Biochimica et Biophysica Acta, 1015 (1990) 223–230
Elsevier

BBABIO 43136

Purification and properties of an antenna-reaction center complex from heliobacteria

Erik Jan van de Meent, Frank A.M. Kleinherenbrink and Jan Amesz

Department of Biophysics, Huygens Laboratory of the State University, Leiden (The Netherlands)

(Received 3 July 1989)
(Revised manuscript received 10 October 1989)

Key words: Antenna-reaction center complex; Electron transport; Energy transfer; Bacteriochlorophyll *g*; (*H. chlorum*) (*Hb. mobilis*)

Antenna-reaction center complexes were prepared by incubation of membranes of *Heliobacterium chlorum* and *Heliobacillus mobilis* with the detergent sulfobetaine-12, followed by sucrose gradient centrifugation in the presence of sulfobetaine-12 and sodium cholate. Similar results were obtained with *n*-octyl β-D-glucopyranoside. Further purification was obtained by size-exclusion HPLC. From the elution volume, the molecular masses for the purified complexes solubilized with sulfobetaine-12 were estimated to be 335 kDa for both species, not corrected for the weight of the associated detergent molecules. SDS-polyacrylamide gel electrophoresis showed the presence of a dominant peptide located at 94 kDa, together with minor and variable bands near 50 kDa and above 100 kDa. The spectral properties of the isolated complexes were very similar for the two species and also almost identical to those of the membranes used as starting material. Fluorescence emission and excitation spectra showed efficient energy transfer within the complexes. The reaction center activity, measured by the yield and extent of photo-oxidation of the primary electron donor P-798, was completely conserved. These observations indicate that the structure of the antenna and of the reaction center was fully retained during solubilization and isolation. It is concluded that the complexes probably contain the complete pigment complement of a single photosynthetic unit, together with the associated reaction center. Although the estimated molecular weights of the antenna-reaction center complexes are similar to that of the Photosystem I core, the peptide composition appears to suggest a basically different structure.

Introduction

The heliobacteria are strictly anaerobic, nitrogen fixing photosynthetic bacteria that were discovered only quite recently [1]. They are distinguished from other classes of photosynthetic bacteria by the possession of a new type of bacteriochlorophyll, BChl *g* [1,2]. The first species of this group, *Heliobacterium chlorum*, was isolated from a soil sample of the Indiana University [1]. Other species, like *Heliobacillus mobilis* [3] have been isolated from rice paddies in Thailand and elsewhere (Ormerod, J., unpublished data).

Studies on the molecular mechanism of the photosynthetic process in heliobacteria have been confined so far to *H. chlorum*. The primary electron donor of *H. chlorum.*, P-798 [4–6], is probably a dimer of BChl *g* [5,7]. Studies on the electron acceptor chain suggested that the properties of the reaction center of heliobacteria are more similar to those of green sulfur bacteria and PS I of green plants than to those of purple bacteria and PS II. Flash spectroscopic measurements indicated that the primary electron acceptor, as in green sulfur bacteria, absorbs near 670 nm, and is presumably a BChl *c*- or Chl *a*-like pigment [6,8]. There is also evidence that an iron-sulfur center may act as secondary electron acceptor, but it is not clear if it is part of the main electron transfer chain [7,9].

Abbreviations: A-RC, antenna-reaction center; BChl, bacteriochlorophyll; HPLC, high-performance liquid chromatography; OGP, *n*-octyl β-D-glucopyranoside; P-798, primary electron donor; PAGE, polyacrylamide gel electrophoresis; PS, photosystem; PMS, *N*-methylphenazonium methosulfate; SB-12, *n*-dodecyl-*N*,*N*-dimethylammoniopropylsulphonate (sulfobetaine-12); SDS, sodium dodecyl sulfate; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.

Correspondence: E.J. van de Meent, Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden, The Netherlands.

Little is known about the structure of the photosynthetic membrane and of the antenna. Low temperature absorption spectra showed the presence of three spectral forms of BChl *g*: BChl *g*-778, BChl *g*-793 and BChl *g*-808 [10], suggesting a fairly complicated antenna structure. Measurements of fluorescence [10] and flash-induced absorbance changes [6,8] indicated rapid energy transfer from the short-wavelength-absorbing BChls to BChl *g*-808, even at cryogenic temperatures.

In this paper we report the isolation and purification of an antenna-reaction center complex by means of detergent treatment of membranes of *H. chlorum* and *Hb. mobilis*. The complexes appear to be functionally and structurally intact. They have an estimated molecular mass of 310 kDa and probably consist of a single photosynthetic unit, together with the associated reaction center.

Materials and Methods

Heliobacterium chlorum and *Heliobacillus mobilis*, both obtained from the American Type Culture Collection (ATCC), were grown batchwise under a nitrogen atmosphere at 37°C, at a light intensity of 5000 lux from incandescent lamps. For both species medium No. 1552 of the ATCC was used, containing 2.5 mM sodium ascorbate, since growth of *H. chlorum* was found to be more rapid in this medium than in medium No. 112, originally used by Gest and Favinger [1]. Near the end of the exponential growth phase the cells were harvested by centrifugation and resuspended in a buffer containing 10 mM sodium ascorbate and 10 mM Tris (pH 8.0). Membrane fragments were prepared by sonication followed by two centrifugation steps, each of 20 min at $20000 \times g$ to remove unbroken cells and large cell fragments. The membranes were further purified by centrifugation of the supernatant at $265000 \times g$ for 1 h on a 30% (w/v) sucrose cushion in buffer. In this way a concentrated membrane suspension was obtained with a typical absorbance of 15 mm^{-1} at 786 nm.

