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Characterization of an Improved Reaction Center Preparation from the Photosynthetic Green Sulfur Bacterium *Chlorobium* Containing the FeS Centers F_A and F_B and a Bound Cytochrome Subunit†

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Received October 15, 1991; Revised Manuscript Received December 23, 1991

ABSTRACT: A photosynthetic reaction center complex was prepared from the green sulfur bacterium *Chlorobium* by solubilization of chlorosome-depleted membranes with lauryl maltoside, followed by anion-exchange chromatography and molecular sieve chromatography. The purified complex was characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, optical spectroscopy, and EPR spectroscopy. The major bands migrated at apparent molecular masses of 50, 42, and 32 kDa (heme-staining) and additional weaker bands at 22, 15, and 12 kDa. The isolated reaction center complex contained about 40 bacteriochlorophyll *a* molecules per primary electron donor, P_{840}, assayed by photooxidation. It was competent in stable low-temperature photoreduction of the FeS centers F_A and F_B. The spectra of these acceptors and their low-temperature photochemistry in the purified complex were the same as found in intact *Chlorobium* membranes and similar to what had been described for photosystem I from plants. Membrane-bound cytochrome c_{553} copurified with the reaction center complex. A ratio of about four hemes per P_{840} was determined. This result indicates that cytochrome c_{553} that is closely associated with the reaction center is a tetraheme cytochrome, as described for some purple bacteria.

The first published data pointing toward a relatedness of the green sulfur bacterial reaction center to photosystem I (PSI)[1] date back to 1968 (Buchanan & Evans, 1968). These early results have been supported by (however somewhat controversial) results suggesting that one or more FeS centers might participate in stabilization of light-induced charge separation within the reaction center (Jennings & Evans, 1977; Swarthoff et al., 1981).

† Supported by the Leibniz-Programm (to H.M.) of the Deutsche Forschungsgemeinschaft (DFG), the Max-Planck Gasellschaft (U.F. and H.M.), and the DFG (W.N.). Supported in part by the CNRS (URA 1290).

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[1] Abbreviations: BChl, bacteriochlorophyll; Chaps, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; EDTA, ethylenediaminetetraacetic acid; FeS center, iron–sulfur center; kDa, kilodalton; LDAO, *N,N*-dimethyldodecylamine *N*-oxide; LM, lauryl β-D-maltoside; Mega-9, decanoyl-*N*-methylmaltosylamine; MW, molecular weight; OTG, *n*-octyl β-D-thioglucoside; P_{840}/P_{700}, the primary electron donor in green sulfur bacteria/PSI; PSI, photosystem I; RC, reaction center; SB12, *N*-dodecyl-*N,N*-dimethylammonio-3-propanesulfonate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/92/0431-2608$03.00/0

Recently it was shown that actual similarities between the green sulfur bacterial reaction center and PSI go much further than previously assumed (Nitschke et al., 1990a). The presence of the FeS centers F_A, F_B, and F_X has been demonstrated. Both the primary donor and the three FeS centers are oriented almost exactly as in PSI. Furthermore, indirect evidence for the existence of a quinone-type acceptor, analogous to A_1 in PSI, has been presented. Together with the finding that the primary acceptor in green sulfur bacteria is a BChl molecule (Nuijs et al., 1985; Braumann et al., 1986), these results demonstrate that the green sulfur bacterial reaction center contains the same set of electron acceptors as PSI.

However, some properties of these acceptors seem to be different (Nitschke et al., 1990a): (a) The FeS center F_B is the terminal acceptor predominantly photoreduced at low temperature; (b) the midpoint potentials of centers F_A and F_B are considerably more negative than those of their counterparts in PSI. Still, the reducing potential of the excited singlet state P_{840}* is calculated to be rather similar to that of P_{700}*, and therefore it was proposed that the kinetics of the individual electron-transfer steps between the acceptors might be different in both systems (Nitschke et al., 1990a). Indeed, electron transfer from the primary acceptor to the subsequent acceptor seems to be slower by a factor of about 20 in green sulfur bacteria (Nuijs et al., 1985) as compared to PSI (Nuijs et al., 1986; Shuvalov et al., 1986).

Unfortunately, almost no information is available on the kinetics of electron transfer beyond this secondary acceptor or on its chemical nature. On one hand, this is due to the fact that in intact systems (e.g., cells, membranes) the extremely large antenna systems of green sulfur bacteria (the "chlorosomes") preclude time-resolved optical studies due to high background absorption. Preparations enriched in reaction centers, on the other hand, have so far always shown impaired secondary electron transfer (Swarthoff & Amesz, 1979; Feiler, 1987; Nitschke et al., 1987).

