Menaquinone-7 in the Reaction Center Complex of the Green Sulfur Bacterium *Chlorobium vibrioforme* Functions as the Electron Acceptor A_1[†]

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ABSTRACT: Photosynthetically active reaction center complexes were prepared from the green sulfur bacterium *Chlorobium vibrioforme* NCIMB 8327, and the content of quinones was determined by extraction and high-performance liquid chromatography. The analysis showed a stoichiometry of 1.7 molecules of menaquinone-7/reaction center. No other quinones were detected in the isolated reaction centers, whereas membrane preparations also contained chlorobiumquinone. The possible involvement of quinones in electron transport was investigated by electron paramagnetic resonance (EPR) spectroscopy. A highly anisotropic radical was detected by Q-band EPR spectroscopy in both membranes and isolated reaction centers following dark reduction with sodium dithionite and photoaccumulation at 205 K. At 34 GHz, the EPR spectrum is characterized by a g tensor with $g_{xx} = 2.0063$, $g_{yy} = 2.0052$, $g_{zz} = 2.0020$ and ΔB of 0.7 mT, consistent with its identification as a quinone. This spectrum is highly similar in terms of g values and line widths to photoaccumulated A_1^- in photosystem I of *Synechococcus* sp. PCC 7002. The results indicate that menaquinone-7 in the green sulfur bacterial reaction center is analogous to phylloquinone in photosystem I.

Green sulfur bacteria contain a single type of photosynthetic reaction center that resembles photosystem I of cyanobacteria and plants and the reaction center of heliobacteria (*1*−*3*). These reaction centers are characterized by the presence of low potential iron−sulfur clusters as the terminal electron acceptors. The reaction center complex of green sulfur bacteria contains two iron−sulfur clusters bound to the PscB protein which are analogous to the F_A and F_B clusters of photosystem I (*4*−*11*). In heliobacteria similar iron−sulfur clusters have been characterized by electron paramagnetic resonance (EPR)[1] spectroscopy (*12*), but the protein which binds the clusters has not been identified. In iron−sulfur-type reaction centers, the primary donor and the initial acceptors are located on a dimer of pigment−proteins with a molecular mass of 68−82 kDa. In photosystem I, this dimer is comprised of the homologous subunits PsaA and PsaB. In the reaction center complexes of green sulfur bacteria and heliobacteria, the core dimer is homodimeric and made up of the PscA and PshA proteins, respectively. Both PscA and PshA have sequence similarity to PsaA and PsaB (*13*, *14*). The primary electron acceptor in green sulfur bacteria was originally designated bacteriochlorophyll (BChl) 663 and has been identified as a chlorophyll *a*-like molecule (*15*, *16*). Thus, the reaction centers of green sulfur bacteria and photosystem I are very similar with respect to the primary acceptor and the terminal acceptors.

There are, however, some puzzling differences. The iron−sulfur cluster F_X is easily detected in photosystem I by low-temperature EPR spectroscopy, but except for an indication of a weak highfield resonance (*7*, *11*, *17*), F_X has not been unambiguously identified in green sulfur bacteria. Yet, the domains of the PsaA and PsaB proteins that appear to bind the F_X cluster in photosystem I are conserved in the PscA protein (*13*), and biochemical studies have shown the presence of acid-labile sulfide in the isolated reaction center protein (B. Kjær and H. V. Scheller, unpublished).

Another possible difference in the electron transport chains is in the quinone acceptor. In photosystem I, A_1, the intermediate acceptor between A_0 and F_X, is identified as phylloquinone (vitamin K_1; 2-methyl-3-phytyl-1,4-naphthoquinone). Photosystem I contains two phylloquinones, but apparently only one participates in electron transport (*17*, *18*). Several quinones are known to be present in green sulfur bacteria: menaquinone-7, chlorobiumquinone (1′-oxomenaquinone-7) and a polar menaquinone (probably 1′-hydroxy-menaquinone-7) (*19*). Chlorobiumquinone has been proposed to function in the redox regulation of excitation

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[1] Abbreviations: BChl, bacteriochlorophyll; CP1, chlorophyll *a*-protein 1; EPR, electron paramagnetic resonance; HPLC, high-performance liquid chromatography.