Detergent incubation was performed with SB-12 (Serva Feinbiochemica, Heidelberg) at a concentration of 1.32% (w/v) for 30 min at 4°C in the dark. The absorbance of the suspension was 10 mm^{-1} at 786 nm during incubation. The incubation mixture was then loaded on top of a 20–55% (w/v) sucrose gradient with a 75% sucrose layer on the bottom. The sucrose solutions were prepared with buffer containing 0.1% SB-12 and 0.1% sodium cholate. Centrifugation was performed at $175000 \times g$ for 16 h in a Ti-70 fixed-angle rotor.

The apparatus used for size exclusion-HPLC consisted of a Spectroflow 450 programmer, two Spectroflow 400 pumps and a Spectroflow 757 UV-detector, from Applied Biosystems. The column was a TSK G4000 SWG (21.6 × 300 mm) from LKB, used at a flow rate of 2 $ml \cdot min^{-1}$ with an eluent of pH 6.9 containing 0.1 M NaH_2PO_4, 10 mM sodium ascorbate, 0.1 M NaCl and 0.1% (w/v) SB-12. The high-molecular-mass standards alcohol dehydrogenase (150 kDa), aldolase (158 kDa), β-amylase (200 kDa), ferritin (440 kDa) and apoferritin (443 kDa) were obtained from Pharmacia and Sigma.

SDS-PAGE experiments were performed under fully denaturing conditions following the method of Laemmli [11] with a few modifications. 1.5 mm thick gels were used. Stacking gel was 3% acrylamide; running gel 12.5% acrylamide. Prior to electrophoresis the samples were heated at 53°C for 35 min in 1.9% (w/v) SDS with 4.8% (v/v) β-mercaptoethanol, 3.2% (v/v) glycerol, 0.39 M urea and 40.2 mM Tris (pH 6.8) in the presence of Bromophenol blue. Gels were run overnight at a fixed current of 8 mA per gel until the Bromophenol blue reached the bottom of the gel. Gels were fixed and stained in 10% methanol, 7% acetic acid and 0.1% Coomassie brilliant blue for at least 1 h. The molecular mass standards, obtained from Bio-Rad Laboratories, were phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.7 kDa), carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa).

Absorbance, fluorescence emission and excitation spectra were recorded on a single-beam spectrophotometer [12]. In some cases room-temperature absorbance spectra were recorded with a Shimadzu UV-360 spectrophotometer. Flash-induced absorbance kinetics and difference spectra were measured as described in Ref. 9. Actinic flashes were provided by a Q-switched, frequency-doubled Nd-YAG laser (15 ns half width, 532 nm). Measurements with isolated membranes and with fractions obtained by sucrose gradient density centrifugation were done in Tris-ascorbate buffer with sucrose (membranes 1%, the pigment-protein complexes 10%); measurements on HPLC fractions in the eluent solution mentioned above. For low-temperature experiments 66% (v/v) glycerol was added to obtain clear samples.

Results and Interpretation

Detergent solubilization

Membranes of *Heliobacterium chlorum* were incubated with various concentrations of ionic, neutral and zwitterionic detergents. Fig. 1 shows the absorption spectrum of the membranes used as starting material. It can be seen that, due to improved growth conditions, the amplitude of the band near 670 nm is considerably lower relative to the BChl *g* Q_y-band at 786 nm than in earlier published spectra [1,10] and similar to the recently published absorbance spectrum of *Heliobacillus mobilis* [13]. The same absorption spectrum was obtained with whole cells taken directly from the culture. In all detergent experiments the incubation time was 30 min, at a temperature of 4°C in the dark. The incuba-

TABLE I

Properties of the fractions obtained after SB-12 solubilization of membranes of heliobacteria

The upper, middle and lower fractions refer to the bands obtained upon sucrose gradient density centrifugation (see text). The yield is expressed as recovery of BChl *g* as determined from the amplitude of the Q_y-absorption bands. For each species, the last column gives the activity for P-798 photo-oxidation, as determined from the relative amplitude of the bleaching at 798 nm, induced by a saturating laser flash.

Material	*Heliobacterium chlorum*			*Heliobacillus mobilis*		
	A_{max} (nm)	yield (%)	$A_{max}/\Delta A_{798}$	A_{max} (m)	yield (%)	$A_{max}/\Delta A_{798}$
Membranes	786	–	21	787	–	22
Upper fraction	756	1	300	759	1	270
Middle fraction	785	61	20	786	26	20
Lower fraction	786	38	20	786	73	22
HPLC fraction	785	–	21	786	–	22

tion mixture was then layered on top of a sucrose gradient (see Materials and Methods) containing a low concentration of detergent. The standard method adopted, with SB-12 and cholate, is described in Materials and Methods.

