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PHOTOCHEMICALLY ACTIVE PIGMENT-PROTEIN COMPLEXES FROM THE GREEN PHOTOSYNTHETIC BACTERIUM *PROSTHECOCHLORIS AESTUARII*

T. SWARTHOFF and J. AMESZ

Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden (The Netherlands)

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Summary

Photochemically active pigment-protein complexes were prepared from a bacteriochlorophyll *a* containing membrane preparation of the green photosynthetic bacterium *Prosthecochloris aestuarii*. The preparations contained about 75 and 35 bacteriochlorophyll *a* molecules per reaction center and had molecular weights of $6 \cdot 10^5$ and $3.5 \cdot 10^5$, respectively. Some of the other properties of these preparations are described.

Recent advances in the study of bacterial photosynthesis, and in particular of primary and associated reactions, are largely due to experiments with purple bacteria. Data on the photosynthetic reactions in green bacteria are scarce, and attempts to obtain subcellular fractions that are photochemically active have met with limited success. Fowler et al. [1] and Olson et al. [2] have reported the isolation of membrane vesicles that are nearly free of chlorobium chlorophyll and are photochemically active. However, attempts to remove antenna BChl *a* resulted in loss of most of the activity [3, 4]. This report deals with the results of fractionation experiments based on detergent treatment of the photosynthetic membrane. It will be shown that pigment-protein complexes can be solubilized without loss of reaction center activity.

Prosthecochloris aestuarii, strain 2 K, was grown anaerobically in a mixed culture originally known as *Chloropseudomonas ethylica* [5] as des-

Abbreviations: BChl *a*, bacteriochlorophyll *a*; BChl *c*, bacteriochlorophyll *c*; BPh *c*, bacteriopheophytin *c*; *P*-840, reaction center bacteriochlorophyll *a* absorbing near 840 nm; Triton X-100, polyoxyethylene (10)-*p*-octylphenyl ether; SDS, sodium dodecyl sulfate.

cribed by Holt et al. [6]. The cells were harvested by centrifugation, washed once with a solution containing 10 mM sodium phosphate and 10 mM sodium ascorbate, pH 7.4, and stored as a concentrated suspension in liquid nitrogen until use. All purification steps to be described below were carried out at 0—4° C, unless otherwise indicated.

A vesicle preparation resembling 'Complex I' obtained by Olson et al. [3, 7] from *Chlorobium limicola* was prepared by a modification of the method described by Fowler et al. [1]. A concentrated suspension of this preparation (absorbance 25—30 cm^{-1} at 810 nm) was incubated for 1 h at room temperature with 1% (w/w) Triton X-100. After the incubation the reaction mixture was diluted 3—5 times with phosphate-ascorbate buffer and put on a sucrose gradient containing 0.05% Triton X-100. The gradient consisted of a 10—40% continuous gradient on top of a layer of 50% sucrose. After centrifugation for 16 h at 40 000 rev./min in a SW 41 swing-out rotor, three bands had formed in the gradient. An orange band was situated in the top of the tube, containing carotenoid, pigments absorbing at 410 and 670 nm, and some BChl *a*. A narrow green band at 35% sucrose contained mainly BChl *c*. About 70% of the BChl *a* was present in a greenish-brown fraction situated near the 40—50% interface. Most of the reaction center activity was contained in this fraction, which we call the photosystem-protein complex. The preparation, as judged from the absorption spectrum (Fig. 1) contained much less carotenoid and BChl *c* than Complex I (on basis of BChl *a*). From the elution volume on a Sepharose-Cl-4B column a particle weight of (6.0 ± 0.5) • 10^5 was calculated.

Removal of part of the antenna BChl *a* was obtained by the use of guanidine-HCl. Direct treatment of the photosystem-protein complex with 1 M guanidine-HCl, however, resulted in loss of photochemical activity. Therefore, the complex was diluted with an equal volume of phosphate-ascorbate