In this work we present for the first time a reaction center preparation with low BChl content from *Chlorobium* which in all charge separation properties is similar to intact membranes (as seen by EPR spectroscopy) and contains the FeS centers F_A and F_B. This preparation is well suited for all kinds of optical experiments.

Furthermore, we demonstrated that membrane-bound cytochrome c_{553} copurifies with the reaction center. This cytochrome has already been characterized in membranes (Feiler et al., 1989), and it appears to be similar in many respects to the tetraheme cytochrome subunits found in some purple bacteria. The copurification of cytochrome c_{553} with the reaction center further supports the idea that this cytochrome plays a role comparable to that of the tetraheme cytochrome subunits in purple bacteria and possibly also in *Chloroflexus* (Freeman et al., 1990).

Experimental Procedures

Protein Purification. Chlorobium limicola f *thiosulfatophilum*, strain tassajara, and *Chlorobium phaeobacteroides*, strain 4930, were obtained from Prof. N. Pfennig, Konstanz, FRG. The cells were grown in 1-, 2-, and 10-L bottles under strictly anaerobic conditions in a medium described by Biebl and Pfennig (1978). The trace element solution SL10 of Widdel et al. (1983) was used. After 3–5 days of growth, cells were harvested by a low-speed centrifugation in a continuous-flow centrifuge (Carl Padberg, Lahr, FRG) and were stored at −80 °C until use.

All following steps were carried out at 4 °C. Thawed cells were washed in 50 mM Tris-HCl, pH 8.0. After centrifugation the pellet was resuspended in the same buffer. The cells were disrupted by passing three times through a French pressure cell (20 000 psi) in the presence of DNase. The preparation of chlorosome-depleted membranes followed a method described by Schmidt (1980), which uses the fractionation of chlorosomes and membranes on a sucrose density gradient. The typical BChl*c* to BChl*a* ratio in chlorosome-depleted membranes was 0.5:3 BChl*c*:BChl*a*, as estimated from the absorptions at 746 and 803 nm. The band containing the chlorosome-depleted membranes was diluted with 50 mM Tris-HCl, pH 8.0, 300 mM KCl, and 1 mM EDTA and spun down (100000*g*, 60 min). This membrane pellet was resuspended in 50 mM Tris-HCl, pH 8.0, and solubilized with 0.4% lauryl β-D-maltoside (LM) at a final BChl concentration of 30 μM BChl*a*. After addition of LM the suspension was stirred for 60 min at 4 °C, followed by a centrifugation (100000*g*, 60 min). The supernatant was loaded onto an anion-exchange column [Fractogel TSK DEAE-650 (S), Merck] equilibrated with 20 mM Tris-HCl, pH 9.0, and 0.1% LM. The column was washed with 1 column volume before elution with a linear gradient of 0–800 mM NaCl. The fractions containing the reaction center were pooled and subsequently concentrated in an Amicon cell (PM10) and chromatographed on a molecular sieve column [Fractogel TSK HW55 (S), Merck, equilibrated in 20 mM Tris-HCl, pH 8.0, 0.1% LM, and 5 mM NaCl]. The pooled reaction center fractions were concentrated in Amicon Centriprep-10 and stored at −80 °C until use.

The particle weight of the reaction center complex was determined by using a calibrated molecular sieve column. Dextran (MW = 500 000), cytochrome *c* (horse heart, MW = 12 300), bovine serum albumin (MW = 67 000, runs as a dimer at low ionic strength), catalase (MW = 240 000), myoglobin (MW = 17 8000, runs as a dimer at low ionic strength), ovalbumin (MW = 45 000, runs as a dimer at low ionic strength), and aldolase (MW = 160 000) were used for molecular weight calibration.

Subunit Analysis. SDS–PAGE experiments were performed following the method of Laemmli (1970) modified by Ikeuchi and Inoue (1988). As a molecular mass standard the Amersham Buchler rainbow marker was used. Electrophoresis was carried out with the minigel vertical slab unit from Hoefer Scientific Instruments, as well as with the Pharmacia vertical electrophoresis system. Coomassie-staining and heme-staining methods were performed as described by Cabral and Schatz (1979) and Thomas et al. (1976), respectively.

Optical Spectroscopy. Room temperature absorption spectra were recorded on a Perkin-Elmer UV/vis Lambda 15 spectrophotometer. Light-induced absorbance kinetics were measured on an Aminco DW2000 spectrophotometer (dual wavelength mode). Steady-state photobleaching was performed in the presence of 10 mM ascorbate and 1 μM phenazine methosulfate. Illumination was provided by a 250-W slide projector with a BG780 filter (Schott). The photomultiplier was protected from the actinic light by a BG18 filter (Schott). The measuring wavelengths were 610–540 nm. The chemical bleaching of P_{840} was essentially performed using a method described by Markwell et al. (1980) and recorded on a Shimadzu UV 160 spectrophotometer.