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energy transfer in the chlorosome antenna (*20*). In the membranes, menaquinone-7 probably shuttles electrons between sulfide quinone reductase and the cytochrome *bc* complex (*21*), and it might operate in cyclic electron transport. However, in the green sulfur bacterial reaction center complex there is no previous report of a chemically extractible quinone. Analysis of isolated reaction center complexes from *Chlorobium limicola* (*22*) and *Chlorobium tepidum* (*23*) did not reveal the presence of quinones although these preparations were photochemically active. These extraction studies are in contrast to the report of Muhiuddin et al. (*24*) who showed the presence of an A_1-like EPR signal in the membranes from *C. limicola* and the result of Nitschke et al. (*25*), which suggest the presence of two early electron acceptors prior to the iron−sulfur clusters.

To resolve this discrepancy we decided to undertake a systematic analysis of the content of quinones in reaction center complexes from *C. vibrioforme* and to attempt detection of a photoaccumulated semiquinone anion radical and detection by EPR spectroscopy. Our results show the presence of approximately two molecules of menaquinone-7 per P840, and Q-band EPR studies indicate the presence of a photoreducible quinone in the *C. vibrioforme* reaction center complex.

EXPERIMENTAL PROCEDURES

Chlorosome-depleted membranes and photosynthetically active reaction center complexes were prepared from *Chlorobium vibrioforme* NCIMB 8327 under anaerobic conditions as previously described (*6*).

The membranes and reaction centers were extracted with chloroform and analyzed by high-performance liquid chromatography (HPLC) as previously described (*20*) with the following modifications. The vacuum-dried samples were dissolved in acetone:methanol (4:1 v/v) before injection onto the HPLC column. The elution gradient was optimized for quinone separation and was programmed as follows: 30% solvent B at the time of injection, a linear increase to 65% B after 30 min, to 75% after an additional 35 min, to 85% after 20 min, to 100% after 11 min and constant at 100% for 10 min before returning to 30% B in 2 min. Peaks were identified on basis of the absorption spectrum and comparison of the retention time with known samples. Using this method, the polar menaquinone, chlorobiumquinone and menaquinone-7 eluted at 52.9, 54.5, and 77.1 min, respectively. The relative contents of pigments and quinones were determined by integration of the peaks. Absorption coefficients at 270 nm were estimated from experimental data and literature values. The following values (in inverse millimolarity centimeters) were used: BChl *a*, 30 (*27*); BChl 663, 22 [as for chlorophyll *a* (*27*)]; chlorobactene, 20 (*28*); chlorobiumquinone, 11.0 (*20*); and menaquinone-7, 16.8 (*20*).

EPR studies were performed at X-band using a Bruker ECS 106 spectrometer equipped with an ER/4102 ST resonator and at Q-band using a Bruker ER300E spectrometer equipped with an ER 5106 QT-W1 resonator. Cryogenic temperatures were maintained with an ER4111 liquid nitrogen cryostat and an ER4118CV liquid nitrogen cryostat, both controlled with an ER4121 temperature control unit. The microwave frequency was measured with a Hewlett-Packard 5352B frequency counter, and the magnetic field was measured with a Bruker ER035M NMR gaussmeter. The magnetic field was calibrated at X-band using α,α′-diphenyl-β-picryl hydrazyl (DPPH) and at Q-band using γ,γ-bisdiphenylene-β-phenylallyl (BDPA) complexed 1:1 with benzene. EPR spectral simulations were carried out on a Power Macintosh 8500/233 computer using a Windows 3.1 emulator (SoftWindows 3.0, Insignia Solutions, U.K.) and SimFonia software (Bruker Analytik GMBH).

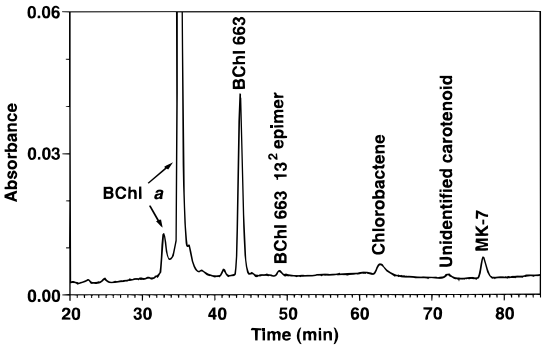

FIGURE 1: HPLC analysis of pigments and quinones in the isolated reaction center complex. The chromatogram shown was recorded at 270 nm and the identity of the peaks was determined from the absorption spectrum and by comparison of the retention time with authentic samples. The peak labeled MK-7 eluted with the same retention time as menaquinone-7.