Table I gives the result of a typical experiment. Three bands were observed on the gradient after centrifugation. The upper fraction, located at 20% (w/v) sucrose, probably consisted of free pigment in detergent micelles, as judged from its blue-shifted absorption and lack of reaction center activity. The middle fraction, at approx. 35% sucrose, consisted of a solubilized pigment-protein complex. Its spectral properties and photochemical activity, as determined from the light-induced bleaching at 798 nm, due to P-798 photo-oxidation, were virtually unchanged as compared to those of isolated membranes. This material, which we shall call the antenna-reaction center (A-RC) complex was further purified and characterized as will be described below. A fraction enriched in cytochrome *c*-553 was recovered from just above the middle fraction. The lower fraction consisted of material which settled on top of the 75% sucrose layer and presumably consisted of non-solubilized membranes. Similar results were obtained with membranes of *Hb. mobilis,* but the yield of the middle fraction was lower (Table I).

A variety of other detergents was also used. Incubation of *H. chlorum* membranes with 90 mM OGP, followed by centrifugation on a sucrose gradient containing 30 mM OGP, yielded a similar middle fraction as obtained with SB-12 and sodium cholate, with a yield of approx. 70%. Results obtained with other detergents were less satisfactory. Mild detergents, like lauryl maltoside and CHAPS, gave a very low degree of solubilization, even when applied at relatively high concentrations of up to 90 mM. Other detergents, like SDS, lithium dodecyl sulfate, *n*-dodecyl-*N*,*N*-dimethylamine oxide (LDAO), Triton X-100, sodium cholate and sodium deoxycholate, led to a loss of reaction center activity and (except for Triton X-100) to an approx. 30 nm blue shift of the Q_y absorption of the solubilized complex, indicating denaturation of the protein. In most cases this was accompanied by an increase of the 670 nm absorption.

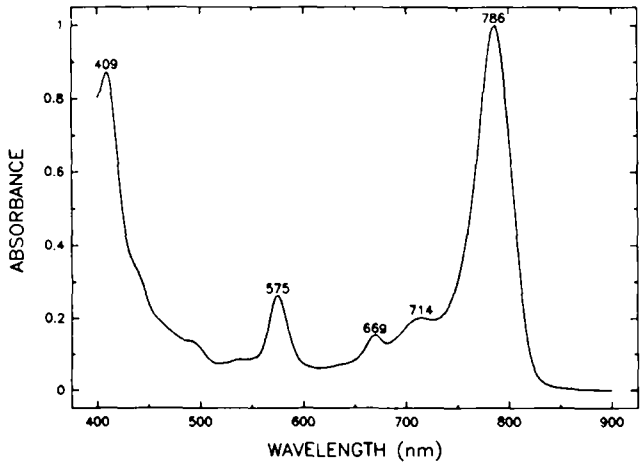

Fig. 1. Absorption spectrum, measured at room temperature, of membranes of *H. chlorum.*

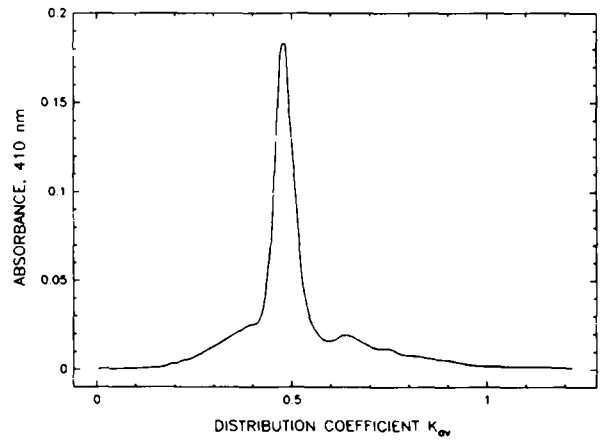

Fig. 2. Chromatogram obtained by size-exclusion HPLC of the SB-12-solubilized A-RC complex of *H. chlorum,* monitored at 410 nm. The horizontal axis gives the distribution coefficient, defined as $K_{av} = (V_e - V_0)/(V_t - V_0)$, where V_e is the elution volume, V_0 is the void volume and V_t is the total volume of the packed bed. For conditions see text.

Purification and peptide composition

The A-RC complexes of *H. chlorum* and *Hb. mobilis* were further purified by size-exclusion HPLC. The chromatogram of a preparation from *H. chlorum*, monitored at 410 nm, is shown in Fig. 2. The main colored band, which we shall call the purified A-RC complex, eluted with a distribution coefficient $K_{av} = 0.48$, corresponding to approx. 335 kDa, on the assumption that there are no differences in shape between the proteins used as molecular weight markers and the protein-detergent micelles which constitute the middle fractions. Some colored material with $K_{av} = 0.64$ was also eluted. In addition to this, a considerable amount of colorless material with absorption at 280 nm was eluted, with $0.78 < K_{av} < 1.0$. This fraction presumably consisted mainly of relatively low-molecular-mass proteins. It did not contain a significant amount of BChl *g*. Essentially the same results were obtained with the middle fraction of *Hb. mobilis*. The middle fraction of *H. chlorum* obtained after solubilization with OGP showed a somewhat different elution pattern. Upon elution in the presence of 30 mM OGP, a colored peak was obtained with $K_{av} = 0.52$ corresponding to an approximate molecular mass of 210 kDa. Again, a considerable amount of colorless material with a high K_{av} was eluted.

