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ORIENTATION OF PIGMENTS AND PIGMENT-PROTEIN COMPLEXES IN THE GREEN PHOTOSYNTHETIC BACTERIUM *PROSTHECOCHLORIS AESTUARII*

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Summary

The orientation of pigments and pigment-protein complexes of the green photosynthetic bacterium *Prosthecochloris aestuarii* was studied by measurement of linear dichroism spectra at 295 and 100 K. Orientation of intact cells and membrane vesicles (Complex I) was obtained by drying on a glass plate. The photochemically active pigment-protein complexes (photosystem-protein complex and reaction center pigment-protein complex) and the antenna bacteriochlorophyll *a* protein were oriented by pressing a polyacrylamide gel. The data indicate that the near-infrared transitions (Q_y) of bacteriochlorophyll *c* and most bacteriochlorophyll *a* molecules have a relatively parallel orientation to the membrane, whereas the Q_y transitions of the bacteriochlorophyll *a* in the antenna protein are oriented predominantly perpendicularly to the membrane. Carotenoids and the Q_x transitions (590—620 nm) of bacteriochlorophyll *a*, not belonging to the bacteriochlorophyll *a* protein, have a relatively perpendicular orientation to the membrane. The absorption and linear dichroism spectra indicate the existence of different pools of bacteriochlorophyll *c* in the chlorosomes and of carotenoid and bacteriopheophytin *c* in the cell membrane. The results suggest that the photosystem-protein and reaction center pigment-protein complexes are oriented with their short axes approximately perpendicular to the plane of the membrane. The symmetry axis of the bacteriochlorophyll *a* protein has an approximately perpendicular orientation.

Abbreviations: LD, linear dichroism; BChl *a*, bacteriochlorophyll *a*; BChl *c*, bacteriochlorophyll *c*; BPh *c*, bacteriopheophytin *c*; PP complex, photosystem-pigment complex; RCPP complex, reaction center pigment-protein complex.

Introduction

The study of linear dichroism is a valuable method to obtain insight in the orientation of electronic transition moments. Information has been obtained in this way about the orientation of pigments in the photosynthetic membrane of various organisms, including purple bacteria [1—5], algae [6] and higher plants [7—10]. However, data on the orientation of pigments in green photosynthetic bacteria were not available as yet. In this paper we present the results of measurements of linear dichroism spectra of various types of preparations and intact cells of the green bacterium *Prosthecochloris aestuarii.*

P. aestuarii contains two distinct antenna chlorophylls: bacteriochlorophyll *c*, which is located exclusively in the chlorosomes, the so-called chlorobium vesicles [11,12], and bacteriochlorophyll *a*, located in or attached to the cell membrane. About half of the BChl *a* is present in a water-soluble BChl *a*-protein complex [13,14], which seems to be situated between the chlorosome and the cell membrane [15].

Two photochemically active pigment-protein complexes have been obtained [16] from the membrane vesicle preparation Complex I [17,18]. The photosystem-pigment (PP) complex has a particle weight of about $6 \cdot 10^5$ and contains about 75 BChl *a* molecules per reaction center. By removal of the antenna BChl *a*-protein complex, the reaction center pigment-protein (RCPP) complex was obtained. This pigment-protein complex has a particle weight of about $3.5 \cdot 10^5$ and contains about 35 BChl *a* molecules per reaction center [16].

The data reported in this paper allow a comparison of the LD spectra, measured at 295 and 100 K and the absorption spectra, measured at 100 and 4 K of intact cells of *P. aestuarii* and of various subcellular preparations of decreasing size and complexity.

Materials and Methods

Prosthecochloris aestuarii, strain 2 K, was grown anaerobically in a mixed culture originally known as *Chloropseudomonas ethylica* [19] as described by Holt et al. [20]. The membrane preparation Complex I, and the reaction center preparations PP and RCPP complex were prepared as previously described [16]. The BChl *a* protein was purified according to Ref. 21. Low temperature absorption spectra were measured with a single beam spectrophotometer constructed in the laboratory and equipped with an optical Dewar for measurement at 100 K and a helium flow cryostat for measurements at 4 K (Rijgersberg, C.P., unpublished data). Glycerol (50% v/v) was present to prevent crystallization upon cooling.