All measurements were performed in 50 mM Tris-HCl, pH 8.0, and 0.05% LM.

The extinction coefficients $\Delta\epsilon_{552-540}$ = 20 mM^{-1} cm^{-1} for cytochrome (Meyer et al., 1968), ϵ_{809} = 100 mM^{-1} cm^{-1} for

BChl*a* (Olson et al., 1973), $\Delta\epsilon = 30\ mM^{-1}\ cm^{-1}$ for the difference spectrum of P/P_{840}^+ at 610 minus 540 nm (Olson et al., 1976), and $\Delta\epsilon_{700} = 64\ mM^{-1}\ cm^{-1}$ [as for PSI (Hiyama & Ke, 1972)] assumed for P_{840} in the chemical difference spectrum were used.

EPR Spectroscopy. EPR spectra were recorded on Bruker 200 and 300 X-band spectrometers fitted with an Oxford Instruments cryostat and temperature control system. Illumination in the EPR cavity was carried out by using a 800-W tungsten projector providing 16 000 $\mu E\ m^{-2}\ s^{-1}$ of white light at the EPR cavity window after being filtered through 2 cm of water and two Calflex filters (Balzers) to remove infrared radiation.

Results

Detergent Treatment. Several ionic, anionic, and zwitterionic detergents were tested to solubilize chlorosome-depleted membranes of green sulfur bacteria. Lubrol PX was not suited for use since during the solubilization the color of the sample changed drastically from green to brown and subsequently P_{840} oxidation could not be observed any more. The change in color was shown to be due to the appearance of a peak in the spectrum at 670 nm which is known to be mainly due to BPh*c*, a degradation product of BChl*c*. The native BChl*a* peak was also shifted to a maximum at 720 nm. Similar effects of detergent treatments on BChl absorption peaks, attributed to degradation of the reaction center, have been observed by van de Meent et al. (1990) in *Heliobacterium chlorum*, a photosynthetic bacterium discovered recently (Gest & Favinger, 1983). With the detergents Chaps (maximum 5%) and Mega-9 (maximum 2%) only about 20% of BChl*a* could be solubilized. With SB12 (maximum 5%), LDAO (maximum 5%), and OTG (maximum 5%) 60–70% of BChl*a* were extracted from the membrane. The solubilized material differed with respect to the ratio of BChl*c*:BPh*c*:BChl*a*, but in all cases the sample contained more BChl*c* and BPh*c* than BChl*a*.

The best results were achieved using LM. With 0.4% LM 70–80% of BChl*a* was solubilized. Higher amounts of LM did not increase this yield any further. The contribution of BChl*c* in the spectrum was diminished to 0.1–2%, and usually the peak at 670 nm in the absorption spectrum (Figure 1b,c) increased.

The spectrum of P_{840}^+ upon chemical oxidation (Figure 2a) was similar to the light minus dark spectrum reported by Swarthoff and Amesz (1979) and showed a ratio of bleachable P_{840} to total BChl*a* of 1:80–100. Photooxidation yielded a ratio of bleachable P_{840} to total BChl*a* of 1:50–70. Additionally, in the chemical difference spectrum bleaching of cytochrome hemes at 553 nm was observed. The stoichiometry was calculated to be 3.3–4.2 heme groups per P_{840}^+.

Further Purification. (A) Anion-Exchange Column. Figure 1d shows the absorption spectrum of the fraction containing the reaction center after the DEAE column. A cytochrome and material showing a large peak at 670 nm could be separated from the reaction center. Some of the fractions also contained carotenoids. One P_{840} per 40–50 BChl*a* could be bleached upon illumination.

The elution profile of the DEAE column is shown in Figure 3. The reaction center was eluted at 170–250 mM NaCl as a rather broad band compared to the other protein fractions eluted from the column.

The peak of cytochrome c_{553} (as measured by the intensity of the optical α-band) coincides well with the maximum of the reaction center. This result argues for a real association of both proteins instead of just an accidential copurification of otherwise unrelated proteins.

