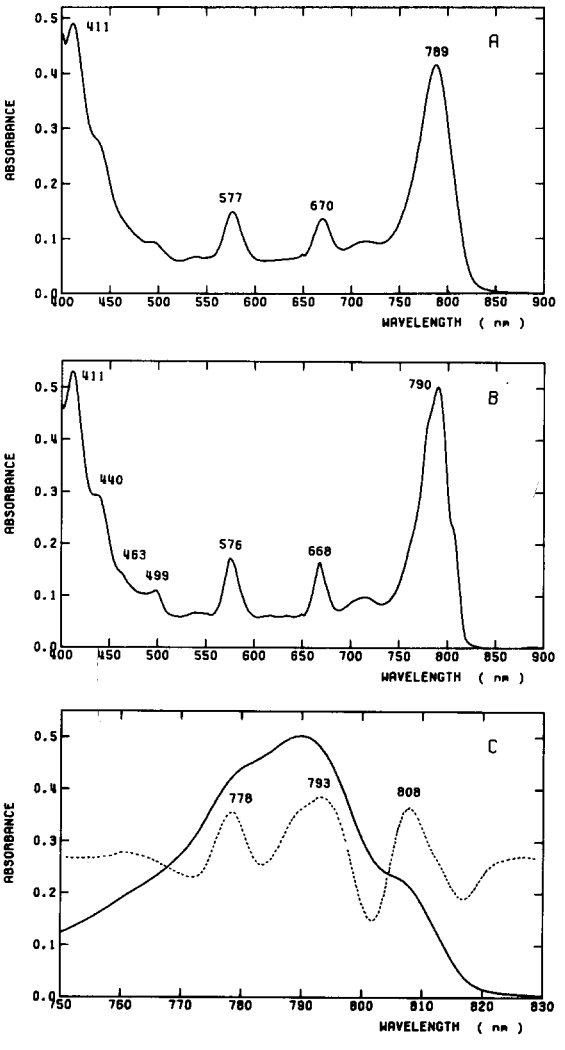

Fig. 1. Absorption spectra of membranes of *Heliobacterium chlorum*. (A) Room temperature, (B) 4 K, (C) the near-infrared region (4 K) on an expanded scale, together with the second derivative spectrum (inverted).

Results and Interpretation

Pigments and energy transfer

Absorption spectra of membranes of *Heliobacterium chlorum* are shown in Fig. 1. The room temperature spectrum (Fig. 1A) is similar to that published by Gest and Favinger [1]. The most prominent features are the near-infrared band at 789 nm, and weaker bands at 577 and 670 nm in the visible region. The first two bands are presumably the Q_y and Q_x bands of BChl *g* [2]. The band at 670 nm is reminiscent of the absorption spectrum of the photosynthetic membranes of green sulfur bacteria [6,7]; in those bacteria this band was assigned to an as yet unidentified BChl *c*-like pigment (Braumann, T. and Vasmel, H., unpublished results). Below 500 nm, bands are visible of carotenoid and, in the Soret region, of BChl *g*.

The absorption spectrum measured at 4 K showed considerably more detail (Figs. 1B and C). The Q_y region of BChl *g* now revealed at least three spectrally different species, with peak positions at 778, 793 and 808 nm, as determined from

the second derivative spectrum (Fig. 1C). The same bands were present in the absorption spectrum of whole cells (not shown). Two different bands were visible near 670 nm (668 and 677 nm). These results show that the pigment organization of the anenna of *Heliobacterium* must be considerably more complex than was suggested by the room temperature spectrum and that the membrane may contain more than one pigment-protein. Pigment extraction, followed by thin-layer chromatography confirmed the suggestion [1] that the major carotenoid of *H. chlorum* is neurosporene. The main carotenoid band on the chromatogram showed absorption maxima at 414, 438 and 468 nm (see Ref. 8) in acetone/methanol (7:2, v/v). It had the same R_F-value (0.71) as neurosporene from the G1C mutant of *Rhodopseudomonas sphaeroides*. The absorption bands at 499, 463, and partly that at 440 nm, in Fig. 1B may thus be ascribed to neurosporene, with a similar in vivo red shift as observed in *Rps. sphaeroides* [9]. The extraction procedure largely converted BChl *g* to BPheo *g* ($R_F = 0.44$) and some more polar degradation products; no BChl *a* was detected.

Circular dichroism spectra showed a conservative signal centered at 793 nm, both at 293 and 77 K (Fig. 2). This indicates that the absorption band at 793 nm is probably composed of two exciton bands that are too close together to be resolved in the absorption spectrum. Bacteriochlorophylls absorbing at other wavelengths do not appear to have significant excitonic interactions. Weak CD signals were visible near 580 and 670 nm; neurosporene showed no detectable CD.