The absorption spectrum of the purified A-RC complex from *H. chlorum* is shown in Fig. 3. In the visible region the spectrum was very similar to that of the starting material. The height of the band near 670 nm was the same as in the membranes and in the (unpurified) middle fraction, with an average A_{670}/A_{786} ratio of 0.14. In the ultraviolet the second Soret band near 370 nm [4,14] can be seen, together with a protein band at 272 nm. The ratio A_{272}/A_{786} of the preparations was 0.60 for the SB-12 solubilized and 0.45 for the OGP solubilized complex. The photochemical activity of the preparations was fully retained during the HPLC fractionation (Table I).

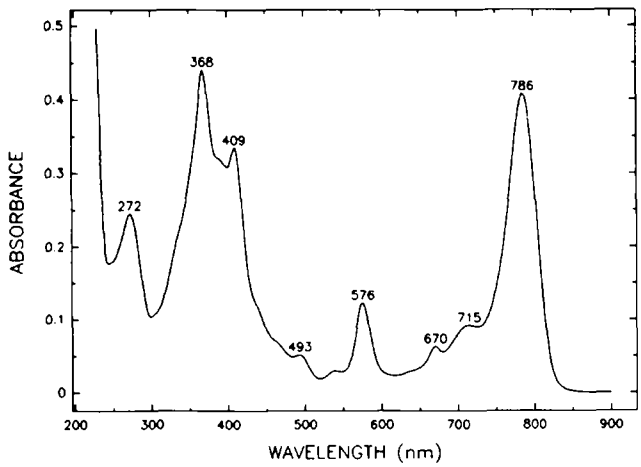

Fig. 3. Room temperature absorption spectrum of the antenna-reaction center complex from *H. chlorum*, after purification by means of size-exclusion HPLC. The spectrum was measured in the absence of ascorbate.

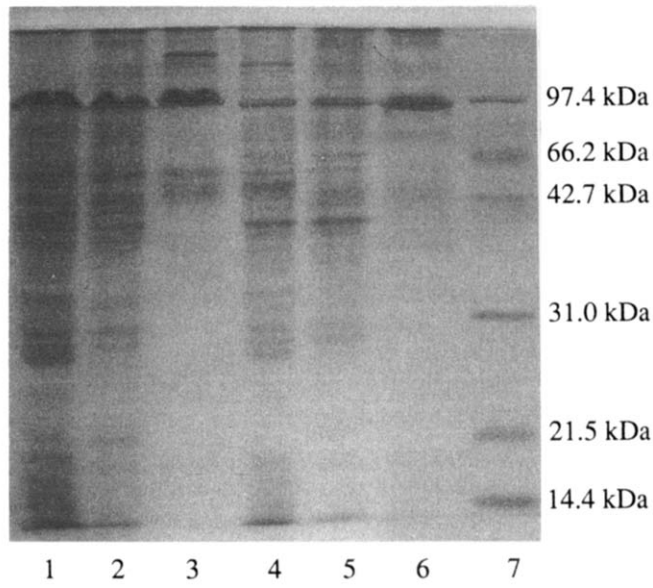

Fig. 4. Polyacrylamide gel electrophoresis of *H. chlorum* and *Hb. mobilis* preparations. Lane 1, *H. chlorum* membranes; lane 2, *H. chlorum* SB-12 middle fraction; lane 3, *H. chlorum* HPLC fraction; lane 4, *Hb. mobilis* membranes; lane 5, *Hb. mobilis* SB-12 middle fraction; lane 6, *Hb. mobilis* HPLC fraction; lane 7, standards. Conditions and standards, see Materials and Methods.

Fig. 4 shows the results of SDS-PAGE, obtained under denaturing conditions, of membranes, the middle fractions and the complexes obtained after purification with size-exclusion HPLC. The purified A-RC complexes of both species showed a dominant peptide band of 94 kDa and weaker and variable bands above 100 kDa. For *H. chlorum*, two weak bands, at 45 and 55 kDa, are also visible, but the intensity of these bands varied, and in some preparations they were not resolved. No evidence was found for the presence of smaller peptides. Weak and variable bands in the range 40–90 kDa were seen in the *Hb. mobilis* complex. The 94 kDa peptide was also the strongest component in the membranes and the middle fractions, but these preparations also yielded numerous bands of lower molecular weight, as was observed for membranes of *H. chlorum* already by Fuller et al. [4]. Similar results were obtained upon incubation with SDS at 20 °C instead of 53 °C (see Materials and Methods), except that the band located on top of the gel was absent, suggesting that it may be due to an aggregation product. These results suggest that the complexes may consist of 94 kDa subunits only, perhaps in a dimeric or trimeric form.