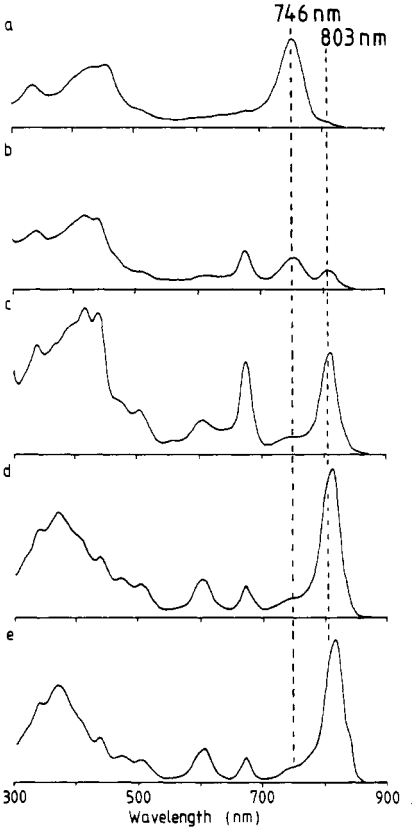

FIGURE 1: Room temperature absorption spectra of (a) crude membranes, (b) chlorosome-depleted membranes, (c) solubilized membranes, (d) the pooled fractions containing the RC complex after an anion-exchange column, and (e) the pooled fractions containing the RC complex after a molecular sieve column.

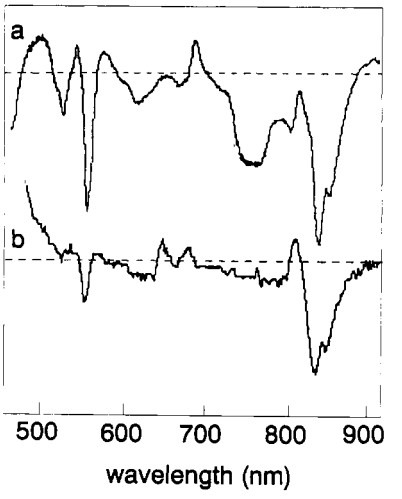

FIGURE 2: Chemically induced difference spectrum of solubilized membranes (a) and the purified reaction center (b) of *Chlorobium*, performed according to Markwell et al. (1980).

(B) Molecular Sieve Column. Figure 1e shows the absorption spectrum of the pooled reaction center fractions after the molecular sieve column. Again, carotenoids, pigments showing an absorption at 670 nm, and antenna BChl*a* could be separated from the reaction center. Photooxidation measured in this purified reaction center complex yielded a ratio of 40:1 BChl*a*:P_{840}.

Reduced minus oxidized chemical difference spectra in the reaction center containing fractions after the molecular sieve column (Figure 2b) demonstrate that cytochrome c_{553} is still present in this sample. Additionally, a protein band at 32 kDa on SDS–PAGE with heme-staining activity was detected.

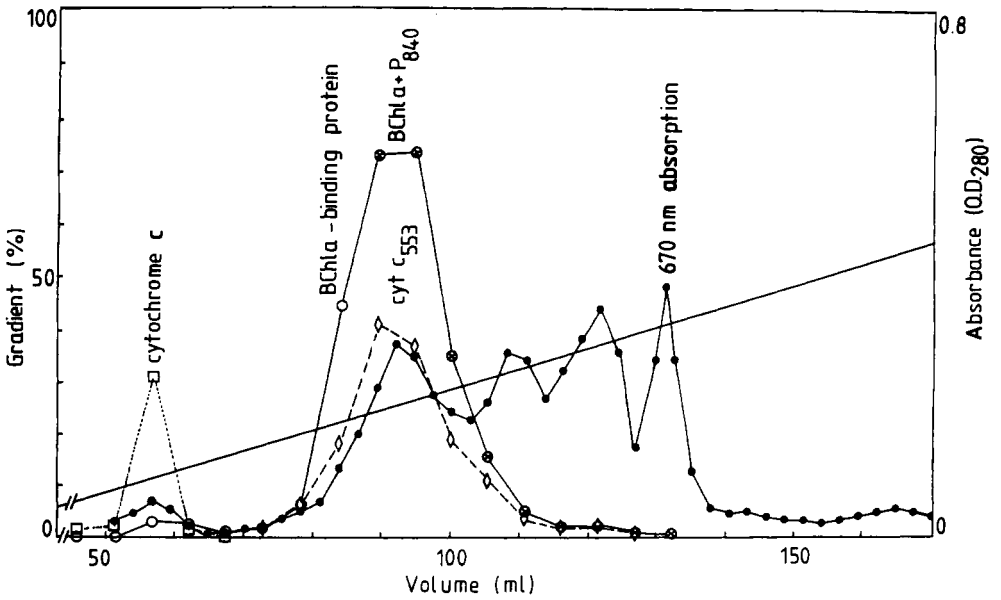

FIGURE 3: Elution profile from the DEAE ion-exchange column. The following symbols and description denote the different parameters assayed: (●) total protein contents measured by the absorption at 280 nm (the protein peak elution between 130 and 135 mL was characterized by the presence of a pigment absorbing at 670 nm, indicated as 670-nm absorption); (○) BChl*a* binding protein measured at 810 nm; (○ with ×) BChl*a* absorption measured at 810 nm with a shoulder at about 840 nm, indicating the RC; (□) cytochrome α-band absorption measured at 551 nm; (◇) cytochrome α-band absorption measured at 553 nm.