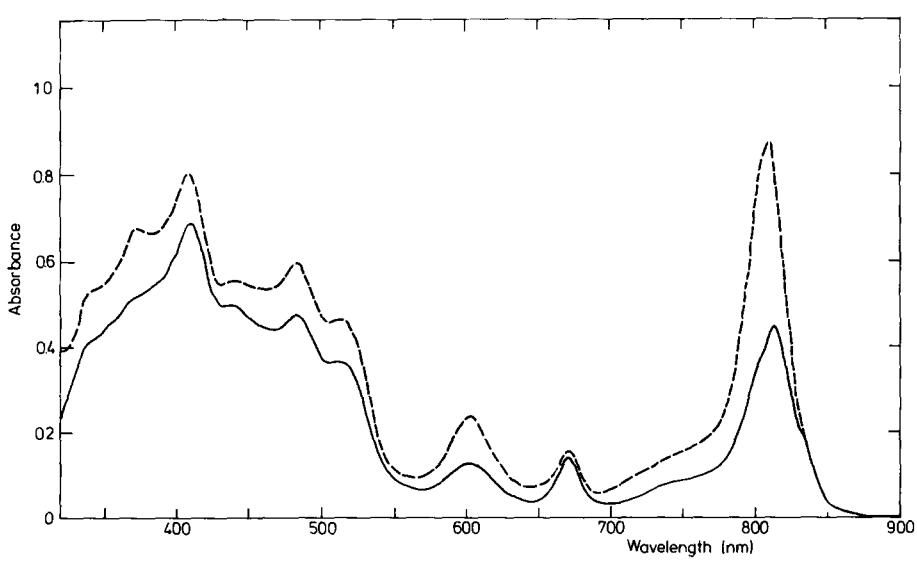

Fig. 1. Absorption spectra measured at room temperature of the photosystem-protein complex (- - -) and of the reaction center pigment-protein complex (—).

buffer to reduce the sucrose concentration to 20%, and put on a discontinuous sucrose gradient consisting of layers of 25, 30, 40 and 50% sucrose, respectively. The 30% sucrose layer contained 1 M guanidine-HCl, pH 7.4. After centrifugation in a Beckman SW 41 rotor (40 000 rev./min, 2 h) two bands had formed: a blue-green one in the 25% layer and a brownish-green band at the 40—50% interface. The amount of BChl *a* contained in the two bands was approximately equal. The blue-green band consisted of the light-harvesting BChl *a* protein described by Olson [8] and Fenna and Matthews [9]. The reaction center activity was contained in the brownish-green band. We call this preparation the reaction center pigment-protein complex. Its absorption spectrum is shown in Fig. 1; compared to the photosystem-protein complex, the absorbance had decreased at 340, 370, 603 and 810 nm, relative to the shoulder at 835 nm, indicating a specific removal of antenna BChl *a*. In the near-infrared region, the spectrum is very similar to that of 'Complex II' from *Chlorobium limicola* [3], a preparation obtained from Complex I by the action of guanidine-HCl, which has, however, little detectable photochemical activity [4]. The relatively high sedimentation velocity and a slight turbidity indicated aggregation when suspended in phosphate-ascorbate buffer. The turbidity disappeared upon addition of Triton X-100, and a particle weight of $(3.5 \pm 0.5) \cdot 10^5$ could be calculated from the elution volume on the Sepharose column in the presence of 0.05% Triton X-100.

A large number of peptide bands were observed upon SDS-gel electrophoresis of the reaction center pigment-protein complex. The major components had molecular weights of 26, 30, 36, 43, 45 and $46 \cdot 10^3$. The pattern observed with the photosystem-protein complex was identical, except that an additional intense band of $42 \cdot 10^3$ was present, apparently due to the subunits of the antenna BChl *a* protein (see Olson [8]).

Low temperature absorption spectra of the pigment-protein complexes are shown in Fig. 2. The spectra at 4.2 K showed the same general features

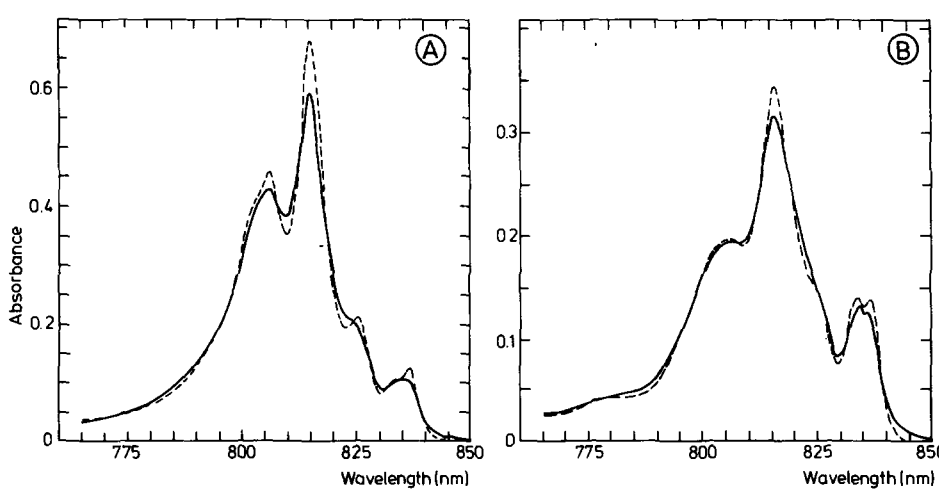

Fig. 2. (A) Absorption spectra of the photosystem-protein complex at 77 K (——) and 4.2 K (- - - - -). (B) Absorption spectra of the reaction center pigment-protein complex at 77 K (——) and 4.2 K (- - - - -). Glycerol (50% v/v) was present to prevent crystallization upon cooling.