Linear dichroism (LD) spectra, measured with a single beam apparatus constructed in the laboratory as described elsewhere (De Grooth, B.G., unpublished data), were recorded by measuring the rotation of the plane of polarization of the measuring light by a dichroic sample, as described by Breton et al. [7]. The apparatus was equipped with an optical Dewar for measurements at 100 K.

Orientation of bacteria and of Complex I was obtained by using the air-drying technique [1,7,8]. The LD spectra were measured with the glass plate tilted by an angle of 45° with respect to the measuring beam. The linear dichroism induced by the glass plate was negligible. The PP and RCPP complex, the

BChl *a* protein, and in some cases intact bacteria were oriented by pressing a polyacrylamide gel in a similar way as described by Abdourakhmanov et al. [22]. The gel was prepared by mixing acrylamide, final concentration 9% (w/v), *N,N'*-methylene-bisacrylamide 0.25% (w/v), glycerol 45% (v/v), *N,N,N',N'*-tetramethylethyldiamine 0.014% (v/v) and ammonium persulfate 0.045% (w/v) in 40 mM phosphate, pH 7.4. Gels were pressed in a cuvette with an optical pathlength of 1 cm, thereby squeezing the gel in one dimension perpendicular to the direction of pressing whereas the other dimension was fixed by the cuvette. For the measurements of the LD spectra, the relative decrease of the height of the gel varied from about 0.2 for the BChl *a* protein to about 0.4 in case of intact cells and the PP and RCPP complexes. Apart from a few cracks, the gels remained optically clear upon cooling to 100 K. In case of air-drying, $A_\perp$ was defined as the absorbance of the measuring light polarized perpendicularly to the plane of the glass plate. In case of orientation in a gel, $A_\perp$ was defined as the absorbance of the measuring light polarized parallel to the direction of pressing of the gel.

Results and discussion

Orientation of pigments

Figs. 1 and 2 show the absorption spectra, measured at 100 and 4 K and the LD spectra measured at 295 and 100 K of intact cells of *Prosthecochloris aestuarii* and of Complex I, a membrane vesicle preparation, oriented by air-drying. We assume that, like with chloroplasts [7,8] and intact cells and chromatophores of purple bacteria [1,2], the membranes are preferably oriented parallel to the glass plate by the drying process. Therefore the LD spectra yield information about the orientation of the pigments relative to the plane of the cell membrane. The absorption spectra were also measured at 4 K in order to obtain better resolution and thus to facilitate comparison with the LD spectra.

The spectra obtained with whole cells are dominated by the absorbance of BChl *c*. The position of the Q_y band of BChl *c* in the absorption spectrum shifted from 745 nm at room temperature [23] to 753 nm at 100 K, whereas the LD spectrum at room temperature showed a positive band at 753 nm, which was resolved in two bands at 744 and 758 nm at 100 K. These data indicate the existence of at least two pools of BChl *c* of different size and orientation. For the Q_y transitions of BChl *c* the angles calculated relative to the orientation axis, which is normal to the membrane, were larger than the magic angle of 54.7°, indicating that these transitions (which are not necessarily those of the monomers) are oriented more or less parallel to the membrane. The positive band around 465 nm, where part of the absorption may be due to the B_x transition of BChl *c*, also indicates a relatively parallel orientation. These results suggest that the tetrapyrrolic rings of at least part of the BChl *c* molecules are oriented more or less parallel to the cell membrane.

In the region of BChl *a* absorption in the near-infrared (770—850 nm), the spectra obtained with whole cells appear to be similar to those of Complex I, a membrane vesicle preparation that is practically devoid of BChl *c*. In particular, both preparations show maxima at 805 and 815 nm in the low temperature absorption spectra, and a clear negative band at about 815 nm in the LD spec-