Prior to the photoaccumulation, the pH of the sample was adjusted to 10.0 and sodium dithionite was added to a final concentration of 100 mM. After incubation for 30 min, the sample was placed in a dewar containing dry ice-ethanol at a temperature of 205 K and illuminated by a focused 100 W quartz-tungsten lamp through a 2.5 cm water filter. The photoaccumulation times are indicated in the text. The sample was immediately frozen in liquid nitrogen, transferred to the cryostat, and the spectrum was acquired at 100 K.

P840 and cytochrome *c* was determined from the ferricyanide-oxidized minus ascorbate-reduced spectrum using extinction coefficients at 830 nm of 100 mM^{-1} cm^{-1} and at 551 nm of 20 mM^{-1} cm^{-1}, respectively (*29*).

RESULTS

The HPLC analysis of a reaction center preparation from *C. vibrioforme* is shown in Figure 1. The peak labeled MK-7 had a retention time identical to that of authentic menaquinone-7 and well resolved from the retention times of the other quinones known to be present in *Chlorobium*. The absorption spectrum of the collected peak, shown in Figure 2, was also indistinguishable from that of authentic menaquinone (*30*). The spectrum was aquired in isooctane, which enables a very clear distinction between different quinones (*30*). On the basis of the HPLC analysis of the reaction center preparations, we determined a content of 9.1 mol of BChl 663, 1.7 mol of menaquinone-7, <0.1 mol of chlorobiumquinone, and 1.3 mol of chlorobactene/mol of P840. From the reduced-minus-oxidized absorption spectrum of the intact reaction center preparations, we deduced a 1.1 molar ratio of cytochrome c_{551} to P840. Analysis of three separate reaction center preparations gave essentially the same results. From a similar HPLC analysis, we find that chlorosome-depleted membranes contain approximately 200 mol of

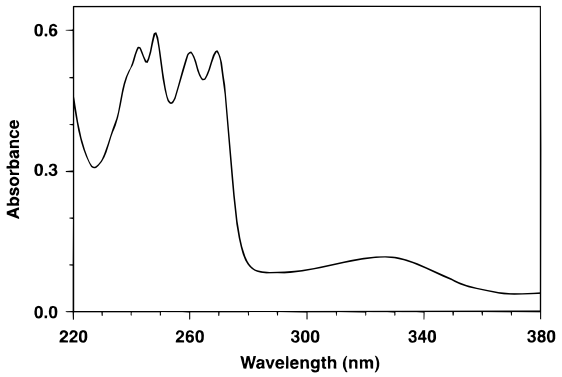

FIGURE 2: Absorbance spectrum in isooctane of menaquinone-7 isolated from a reaction center preparation by HPLC (Figure 1, peak MK-7). The spectrum is indistinguishable from that of authentic menaquinone-7.

menaquinone-7 and 40 mol of chlorobiumquinone/mol of P840, (assuming a ratio of 100 BChl *a* per P840). The polar menaquinone, which is only found in small amounts under certain growth conditions (*19*; N.-U. Frigaard, unpublished results), was neither detected in our chlorosome-depleted membranes nor in our reaction center preparations.

Because the *g* tensor of A_1 is poorly resolved at 9.4 GHz, EPR experiments of photoaccumulated membranes at X-band led to a spectrum in which A_1^- could not be clearly distinguished from A_0^-. We therefore conducted experiments at 34 GHz, a microwave frequency where the *g* anisotropy of the quinone leads to a much broader peak-to-trough line width for A_1. By photoaccumulating *C. vibrioforme* membranes for periods up to 2 min, an axial-like signal progressively developed with a low-field peak at an apparent *g* value of 2.0060, and a derivative signal centered around $g = 2.0028$ (data not shown). After 30 min of additional illumination at 205 K, the $g = 2.0060$ feature decreased in intensity and disappeared while the $g = 2.0028$ feature increased slightly in intensity. A similar spectrum was found in the purified reaction center preparations except that only 30 s of photoaccumulation was required to achieve the maximum signal intensity (Figure 3A, solid line). After 10 min of further illumination at 205 K, the $g = 2.0060$ feature decreased in intensity and disappeared while the $g = 2.0028$ feature increased slightly in intensity. The overall spectral appearance of the 30 s sample is similar to that of photoaccumulated A_1^- measured in deuterated CP1 particles (*31*). Here, only the low-field portion of the CP1 spectrum represents exclusively A_1^-, while the $g = 20028$ feature is thought to represent largely A_0^-.