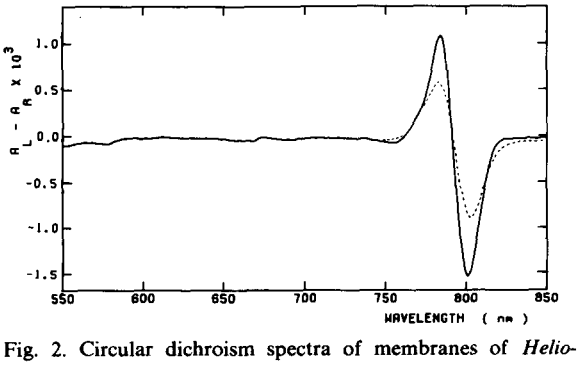

Fig. 2. Circular dichroism spectra of membranes of *Heliobacterium chlorum*, measured at 77 K (———) and at 293 K (- - - - - -). The absorbance of the sample, corrected for scattering, was 0.78 at 789 nm (293 K).

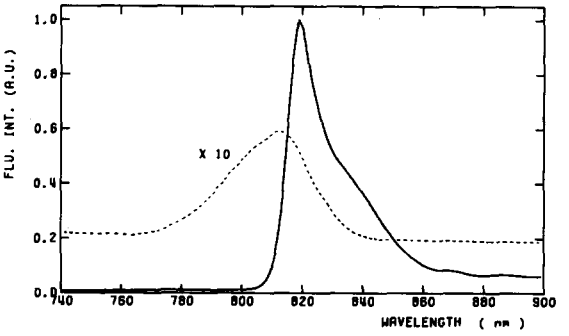

Fig. 3. Fluorescence emission spectra measured at 4 K (———) and at 293 K (- - - - - -), respectively. The latter spectrum is shown on a 10-fold expanded scale. Excitation was at 580 nm. The absorbance of the samples was 0.41 at 789 nm (293 K). A.U., arbitrary units.

Fluorescence emission spectra (Fig. 3) of *H. chlorum* showed a relatively simple structure. Upon excitation at 580 nm an emission band at 813 nm was observed at room tempeorature. Upon cooling, the band intensified considerably, it narrowed and it shifted to 819 nm at 4 K. The same spectra were observed upon excitation at 440 nm. Apparently, at both temperatures all or nearly all of the emission comes from the bacteriochlorophyll absorbing at 808 nm (BChl *g* 808), indicating that light absorbed by the other BChl *g* molecules is transferred with very high efficiency to BChl *g* 808. This is also in agreement with the fluorescence excitation spectrum (see below). Upon excitation at 580 nm a very weak fluorescence band at 4 K was seen at 678 nm (not shown) that can be ascribed to the pigment or pigments absorbing

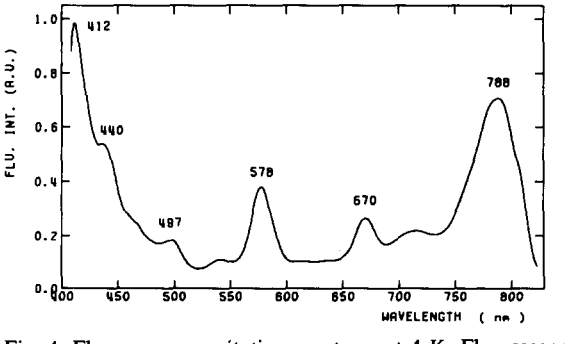

Fig. 4. Fluorescence excitation spectrum at 4 K. Fluorescence was detected at 840 nm. The absorbance at 790 nm was 0.50. A.U., arbitrary units.

near 670 nm. Its amplitude was only 1–2% of the band at 819 nm.

The excitation spectrum for the fluorescence emitted at 840 nm (Fig. 4) showed a high efficiency of energy transfer (90–100%) for light absorbed by BChls *g* 778 and *g* 793 to the long-wave pigment, BChl *g* 808. A similarly high efficiency was observed upon excitation in the Q_x band at 576 nm. Excitations of neurosporene and of the 670 nm pigment were transferred with about 70% efficiency to BChl *g*.

Orientation of pigments

Excitation spectra in polarized light (Fig. 5) showed a strong depolarization of fluorescence for light absorbed by most pigments other than BChl *g* 808. Between 700 and 800 nm the fluorescence polarization (p) was only slightly positive, with a minimum near 780 nm, perhaps corresponding to BChl *g*778. However, the band of BChl *g* 808 was much more prominent in the $F_{\parallel}$ than in the $F_{\perp}$ spectrum, and above 800 nm there was a steep increase of p to a value of about 0.30. This high value suggests either a parallel alignment, or the absence of significant energy transfer between different BChl *g* 808 molecules. The negative polarization at 576 nm ($p \approx -0.05$) agrees with its assignment as the Q_x band of BChl *g*. Carotenoid showed a weak positive polarization ($p \approx +0.10$).