Spectral properties and reaction center activity

As shown in Fig. 5, the low-temperature absorption spectra of the solubilized complexes from *H. chlorum*

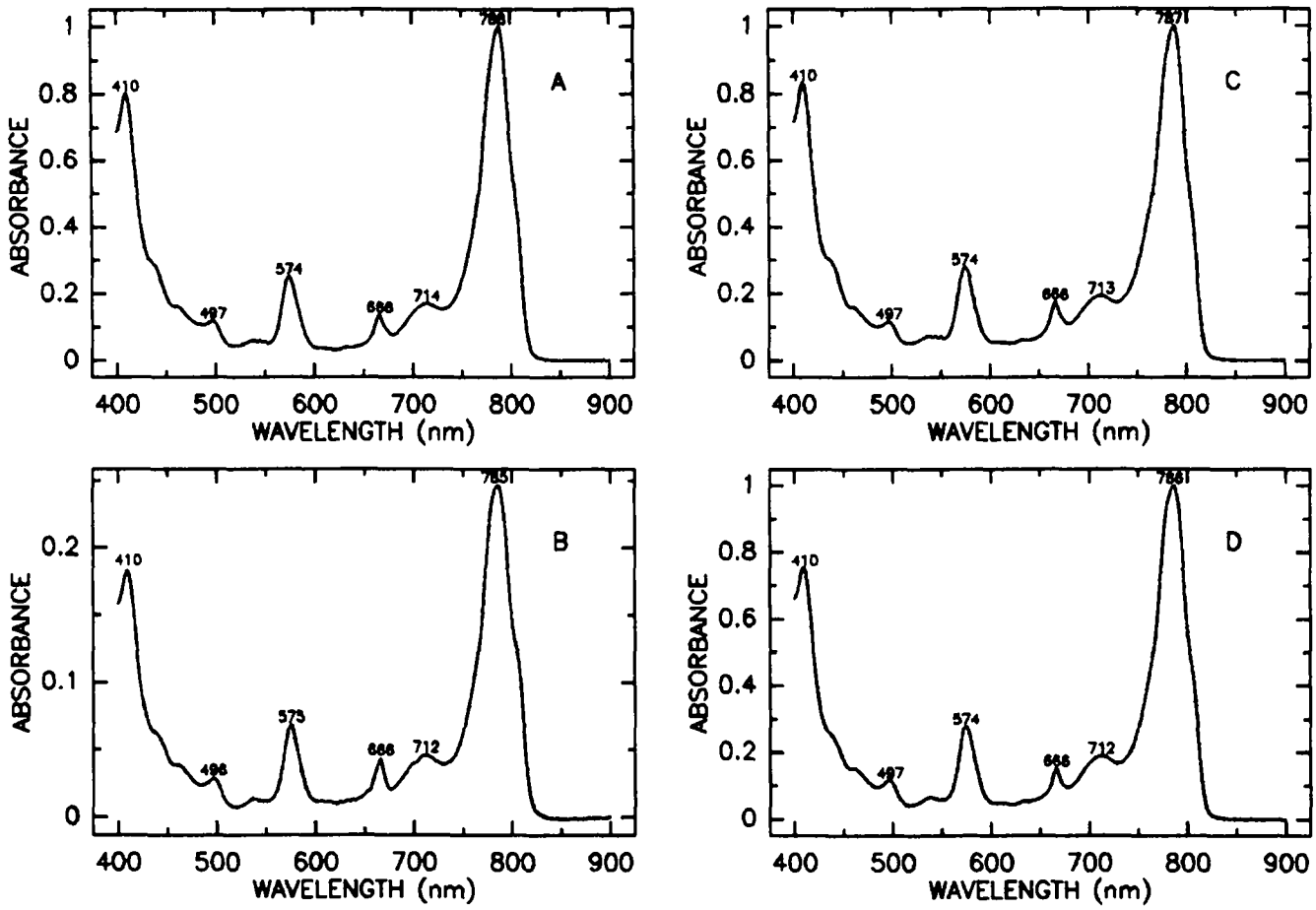

Fig. 5. Absorption spectra, measured at 100 K, of membranes and A-RC complexes. A, membranes of *H. chlorum*; B, the purified A-RC complex from *H. chlorum*; C, membranes of *Hb. mobilis*; D, the A-RC complex from *Hb. mobilis* (middle fraction).

and *Hb. mobilis* were very similar to those of the membranes used as starting material. Although the maximum in the Q_y region was shifted by approx. 1 nm to the blue, the three spectral forms of BChl *g*, BChl *g*-778, BChl *g*-793 and BChl *g*-808 [10], were still observed in the same ratio as in the membranes, albeit in a slightly blue-shifted position as indicated by the second derivatives of the spectra (not shown). The relative height of the band near 670 nm, which may be taken as an indication of the amount of (photo)converted BChl *g* [13] was not significantly changed. The amount of carotenoid (mainly neurosporene [1,10]) was not changed in the solubilized complexes.

The low-temperature fluorescence spectra of the isolated A-RC complexes (not shown) were again similar to those of the membranes. They showed a maximum at 815 nm at 77 K, indicating that most of the fluorescence originated from the long-wave-absorbing BChl *g*-808. Comparison of the absorbance $(1 - T)$ spectrum with the fluorescence excitation spectrum of the long-wave fluorescence (Fig. 6) indicated efficient energy transfer, approaching 100%, from BChl *g*-778 and BChl *g*-793 to BChl *g*-808, as earlier observed in membranes of *H. chlorum* [10]. Efficient energy transfer also occurred from neurosporene to BChl *g*. The efficiency of energy transfer from the 670 nm pigment to BChl *g* was approx. 50%.