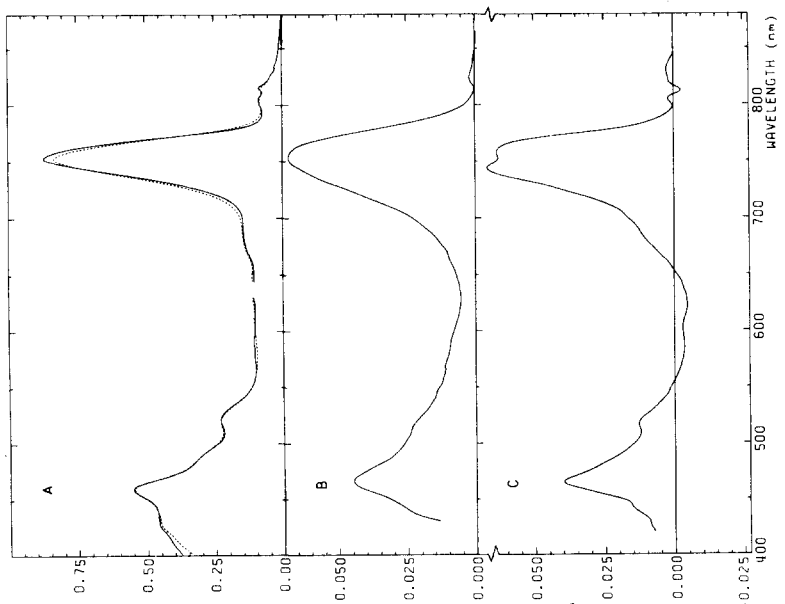

Fig. 1. (A) Absorption spectra of cells of *P. aestuarii* at 100 K (broken line) and 4 K (solid line); A_{745} = 0.80 at room temperature. (B) and (C) Linear dichroism spectra, measured at room temperature and at 100 K, respectively. Orientation by air drying. A_{745} = 0.40 at room temperature, measured with the glass plate perpendicular to the measuring beam.

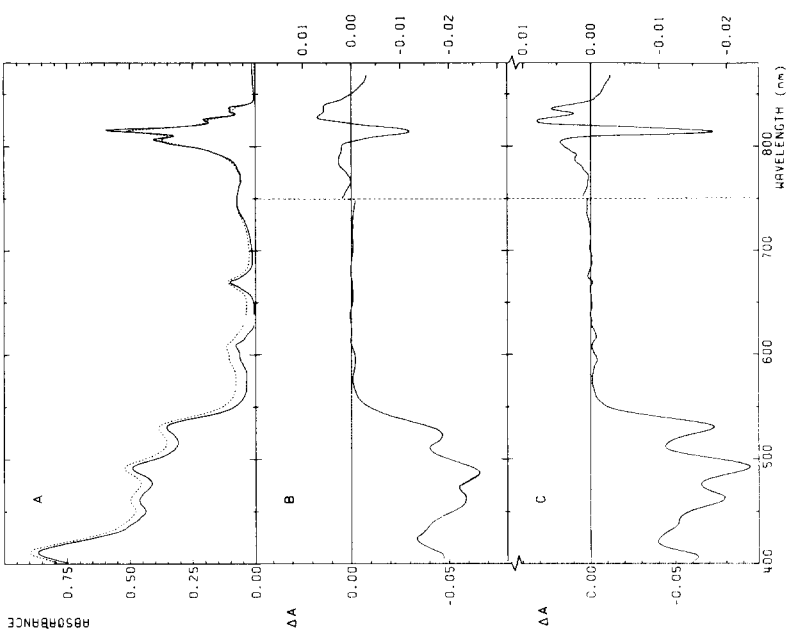

Fig. 2. (A) Absorption spectra of Complex I at 100 K (broken line) and 4 K (solid line); A_{810} = 0.30 at room temperature. (B) and (C) Linear dichroism spectra, measured at room temperature and at 100 K, respectively. Orientation by air drying. A_{810} = 0.15 at room temperature, measured with the glass plate perpendicular to the measuring beam.

tra at 100 K. However, the spectra of Complex I are much better resolved. The LD spectra (Fig. 2) show positive bands at 836, 824, 802–806 and 780–800 nm in addition to a negative band at 815 nm. These bands are in close correspondence to the peaks in the absorption spectra at 100 and 4 K and presumably (cf. Ref. 24) belong to the Q_y transitions of BChl *a*. The Q_x transition of BChl *a* is resolved in three bands at about 595, 609 and 618 nm in the absorption spectrum at 4 K, whereas the LD spectrum shows negative bands at 596 and 618 nm. These data can be interpreted by assuming that the Q_y transitions of most BChl *a* molecules make a relatively small angle with the plane of the membrane, while the Q_x transitions and also the Q_y transition of the BChl *a* molecules absorbing at 815 nm have relatively perpendicular orientation. As will be shown below, the latter band is mainly due to the antenna BChl *a* protein.