The linear dichroism spectrum obtained at room temperature of membrane fragments of *H. chlorum* is shown in Fig. 6. Orientation was obtained by suspending the membranes in gelatin and squeezing the gel in one direction. A polyacrylamide gel [10] could not be used for these experiments, because of a strong bleaching of BChl *g* during the polymerization process. In the Q_y region, the LD spectrum showed a maximum at 795 nm, and comparison with the absorption spectrum showed an increasing dichroic ratio $(A_{\parallel} - A_{\perp})/A$ (where A is the absorbance) with increasing wavelength, indicating that the Q_y transitions of BChl *g* 808 make a smaller angle with the membrane plane than those absorbing at shorter wavelengths. Maxima are also observed at 678 and 450 nm; minima at 573 and 409 nm. It should be noted that the LD values observed were relatively small. The spectrum of Fig. 6 was obtained with a gel that was pressed to a thickness of one-fourth of the original one. The dichroic ratio was a linear function of the compression factor, but even when extrapolated to zero thickness, the dichroic ratio was only 0.16 at 820 nm as compared to a maximal dichroic ratio of 1.5 in our arrangement for dipole moments parallel to the plane of orientation. This suggests a rather low degree of orientation of the sample. The Q_y transitions at shorter wavelengths appear to be oriented fairly close to the magic angle, or, more likely, to have several different orientations resulting in an effectively small LD. The fluorescence polarization data are in general agreement with this notion.

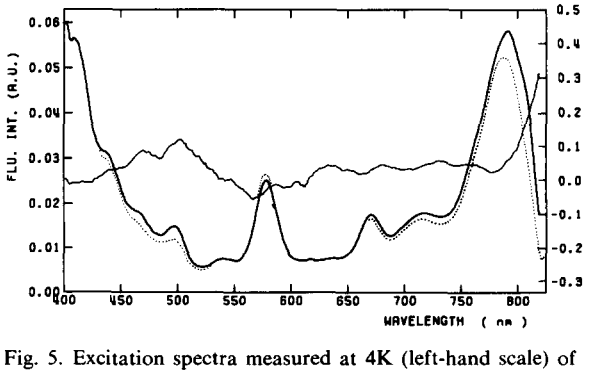

Fig. 5. Excitation spectra measured at 4K (left-hand scale) of fluorescence polarized either parallel (———) or perpendicular (·····) to the polarization of the excitation beam. The thin solid line (right-hand scale) shows the degree of polarization, $p = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$, as a function of wavelength. The absorbance was 0.50 at 790 nm. A.U., arbitrary units.

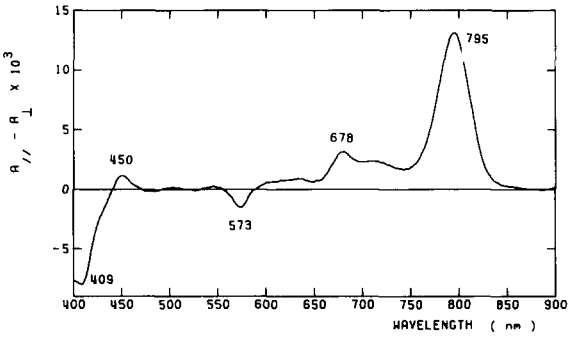

Fig. 6. Linear dichroism spectrum, measured at room temperature, of membranes of *Heliobacterium chlorum* contained in a pressed gelatin gel (see Materials and Methods). The absorbance was 0.20 at 789 nm.

Discussion

Our results show that the structure of the antenna system of *Heliobacterium chlorum* is considerably more complex than was suggested by the room temperature absorption spectra published earlier [1,3]. At least three different Q_y bands of BChl *g* (BChl *g* 778, 793 and 808) can be discerned in the absorption spectra of intact cells and membrane fragments at low temperature. The fluorescence excitation spectrum shows that energy absorbed by the shortwave bacteriochlorophylls is transferred with a high efficiency, approaching 100%, to BChl *g* 808.

In addition to bands of BChl *g* and carotenoid, the absorption spectrum of *H. chlorum* also contains a conspicuous band near 670 nm, which upon cooling is resolved in two bands at 668 and 677 nm. This absorption is clearly not due to an artefact produced by photooxidation of BChl *g* [3], since it is present in the same relative amount in intact cells and membrane fragments. It may be related to a pigment absorbing at about the same wavelength in membranes and isolated pigment-protein complexes of the green sulfur bacterium *Prosthecochloris aestuarii*, which was recently shown to be related to BChl *c* (Braumann, T. and Vasmel, H., unpublished experiments). In this connection it is of interest to note that recent results obtained by picosecond flash spectroscopy in our laboratory (Nuijs, A.M. et al., unpublished data) show that the primary electron acceptor in *H. chlorum* is a pigment absorbing near 670 nm, like that in green sulfur bacteria [11]. Comparison with absorption and excitation spectra of *P. aestuarii* membranes [6] indicates that the absorption at 440 nm is, at least in part, due to the Soret band of the 670 nm pigment. The overall efficiency of energy transfer from this pigment to Bchl *g* (70%) is comparable to that observed for *P. aestuarii* at low temperature [6]. The LD and fluorescence polarization spectra indicate that the two spectroscopically different forms have different orientations.

The heterogeneous structure of the pigment system of *H. chlorum* is also reflected by the other spectral data. Significant circular dichroism was only observed for BChl *g* 793, and the fluorescence polarization as well as the LD spectra show different orientations for the various BChl *g* Q_y and Q_x transition dipoles. Thus it appears that *H. chlorum* either contains more than one pigment protein complex, like most other photosynthestic bacteria, or that it contains one antenna protein that binds several spectrally distinct BChl *g* molecules.

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