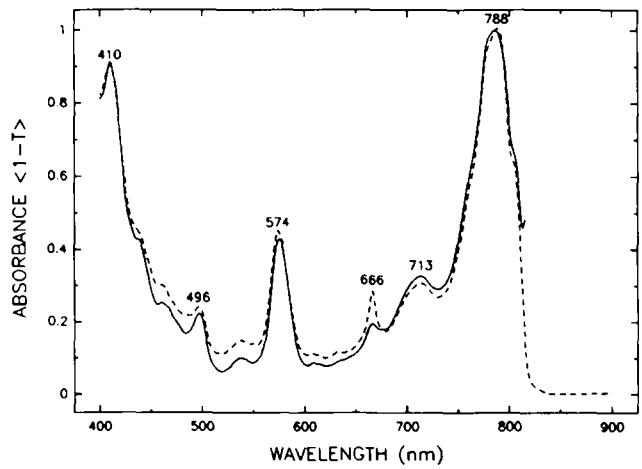

Fig. 6. Fluorescence excitation spectrum, monitored at 820 nm, (solid line) of the A-RC complex (middle fraction) from *H. chlorum*, measured at 5 K. The broken line shows the absorbance $(1 - T)$ spectrum. The two spectra are normalized at the Q_y maxima, the excitation spectrum is plotted in arbitrary units. The absorbance was 0.75 at 788 nm.

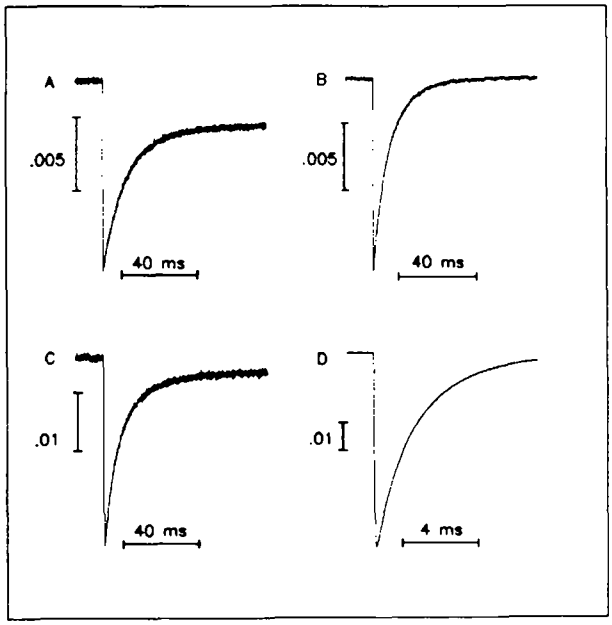

Fig. 7. Kinetics of absorbance changes due to P-798, induced by a saturating laser flash at 532 nm (intensity 10 mJ·cm^{-2}), measured at 798 nm (A–C) and 798 nm (D), respectively. Recording (A), purified A-RC complex of *H. chlorum*; (B) complex with additional 40 μM PMS; (C) membranes; (D) complex at 5 K. The absorbances of the samples in the Q_y maximum were 0.30 (A, B); 0.78 (C) and 0.83 (D), respectively. The vertical bars indicate the change of absorbance (ΔA).

These results strongly indicate that the structure of the antenna complexes was completely conserved upon solubilization. The same applied to the reaction center activity, as already indicated in Table I. Fig. 7 shows the bleaching of P-798 in the *H. chlorum* complex upon excitation with saturating laser flashes. The amount of P-798 oxidized, relative to the amount of BChl *g*, was essentially the same as in membranes, in saturating (Fig. 7) as well as in non saturating flashes. This shows that not only the amount of active reaction centers was unchanged, but also the efficiency of charge separation and of energy transfer to the reaction center. The overall rate of reduction of P-798$^+$ after a flash was lower in the complexes than in the membranes. In the presence of ascorbate the kinetics were multiphasic, with time constant of 10 and 25 ms, together with a much slower decay phase. A dark time of several seconds between the flashes was needed to approach complete recovery of the reduced form. The reduction of P-798$^+$ was considerably speeded up upon addition of the redox mediator PMS (Fig. 7B). At 5 K, the time constant for the back reaction of P-798$^+$ was 2.3 ms (Fig. 7D), the same as in the isolated membranes [15]. Similar results were obtained with the complex from *Hb. mobilis*. These results show that at least part of the electron-acceptor chain was still functioning in our preparations, since otherwise a rapid back-reaction with a time constant of tens of nanoseconds would be expected to occur [9]. The relatively slow decay kinetics of P-798$^+$ at room temperature may at least in part be related to the absence of cytochrome *c*-553 oxidation which was detached from the complex by the detergent.

The difference spectra for P-798 oxidation in the A-RC complexes of *H. chlorum* and *Hb. mobilis* are shown in Fig. 8. Both spectra are virtually identical, and they were also indistinguishable from the difference spectrum obtained with membranes of *H. chlorum*. This indicates that the structure of the reaction center was not changed.

Discussion

Our results demonstrate that detergent solubilization of membranes of *H. chlorum* and *Hb. mobilis* and subsequent fractionation of the solubilized material yields an antenna-reaction center complex which is structurally and functionally intact. The optical properties of the complexes are virtually identical to those of the pigment system in vivo and the reaction center activity is fully retained.