The LD spectrum at 100 K of Complex I shows negative bands at 462, 491 and 530 nm, corresponding to bands at the same wavelength in the absorption spectra, which belong to carotenoid. It is concluded, that at least part of the carotenoids are oriented with their long axis more or less perpendicularly to the membrane; a similar orientation was observed in purple bacteria [3–5]. The long wavelength band of carotenoid at 514 nm in the absorption spectrum at room temperature [16] shifted to 530 nm upon cooling to 100 K, whereas a smaller shift, from 523 to 530 nm, occurred in the LD spectra. This suggests the existence of at least two different pools of carotenoid: a relatively small pool which is oriented more or less perpendicular to the membrane and another pool which is oriented randomly or near the magic angle. At room temperature, the latter pool absorbs at shorter wavelengths than the other one; it shows a larger red shift upon cooling.

The band in the absorption spectra situated at 670 nm is probably due to BPh *c*. Chromatography of an acetone-methanol extract of the PP complex on a silica gel plate yielded amongst other things a green spot, the absorption spectrum of which was characteristic for BPh *c* [25]. The absorption spectra at 4 K of Complex I and also of the PP and RCPP complexes (Figs. 4 and 5) suggest the existence of three different forms of BPh *c* with Q_y transitions absorbing at about 665, 670 and 675 nm, respectively. The LD spectra show positive bands at 665 and 675 nm and a negative band at 670 nm, indicating that the Q_y tran-

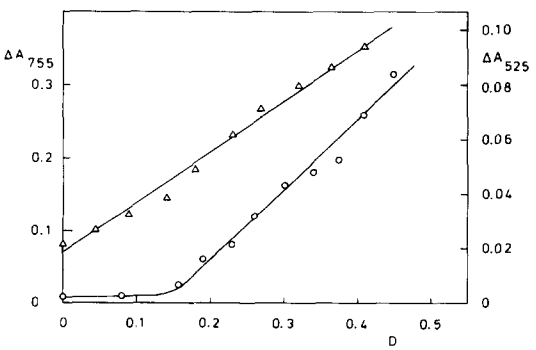

Fig. 3. Linear dichroism ($A_{/\!/} - A_{\perp}$) at room temperature as a function of *D*, the relative decrease of height of the pressed gel. Circles (left hand scale): intact cells, $A_{745} = 0.80$ measured at 755 nm; triangles (right hand scale): RCPP-complex, measured at 525 nm. $A_{813} = 0.25$.

sition of the BPh *c* molecules absorbing at 665 and 675 nm makes a relatively small angle with the plane of the membrane, while that of the BPh *c* absorbing at 670 nm points out of the membrane. These bands are most clearly seen in the low temperature LD spectra of the RCPP complex, where a relatively dense sample was used to obtain larger signals in the region 550—700 nm, but they can also be observed in the other preparations.

From the $\Delta A/A$ values of intact cells and Complex I oriented by air-drying deviations from the magic angle could be calculated, which were small (2—5%). This is partly due to imperfect orientation of the sample: similar spectra but with higher $\Delta A/A$ values were obtained for intact cells by means of the gel pressing method. Since the latter measurements were done with a Cary 14, modified to collect the light at a large solid angle, this indicates that the effects of selective scattering (cf. Ref. 26) on the linear dichroism spectra was very small, even for intact cells, for which the effect should be most severe [27]. The observed relation between ΔA and the deformation of the gel, shown in Fig. 3 (circles), suggests that even larger ΔA values would have been obtained by more extensive deformation of the gel.