These results further support an association of cytochrome c_{553} to the reaction center. The ratio of cytochrome c_{553}:P_{840} has decreased from 3.3–4.2 measured in the sample directly after solubilization to 1.7 measured in this sample. The detailed study of the biochemical and biophysical properties of cytochrome c_{553} as well as of its association to the reaction center will be published in a separate article. The chemically induced difference spectra in Figure 2 show all spectral features which were observed in the light-induced difference spectra of Swarthoff and Amesz (1979). Furthermore, our spectrum obtained in solubilized material contains an additional broad band around 750 nm, which is most probably due to unspecific oxidation of BChl*c* induced by the oxidant. Swarthoff and Amesz report a stoichiometry of about 3 cytochromes per P_{840} in the membranes, which goes down to 0.5–1.5 in the partially purified complex (i.e., the so-called PP complex). An inspection of the spectra shown by Swarthoff and Amesz (1979) reveals a further significant decrease of photooxidizable cytochrome c_{553} per P_{840}^+ in the RCPP sample as compared to the PP sample.

The "molecular weight" of the isolated reaction center complex (including the detergent micelle) was determined with the calibrated molecular sieve column to be about 330 000. This apparent molecular weight is similar to the 300 000 determined for the PSI core complex of *Synechocystis* PCC 6803 (Rögner et al., 1990) and to the purified reaction center complex of heliobacteria estimated to be 335 000 (Van de Meent et al., 1990). The heliobacterial reaction center was found to be a PSI-type reaction center as well (Nitschke et al., 1990b).

During the whole purification the BChl*a* base-plate protein (Matthews et al., 1979) could not completely be separated from the reaction center (see Molecular Weight Analysis). This copurification of the BChl*a* base-plate protein, probably as a light-harvesting protein, with the reaction center complex indicates a physical association. All attempts to further purify the reaction center complex resulted in loss of activity.

Optical Characteristics during Purification. Figure 1 shows the absorption spectra of (a) membranes, (b) chlorosome-depleted membranes, (c) solubilized membranes, (d) the fraction containing the reaction center after DEAE chromatography, and (e) the fraction containing the reaction center after the molecular sieve column. The membrane spectrum (a) is identical with the spectrum of whole cells. During purification the 746-nm peak of BChl*c* was diminished, and the BChl*a* peak containing the P_{840} shoulder became clearly visible. A progressive red shift from 803 to 816 nm indicated the increasing purity of the reaction center with respect to antenna proteins. The 672-nm peak is probably mostly due to BPh*c* (Olson, 1981) with a minor contribution from the BChl*c*-like pigment described by Nuijs et al. (1985) which is proposed to act as the primary electron acceptor. The absorption maximum at 603 nm corresponds to the Q_x band of BChl*a*. This peak is asymmetric in purified reaction center samples. This asymmetry possibly reflects the presence of a heterogeneous population of BChl*a* molecules, located in slightly different protein environments as is also suggested by the low-temperature absorption spectrum of the BChl*a* Q_y peak (Olson et al., 1973; Fowler et al., 1973; Hurt & Hauska, 1984). In the wavelength range from 440 to 505 nm carotenoids [presumably rhodopin and/or hydroxychlorobactene (Braumann et al., 1986)] absorb. A wide variety of Soret bands were observable throughout the preparation. The 370-nm peak is due to the Soret band of BChl*a*, the 416-nm absorption (most clearly seen in spectrum c, Figure 1) could be attributed to the Soret band of BPh, and furthermore a peak at 340 nm is due to BChl*a* (Olson, 1981).