From the elution volume on the size-exclusion HPLC column a molecular mass of approx. 335 kDa was obtained for the complexes obtained upon SB-12 solubilization. This number, however, should be corrected for the weight of the attached detergent molecules. It has been assumed that the amount of detergent material is approximately the same for pure micelles and for protein-detergent micelles [16]. If we assume that for SB-12 the number of monomers per micelle is the same (76) as for *n*-dodecyl-*N*,*N*-dimethylamine oxide [16], this gives a correction of 25 kDa for the total mass of the detergent, resulting in a corrected molecular mass of 310 kDa for the antenna-reaction center complex. A

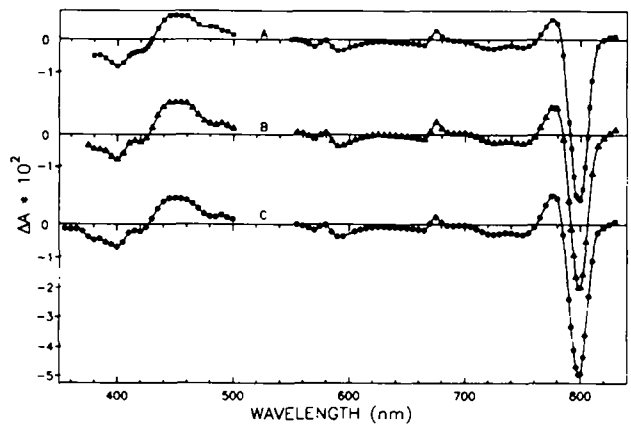

Fig. 8. Absorbance difference spectra of (A) membranes of *H. chlorum*; (B) the purified A-RC complex of *H. chlorum* and (C) the A-RC complex from *Hb. mobilis*. Conditions as for Fig. 7B and C. The spectra were normalized to an absorbance of 1.0 at the Q_y-maximum for each sample. The extent of bleaching was calculated by extrapolating the absorbance change at 0.5 (A) or 2.5 ms (B and C) to $t = 0$.

similar correction for the OGP-solubilized complex (80 monomers per micelle [16]) yields a corrected mass of 190 kDa.

The purified A-RC complexes show a relatively low ultraviolet-absorption in the protein region. Comparison of the relative absorption at 270–280 nm with those of the BChl *a*-protein from green sulfur bacteria [17] and of purified reaction centers of *Rhodobacter sphaeroides* [18] suggests that the BChl content of the SB-12 solubilized complexes is about 10–15% (w/w). Together with the molecular mass of 310 kDa as determined from the HPLC elution volume this indicates that the complexes contain about 40 BChl *g* molecules. The same calculation for the OGP-solubilized complex yields a BChl content of about 15–20%, and a total of 35 BChls. Calculation of the BChl content from the extent of bleaching of P-798 in saturating flashes according to the method of Vos et al. [19], by dividing the integrated absorbance in the Q_y-band by the integrated absorbance difference signal in the Q_y region, yields about 50 BChls per reaction center. We thus conclude that the complexes probably contain the pigment complement of a single photosynthetic unit, together with the associated reaction center.

The fluorescence excitation spectrum shows that, in contrast to the other pigments, the pigments absorbing near 670 nm transfer their excitation energy with an average efficiency of only approx. 50%. Comparison of the low temperature absorption spectrum of *H. chlorum* membranes with the amplitude of flash-induced subnanosecond absorbance changes in the 670 nm region [8], indicates that one-fourth of the pigment molecules absorbing near 670 nm act as electron acceptor in the primary charge separation. We thus conclude that the A-RC complex probably contains four BChl *c*- or Chl *a*-like pigment molecules, one of which is the primary electron acceptor. The transfer efficiency of 50% may suggest that two of these pigment molecules are incorporated in the reaction center. Excitation energy absorbed by these pigments then would be directly used for photochemistry and have a low probability of exciting antenna fluorescence. A similar effect has been observed upon excitation of reaction center BChl or bacteriopheophytin in purple and green filamentous bacteria [20,21].

The similarities of the electron transport pathways in reaction centers of heliobacteria, green sulfur bacteria and PS I might suggest that these systems are structurally related. It is therefore of interest to compare the composition of the antenna-reaction center complexes of heliobacteria with that of the PS I core complex and the core complex of green sulfur bacteria. The number of BChl molecules in the complex of heliobacteria (about 50) is in the same range as the number of chlorophylls or BChls reported to be bound to the PS I core [22,23] and to the core complex of green sulfur bacteria. Also the molecular mass of the heliobacterial A-RC complexes (about 310 kDa) appears to be similar to that of the PS I core, which was recently calculated to be about 330 kDa for the green alga *Dunaliella salina* [24]. However, our results thus far do not indicate a similarity in peptide composition. The PS I core contains two homologous peptides of 82 and 83 kDa, normally running on gels as approx. 65 kDa peptides, together with five or six peptides in the 8–25 kDa range in green plants and three in green algae [22–25]. The core complex of green sulfur bacteria was reported to contain 65 and 32 kDa peptides [26].

Our results suggest that the peptide composition of the complexes from *H. chlorum* and *Hb. mobilis* is basically different. For both species, the dominant peptide migrates on the gels with an apparent molecular mass of 94 kDa, significantly larger than observed for the core complexes mentioned above. In addition, weak and variable bands near 50 kDa were seen, and no evidence for the presence of smaller peptides was obtained. We thus conclude that the present evidence does not seem to indicate a similar structure for the heliobacterial complex and for the other systems.