Orientation of the pigment-protein complexes

Figs. 4—6 show the absorption and LD spectra of the PP complex, the RCPP

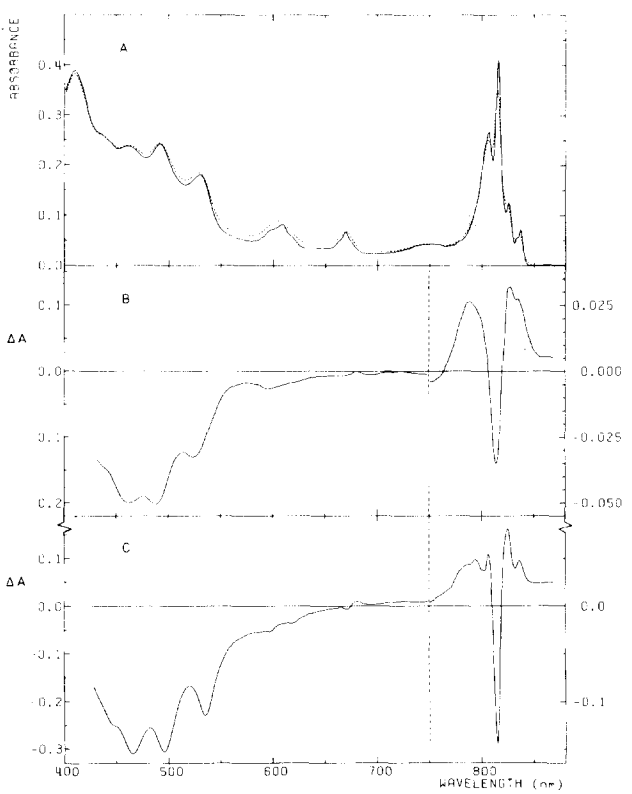

Fig. 4. (A) Absorption spectra of the PP-complex at 100 K (broken line) and 4 K (solid line); A_{810} = 0.20 at room temperature. (B) and (C) Linear dichroism spectra, measured at room temperature and at 100 K, respectively. Orientation in a pressed gel. A_{810} = 0.34 at room temperature in the unoriented sample.

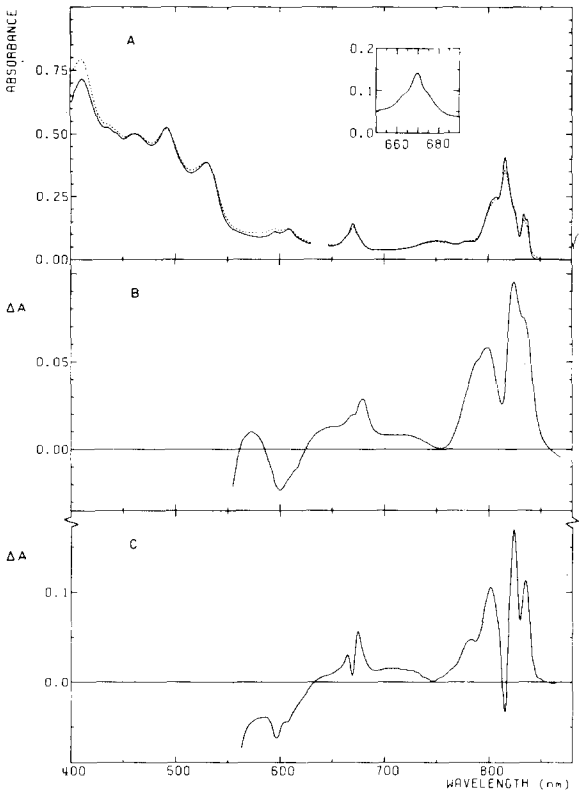

Fig. 5. (A) Absorption spectra of the RCPP-complex at 100 K (broken line) and 4 K (solid line); A_{813} = 0.21 at room temperature. Insert: the BPh *c* band at 4 K with expanded absorbance and wavelength scale. (B) and (C) Linear dichroism spectra, measured at room temperature and at 100 K, respectively. Orientation in a pressed gel. A_{813} = 0.53 at room temperature in an unoriented sample.

complex and the antenna BChl *a* protein oriented in a gel. Over the whole wavelength region, the absorption and LD spectra of the PP and RCPP complex show a close resemblance to those of Complex I. This indicates that the structural integrity of the pigment-protein complexes remained intact during the isolation procedure, as was also indicated by the observation that the photochemical activity is retained [16]. Furthermore, the effect of selective scattering, which is strongly dependent on the size of the particles [27], seem negligible, since Complex I and the pigment-protein complexes have a very different size. We assume that the particles, by pressing the polyacrylamide gel, were oriented with their short axes predominantly parallel to the direction of pressing. Since the LD spectra of Complex I, the PP and the RCPP complex are very similar in shape, it might be concluded that the short axes of these pigment-protein complexes are situated approximately perpendicular to the plane of the membrane. One should, however, consider the possibility that the shape of the complexes is affected by the detergent molecules that are attached to the surface.