Molecular Weight Analysis. Several protein subunits could be detected in the most purified and photoactive fraction by SDS–PAGE (see Figure 4). Some weak high molecular mass bands between 90 and 200 kDa were observed. These bands are probably aggregates of reaction center proteins or aggregates of reaction center proteins with BChl*a* antenna proteins. A band at about 50 kDa, which sometimes migrated as a split band between 60 and 65 kDa (depending on the treatment of the sample), could be seen. It might correspond to the band at about 65 kDa which has been attributed to the apoprotein of P_{840} by Hurt and Hauska (1984). It migrated at roughly the same position as the large subunit of PSI from *Synechococcus* 7002 (see Figure 4). The band at 42 kDa is

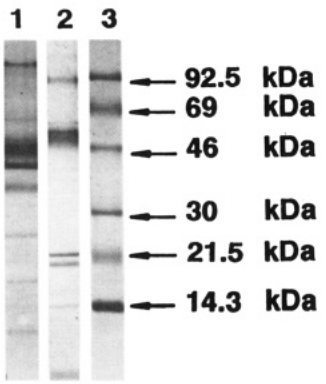

FIGURE 4: SDS–PAGE of the RC complex from *C. limicola* f *thiosulfatophilum* after molecular sieve chromatography (lane 1) compared with the PSI core complex from *Synechococcus* 7002 (lane 2). Lane 3 shows the marker proteins. The PSI samples were kindly provided by G. Tsiotis.

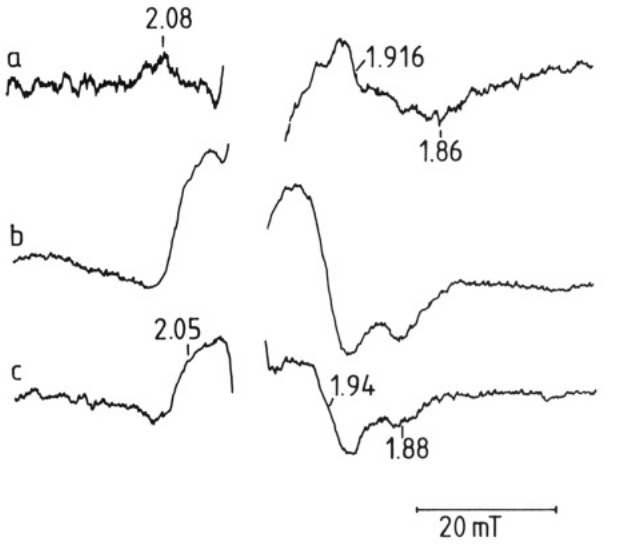

FIGURE 5: Light minus dark EPR difference spectra of the RC complex from *Chlorobium* in the presence of 10 mM dithionite: (a) spectrum of a sample after 10-min illumination at 4 K within the cavity minus the spectrum of a sample frozen after 5-min dark adaptation; (b) spectrum of a sample after 20-min illumination at 200 K in a quartz dewar (containing a mixture of ethanol and dry ice) minus the spectrum of a dark-adapted sample; (c) difference of (b) minus (a). Instrument settings: temperature, 15 K; microwave power, 6.3 mW; frequency, 9.43 GHz; modulation amplitude, 1.6 mT.

due to residual amounts of BChl*a* binding protein, as was demonstrated by N-terminal sequencing (sequence data not shown). The 18 sequenced amino acids of the 42-kDa protein from *C. limicola* f *thiosulfatophilum* showed 77% identity with the corresponding amino acid sequence of *Prosthecochloris aestuarii* (Daurat-Laroque et al., 1986). The intensity of this band diminished during the preparation; however, the most purified sample still contained observable amounts of this protein. The heme-staining cytochrome c_{553} migrated at 32 kDa (Hurt & Hauska, 1984). In some preparations several minor heme-staining bands appeared, which may be proteolytic degradation products of the 32-kDa band since their staining intensity increased after prolonged incubation at room temperature. A similar effect has already been reported for the tetraheme cytochrome subunit from *Chromatium vinosum* (Kennel & Kamen, 1971). The reaction center cytochrome subunits in purple bacteria are by some authors considered to be generally very sensitive to protease degradation (Meyer & Cusanovich, 1989). Further bands could be seen at about 22, 15, and 12 kDa. They may be equivalent to the polypeptides of PSI migrating in the range of ca. 9–22 kDa (Golbeck & Bryant, 1991).