as those measured at liquid nitrogen temperature, except for sharpening and slight shifts of the absorption bands, as was also noted for Complexes I and II [4]: The spectrum of the photosystem protein complex at 77 K was very similar to that of Complex I of *P. aestuarii* published by Olson [8] and Fowler et al. [10]. In agreement with the room temperature spectrum, the reaction center pigment-protein complex showed a clearly enhanced absorption around 835 nm as compared to the absorption at shorter wavelenghts. In both preparations, the 835 nm absorbance was resolved in two peaks, located at 834 and at 836.5 nm at 4.2 K. Comparison of the infrared spectra of the two preparations indicates that the narrow band at 806, the band at 825 and a large part of the band at 815.5 nm in the spectrum of the photosystem-protein complex at 4.2 K are due to the light-harvesting BChl *a* complex, in good agreement with the spectrum of the isolated light-harvesting complex as measured at 77 K by Olson and coworkers [2, 8].

Fig. 3 shows the light-induced difference spectrum of Complex I. Between 740 and 900 nm the spectrum was similar to that of the 'heavy fraction' reported by Fowler et al. [1]. Because of the complexity of the spectrum it is uncertain which peak represents the bleaching of the reaction center BChl *a*. If one assumes that *P*-840 bleaches at 842 nm and has the same specific extinction coefficient (100 $mM^{-1} \cdot cm^{-1}$) as the antenna BChl *a* [2], then there are about 80 antenna BChl *a* per reaction center. The difference spectrum in the visible region reflects the oxidation of *P*-840 and of cytochrome *c*-553 [1]. The amount of photooxidizable cytochrome *c*-553 was about three moles per mole reaction center, if it is assumed that $\Delta\epsilon_{540-553}$ equals 20 $mM^{-1} \cdot cm^{-1}$ [2].

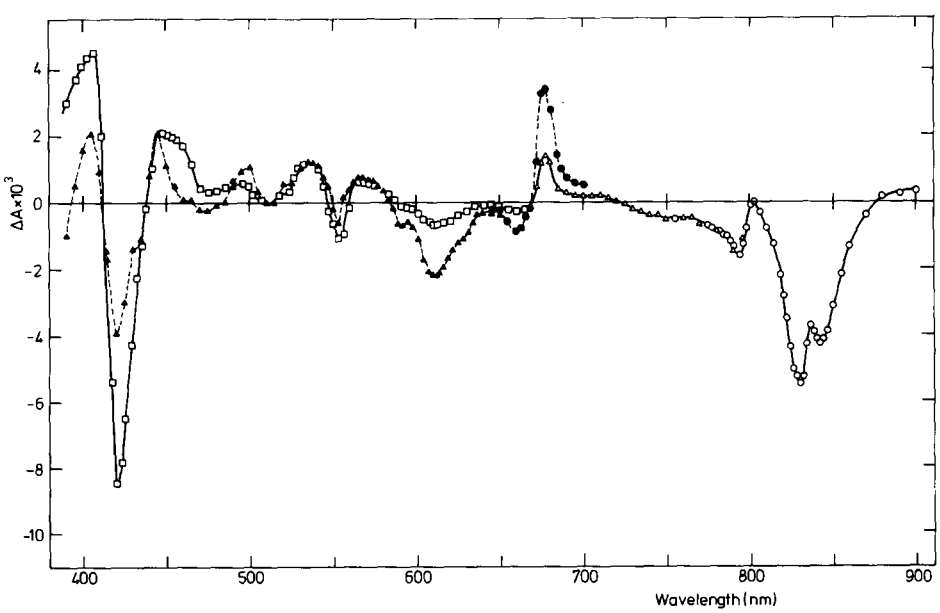

Fig. 3. ——, open symbols: Light-induced difference spectrum of Complex I (A_{810} = 0.37). Actinic illumination: 5 s, 7.5 mW/cm². - - - -, solid symbols: Light-induced difference spectrum of the photosystem-protein complex (A_{810} = 0.85) obtained by 1 s infrared illumination (I = 20 mW/cm²). Different symbols refer to different samples, normalized to an equal absorbance at 810 nm. Illumination: Schott AL 606 interference filter for the infrared and Schott RG 715 for the visible region.