Little is known about the ecology and taxonomy of the heliobacteria. Nevertheless it is quite remarkable that *H. chlorum* and *Hb. mobilis,* in spite of their different habitats and morphology [1,3,13], appear to be virtually identical with respect to the organization of their photosystem. Not only are the optical properties of their antenna systems and of the reaction centers almost indistinguishable, but also the pigment-to-reaction-center ratio appears to be the same in both species. This observation provides some additional evidence for the concept that the heliobacteria contain a single antenna-complex with associated reaction center, in contrast to the purple bacteria, where the presence of a peripheral antenna complex (LH II) in many species results in a much larger variation in photosystem properties.

Acknowledgments

Thanks are due to Ms. M.C. Nieveen for her help in preparing and analyzing the samples, to F.T.M. Zonneveld for assistance in the early phase of the investigation, to A.H.M. de Wit and Ms. M.L. van der Erf for culturing the bacteria and to Ms. E. Tom for her help with some of the experiments. The investigation was supported by the Netherlands Foundation for Scientific Research (NWO) via the Netherlands Foundation for Chemical Research (SON).

Addendum

Trost and Blankenship [27], using Deriphat 160c as detergent, recently reported the isolation of an

antenna-reaction center complex from *Hb. mobilis* which appears to be similar to ours. However, the dominant peptide observed with SDS-PAGE had an apparent molecular mass of 47 kDa, suggesting that the 94 kDa peptide observed by us may be a dimer. This does not affect our conclusions regarding the absence of a similarity with Photosystem I and green sulfur bacteria.

References

1 Gest, H. and Favinger, J.L. (1983) Arch. Microbiol. 136, 11–16.
2 Brockmann, H. and Lipinski, A. (1983) Arch. Microbiol. 136, 17–19.
3 Beer-Romero, P. and Gest, H. (1987) FEMS Microbiol. Lett. 41, 109–114.
4 Fuller, R.C., Sprague, S.G., Gest, H. and Blankenship, R.E. (1985) FEBS Lett. 182, 345–349.
5 Prince, R.C., Gest, H. and Blankenship, R.E. (1985) Biochim. Biophys. Acta 810, 377–384.
6 Nuijs, A.M., Van Dorssen, R.J., Duysens, L.N.M. and Amesz, J. (1985) Proc. Natl. Acad. Sci. USA 82, 6865–6868.
7 Brok, M., Vasmel, H., Horikx, J.T.G. and Hoff, A.J. (1986) FEBS Lett. 194, 322–326.
8 Van Kan, P.J.M., Aartsma, T.J. and Amesz, J. (1989) Photosynth. Res. in the press.
9 Smit, H.W.J., Amesz, J. and Van der Hoeven, M.F.R. (1987) Biochim. Biophys. Acta 893, 232–240.
10 Van Dorssen, R.J., Vasmel, H. and Amesz, J. (1985) Biochim. Biophys. Acta 809, 199–203.
11 Laemmli, U.K. (1970) Nature 227, 680–685.
12 Rijgersberg, C.P., Van Grondelle, R. and Amesz, J. (1980) Biochim. Biophys. Acta 592, 53–64.
13 Beer-Romero, P., Favinger, J.L. and Gest, H. (1988) FEMS Microbiol. Lett. 49, 451–454.
14 Michalski, T.J., Hunt, J.E., Bowman, M.K., Smith, U., Bardeen, K., Gest, H., Norris, J.R. and Katz, J.J. (1987) Proc. Natl. Acad. Sci. USA 84, 2570–2574.
15 Smit, H.W.J., Van Dorssen, R.J. and Amesz, J. (1989) Biochim. Biophys. Acta 973, 212–219.
16 Kühlbrandt, W. (1988) Q. Rev. Biophys. 21, 429–477.
17 Olson, J.M. (1966) in The Chlorophylls (Vernon, L.P. and Seely, G.R., eds.), pp. 413–425, Academic Press, New York.
18 Feher, G. (1971) Photochem. Photobiol. 14, 373–389.
19 Vos, M.H., Klaassen, H.E. and Van Gorkom, H.J. (1989) Biochim. Biophys. Acta 973, 163–169.
20 Wang, R.T. and Clayton, R.K. (1971) Photochem. Photobiol. 13, 215–224.
21 Vasmel, H., Van Dorssen, R.J., De Vos, G.J. and Amesz, J. (1986) Photosynth. Res. 7, 281–294.
22 Malkin, R. (1987) in The Light Reactions (Barber, J., ed.), pp. 495–525, Elsevier, Amsterdam.
23 Nelson, N. (1987) in Photosynthesis (Amesz, J., ed.), pp. 213–231, Elsevier, Amsterdam.
24 Bruce, D.B. and Malkin, R. (1988) Plant Physiol. 88, 1201–1206.
25 Schuster, G., Nechustai, R., Ferreira, P.C.G., Thornber, J.P. and Ohad, I. (1988) Eur. J. Biochem. 177, 411–416.
26 Hurt, E.C. and Hauska, G. (1984) FEBS Lett. 168, 149–153.
27 Trost, J.T. and Blankenship, R.E., reported at the VIIIth International Congress on Photosynthesis, Stockholm 1989.