EPR Spectroscopy. In Figure 5 the spectra of the low-temperature photoreduced FeS centers in purified reaction centers are shown. These spectra are identical to those measured in intact membranes (Nitschke et al., 1990a). At moderately low redox potential (in the presence of 10 mM ascorbate) the EPR signal of the Rieske FeS center, which has been detected under comparable conditions in intact membranes (Knaff & Malkin, 1976), was completely absent if only the fractions from the center of the elution profile peak for the optimally purified reaction center were used. If fractions were pooled for maximal yield of the reaction center, additionally the EPR signal of the Rieske protein was clearly visible. Previously it was suggested (Hurt & Hauska, 1984) that the bc_1 complex is directly associated with the reaction center. However, our data demonstrate that a separation of the reaction center and bc_1 complex is actually possible, but the two enzyme complexes seem to have rather similar properties with respect to purification methods used. Furthermore, in highly reduced, dark-adapted reaction center samples (dithionite, pH 11), no "dark" EPR signals from iron–sulfur centers were observable. The absence of these signals indicated that the pure reaction center preparation was devoid of all the additional FeS center containing enzymes which were not associated with the reaction center but were present in whole membranes. After illumination at 4 K in most reaction center samples, a spectrum with g-values at $g_x = 1.86$, $g_y = 1.916$, and $g_z = 2.08$ appeared (Figure 5a) which is almost identical to that found for the FeS center F_B in membranes (Nitschke et al., 1990a). In Figure 5b a spectrum obtained after illumination at 200 K is shown. A shift of the g-values (g_x, g_y) to lower magnetic field could be seen. The difference spectrum (200 K − 4 K, Figure 5c) is characterized by g-values at $g_x = 1.88$, $g_y = 1.94$, and $g_z = 2.05$ and can be attributed to the FeS center F_A interacting with the FeS center F_B which was prereduced at low temperature (Nitschke et al., 1990a).

All spectral changes could be reversed by thawing the samples and refreezing them in darkness.

In parallel, the yield of the spin-polarized triplet signal was measured to determine the ratio between reaction center complexes which are still capable of low-temperature stable charge separation between P_{840} and the terminal acceptors and damaged complexes which cannot carry out forward electron transfer beyond the primary acceptors. Treatment with dithionite and redox mediators and preillumination at 200 K (Nitschke et al., 1990a) resulted in 100% triplet yield which was used to calibrate the observed yields under moderately reducing conditions. Under these conditions about 20% of the total triplet signal could be photoinduced at 4 K. In principle, it might still be possible that a large fraction of the centers cannot perform charge separation at all and therefore did escape detection. However, the results of the optical photobleaching experiments provide evidence against this possibility, because otherwise the P_{840}:BChl*a* ratio would be significantly diminished. Therefore, 80% of the centers were still able to transfer electrons to the secondary acceptors.

Discussion

As described above, most detergents tried were not suited for the isolation of a photoactive reaction center from the membrane. However, by using LM, a detergent with a large polar headgroup which seems to be suited for solubilization of membrane proteins and to be milder than those with smaller headgroups, it became possible to specifically solubilize the reaction center without a major loss of activity.

Up to now only reaction center preparations from green sulfur bacteria were reported which were apparently devoid of secondary electron acceptors. The very pure reaction center preparation, described by Hurt and Hauska (1984), was still capable of P^+ formation under illumination (Feiler, 1987), but it lacked the terminal electron acceptors, the iron–sulfur centers F_A and F_B (Feiler, 1987; Nitschke et al., 1987). Similar observations have been published by Swarthoff et al. (1981) for a sample solubilized with Triton X-100 (the RCPP complex; Swarthoff & Amesz, 1979). The PP complex, which represents a stage prior to the RCPP complex, was reported to still contain FeS centers (Swarthoff et al., 1981). However, it contained a large number of polypeptides in the range from 26 to 46 kDa, whereas no reaction center band at 50–70 kDa was observed (Swarthoff & Amesz, 1979). Moreover, the illumination protocol applied does not provide unambiguous evidence that the reported EPR signals arise from FeS centers contained within the reaction center [for a detailed discussion of this problem, see Nitschke et al. (1990a)]. The low-temperature photoreduction of center F_B, which provides a reliable criterion for the presence of the terminal electron acceptors (Jennings & Evans, 1977; Nitschke et al., 1990a), was not observed in any of the above-mentioned preparations (Nitschke et al., 1987; Swarthoff & Amesz, 1981). EPR data presented in this work demonstrate that our preparation yields a photoactive reaction center. The spectral parameters of the two terminal FeS centers are similar to those already published for intact membranes. According to the *g*-values and line shape of their spectra, they are equivalent to the centers F_A and F_B of PSI. As in intact membranes, the centers F_A and F_B cannot be reduced by dithionite at high pH (Nitschke et al., 1990a).

A further fact that hinders the work with green sulfur bacterial reaction centers has been the presence of large antenna systems which impaired optical experiments due to the very high background absorbance. During the preparation described, the amount of antenna molecules and nonphotosynthetic proteins is greatly diminished, while the reaction center is maintained intact.

The depletion of antenna pigments (BChl*c* in the chlorosomes and BChl*a* in the base-plate protein) and the enrichment of the reaction center pigments (>800 nm) are nicely demonstrated by the change of the optical spectra during the preparation. The BChl*a*:P_{840} = 40:1 ratio (molecular sieve) is similar to the Chl:P700 = 25–40:1 ratio existing in enriched PSI core complex preparations (Mullet et al., 1980). The interpretation of these ratios is that most of the BChl*a* molecules are part of an internal antenna system associated with the same peptide that binds the primary donor and the early acceptors.