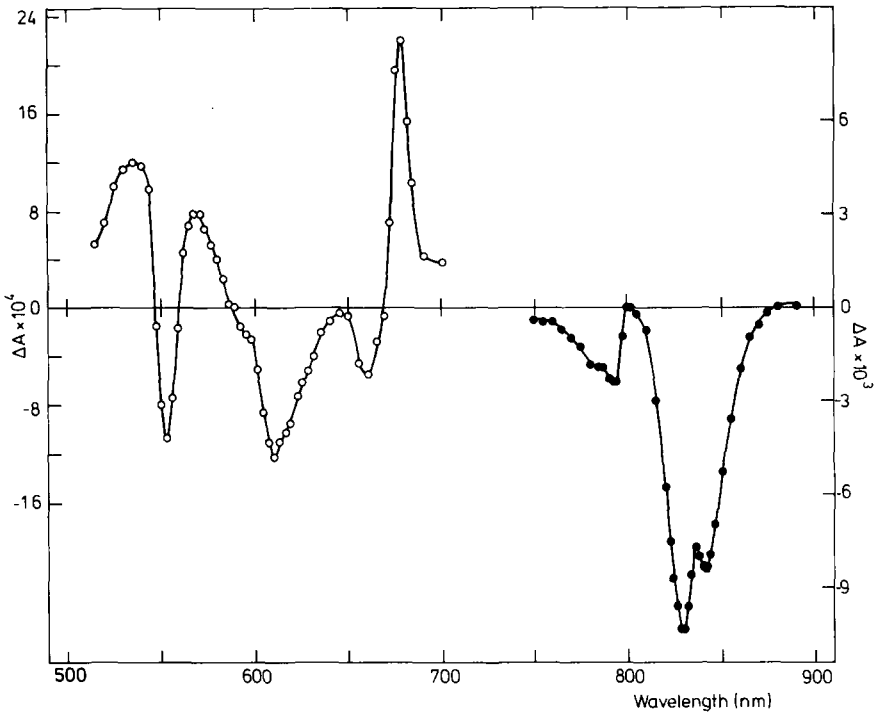

Fig. 4. Light-minus-dark absorbance difference spectrum of the RCPP-complex ($A_{810} \doteq 0.23$). Illumination: 15 s for the region 750—900 nm (right-hand scale) and 5 s for the region 510—700 nm (left-hand scale). Intensity: 20 mW/cm^2. Different symbols refer to different samples, normalized to an equal absorbance at 810 nm. Further conditions as for Fig. 3.

The light-induced difference spectrum in the 740—900 nm region of the photosystem-protein complex (not shown) has the same shape as that of Complex I with an amplitude corresponding to an antenna BChl *a*:*P*-840 ratio of about 75. The visible part of the difference spectrum is shown in Fig. 3 (broken line). The amount of photooxidizable cytochrome *c*-553 in the photosystem-protein complex varied between 0.5 and 1.5 per reaction center for different preparations.

Fig. 4 shows the difference spectrum of the reaction center pigment-protein complex. The amount of photooxidizable cytochrome *c*-553 was about the same as in the photosystem-protein complex. The near-infrared part of the spectrum was again very similar to that of Complex I, indicating that the structure of the reaction center remains intact during the purification procedure. The amplitude had increased by a factor of about 2.3 for equal absorbance at the near-infrared BChl *a* maximum, corresponding to about 35 BChl *a* per reaction center. This indicates that two light-harvesting BChl *a*-protein complexes per reaction center, together containing 42 BChl *a* molecules [9], are released by the treatment with guanidine-HCl. Since the molecular weight of the BChl *a* protein complex is about $1.4 \cdot 10^5$ [8], this is in agreement with the decrease in particle weight from about 6 to about $3.5 \cdot 10^5$.

For each kind of preparation, the kinetics of the light-induced absor-

bance changes at 610 nm were identical to those in the near-infrared region. For Complex I and the photosystem-protein complex a rapid decrease of absorbance, too fast to be measured with the time resolution of the apparatus, was followed by a smaller, slower decrease. The light-on response of the reaction center pigment-protein complex was much slower and resembled that obtained with Complex I at an about 50 times lower actinic light intensity. This is probably not due to a low efficiency of trapping or of the primary charge separation. Measurements of the kinetics of *P*-840 after brief illumination periods and at low temperature suggested that the high intensities or long illumination periods necessary to bring about complete oxidation of *P*-840 are probably due to a low rate of electron transport between primary and secondary acceptors.

In all preparations a negative band at 660 and a positive band at 678 nm were present in the light-induced difference spectrum (Figs. 3 and 4). The kinetics at 678 nm were the same as those of *P*-840. These bands, which have not been reported earlier, may be caused by an electrochromic red shift of the pigment absorbing at 670 nm, which is perhaps BPh *c* [3, 7]. In this respect it is interesting to note that in the pigment-protein complexes this pigment was always present in nearly the same amount per reaction center. A shoulder at 675 nm was also observed in the absorption spectrum of intact cells [11]. This might indicate that the pigment is not a contaminant, as suggested by Olson et al. [3], but a functional component of the reaction center pigment-protein complex.

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