The $\Delta A/A$ values of the different LD bands of the PP and RCPP complex, oriented in a gel, were larger than the corresponding values for Complex I, oriented by air-drying. Fig. 3 (triangles) shows the LD signal of the RCPP com-

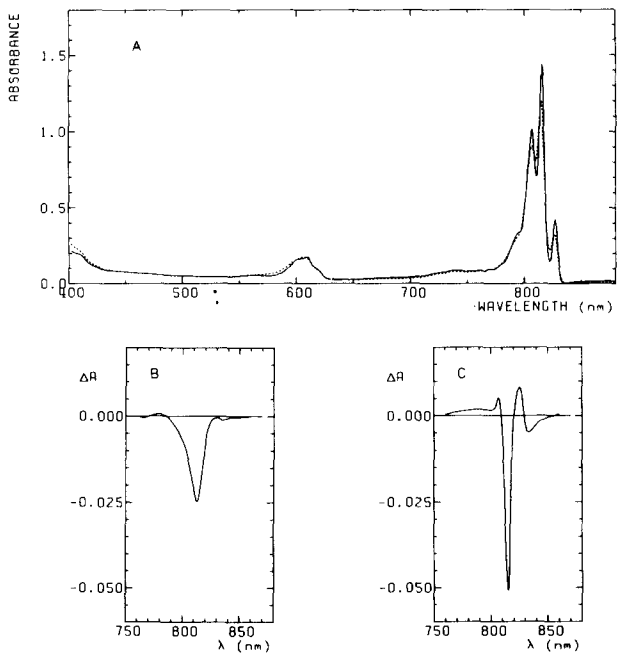

Fig. 6. (A) Absorption spectra of the BChl *a*-protein complex at 100 K (broken line) and at 4 K (solid line). $A_{809} = 0.76$ at room temperature. (B) and (C) Linear dichroism spectra, measured at room temperature and at 100 K, respectively. Orientation in a pressed gel. $A_{809} = 0.76$ at room temperature in an unoriented sample.

plex as function of the degree of deformation of the gel. Because of the unknown dimensions of the particles no meaningful values of the angles between the transition moment and the direction of pressing of the gel could be calculated.

The difference between the LD spectra at 100 K of the PP and RCPP complex is in close agreement with the LD spectra at 100 K of the purified BChl *a* protein, shown in Fig. 6. The same applies to the absorption spectra in the near-infrared, as discussed earlier [16]; the differences are due to the removal of two BChl *a* proteins per PP complex. The LD spectrum at 295 K of purified BChl *a* protein oriented in a gel is similar to the spectrum of the BChl *a* protein oriented in an electric field, as measured by Whitten et al. [28]. No detectable LD signal ($\Delta A < 4 \cdot 10^{-4}$) was obtained in the Q_x region (570—650 nm) of the BChl *a* protein.

The dimensions of the BChl *a* protein trimer are about 5.7 nm along the 3-fold symmetry axis and about 8.3 nm perpendicular to it [14]. This symmetry axis presumably orients preferably parallel to the press direction of the gel. Since the negative peak at 815 nm in the LD spectrum of Complex I like in that of the PP complex presumably belongs to the BChl *a* protein, we conclude that the BChl *a* protein trimer is oriented with the 3-fold symmetry axis approximately perpendicular to the membrane. Inside the BChl *a* protein the average direction of the transition dipoles absorbing near 815 nm makes an angle smaller than the magic angle with the symmetry axis. The low tempera-

ture absorption spectra and the LD spectra of the RCPP complex may suggest that part of the BChl *a* is present in a protein complex similar to the antenna BChl *a* protein complex and with about the same orientation of the BChl *a* core relative to the membrane.

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