The band at about 50 kDa seen with SDS–PAGE migrates at roughly the same position as the large subunit of PSI from *Synechoccocus* 7002. It migrates faster than the 65-kDa protein which was attributed to a P_{840} reaction center apoprotein by Hurt and Hauska (1984). However, the different mobility can be due to different gel conditions, as is found for the reaction center of *Heliobacillus mobilis* (Trost & Blankenship, 1989). It has been suggested by Shirasawa and Sakurai (1990), who measured the cysteine content of several protein bands by SDS–PAGE, that a 42-kDa band is the protein subunit which binds the FeS center F_X. However, by N-terminal sequencing we could show that our 42-kDa protein most likely is the water-soluble BChl*a* base-plate protein of *C. limicola* f *thiosulfatophilum*, since its 18 N-terminal amino acids show a 77% sequence identity to the equivalent protein of *P. aestuarii* (Daurat-Laroque et al., 1986).

The EPR experiments demonstrate that both terminal electron acceptors F_A and F_B are present in the purified sample. It has been shown previously on chlorosome-depleted membranes that both the magnetic interaction and the orientations of the two FeS clusters are very similar to those of PSI (Nitschke et al., 1990a). Therefore, it has been proposed that, in analogy to PSI, these centers reside on an additional small protein subunit. Three low molecular mass peptides can be seen on SDS–PAGE, which could play this role: the 22-, the 15-, and the 12-kDa protein bands. No band is seen at 8.9 kDa, where the F_A/F_B binding subunit (psaC gene product) in PSI approximately migrates (Golbeck & Bryant, 1991). According to the SDS–PAGE data of Shirasawa and Sakurai (1990), a cysteine-rich protein is migrating at 12 kDa, where a protein band is also seen in our preparation. However, as discussed above for the 42-kDa subunit, such an interpretation only based on cysteine data might be misleading. Our results on F_A and F_B (Nitschke et al., 1990a) demonstrate that both terminal acceptors have redox midpoint potentials significantly different from those of their counterparts in PSI. Thus, a different distribution of charged amino acids around the cluster could be anticipated. On a small protein, this could modify the migration properties severely. Therefore, a conclusive identification of the F_A/F_B binding subunit has to await the elucidation of the primary sequences of the 12-, 15-, and 22-kDa protein bands.

The role of the green sulfur bacterial reaction center as a "missing link" between the purple bacterial reaction center and PSI has already been suggested on the basis of EPR data (Nitschke et al., 1990a). The biochemical characterization of the green sulfur bacterial reaction center described in this work corroborates this picture. As outlined above, the chemical nature of the terminal acceptors, the proposed antenna reaction center organization, and the protein composition of the *Chlorobium* reaction center are similar to the PSI reaction center. However, the copurification of a RC-associated cytochrome subunit is reminiscent of some purple bacterial reaction centers (Feiler et al., 1989), which are of the PSII type. Therefore, the photosynthetic reaction center from the green sulfur bacteria assumes an intermediate position between PSI on one side and PSII/purple bacterial reaction centers on the other side. Thus, a common ancestor for all kinds of photosynthetic reaction centers might have existed.

Alternatively, green sulfur bacteria could have acquired a tetraheme cytochrome via lateral gene transfer.

Highly active reaction center preparations of PSI have been studied for many years using optical spectroscopy with high (down to picoseconds) time resolution. Therefore, a wealth of data exists with respect to electron transport within the PSI electron acceptor chain (Golbeck, 1987; Rutherford & Heathcote, 1985). Such experiments were impossible with green sulfur bacteria due to the large antennae. Only the primary step could be characterized so far (Nuijs et al., 1985). Preparation procedures yielding a low BChl:P_{840} ratio, like that described above, enable similar experiments to be performed on the green sulfur bacterial reaction center. Thus reaction center samples purified as described above can be very useful for a further characterization of secondary electron transport in green sulfur bacteria, thereby providing new data which allow an even better comparison with PSI.

ACKNOWLEDGMENTS

We thank Prof. N. Pfennig, Konstanz, FRG, for the stock cultures of *C. limicola* f *thiosulfatophilum* and *C. phaeobacteroides*, Dr. G. Tsiotis for stimulating discussions and

particularly for providing the PSI samples for SDS–PAGE gels, Dr. A. W. Rutherford for interesting discussions, and Dr. I. Sinning for critical reading of the manuscript.

Registry No. Bacteriochlorophyll *a*, 17499-98-8; cytochrome *c*553, 12624-01-0.

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