We interpret the disappearance of the $g = 2.0060$ feature in *C. vibrioforme* as the time-dependent double reduction of a bound quinone to an EPR silent-state (*32*) and the increase of the $g = 2.0028$ feature as the concomitant photoaccumulation of A_0^-. The spectrum in Figure 3A therefore represents an admixture of A_0^- and A_1^-, with the latter as the minority species. We simulated the A_0^- signal, and after suitable scaling, subtracted it from the experimental spectrum in Figure 3A. The result, depicted in Figure 3B (solid line) shows a low-field peak at an apparent *g* value of 2.0060 and a shallow trough at an apparent *g* value of 2.0020. Using a powder spectrum simulation where g_{zz} was taken from the experimental spectrum and g_{yy} and g_{xx} were varied, we obtained the closest match with $g_{xx} = 2.0063$ and $g_{yy} = 2.0052$, and line widths of 0.7 mT for all three *g* tensor components (the g_{zz} component is the least accurate parameter due to subtraction error). The extracted spectrum (Figure 3B, solid line) is a close match to a simulated spectrum of A_1^- in cyanobacterial photosystem I (Figure 3B, dotted line) using the *g* tensor of $g_{xx} = 2.0062$, $g_{yy} = 2.0051$ and $g_{zz} = 2.0022$ obtained from W-band studies of *Synechococcus elongatus* photosystem I trimers (*33*).

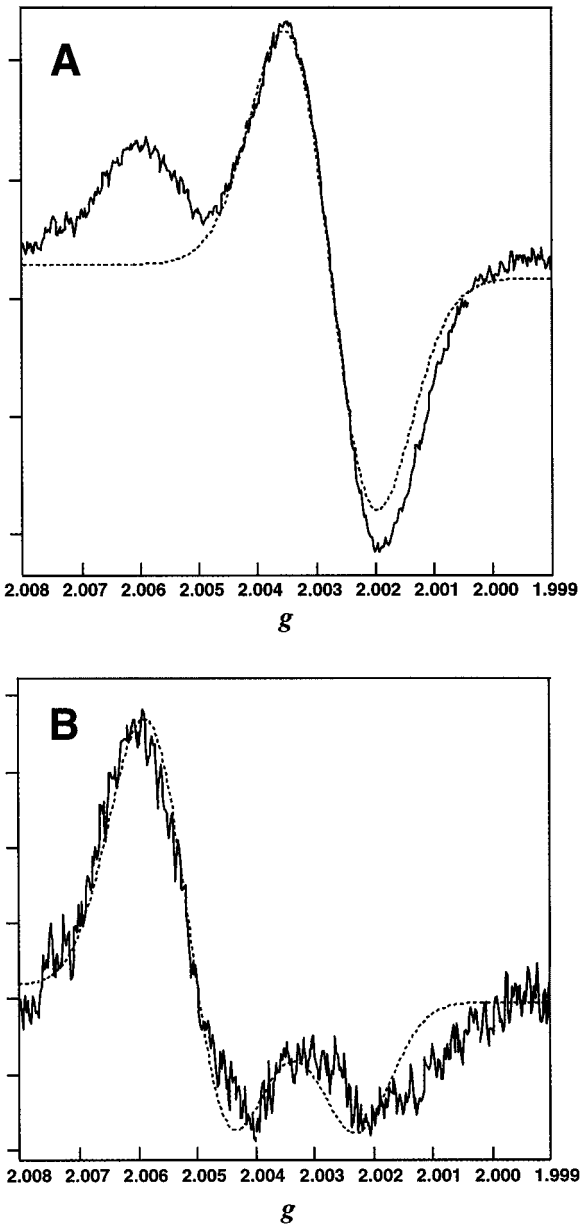

FIGURE 3: Q-band EPR spectrum of photoaccumulated A_1 in *C. vibrioforme*. (A, solid line) Experimental spectrum of reaction centers reduced in darkness with sodium dithionite at pH 10.0 for 30 min and photoaccumulated at 205 K for 30 s. The spectrum represents an average of 10 scans. (A, dotted line) Simulated spectrum of A_0^-, using $g = 2.0028$ and $\Delta B = 1.0$ mT, arbitrarily scaled to visually match the midfield feature. (B, solid line) Extracted spectrum of A_1- obtained by subtracting the simulated A_0- spectrum (A, dotted line) from the experimental spectrum (A, solid line). (B, dotted line) Simulated spectrum of A_1^- using $g_{xx} = 2.0062$, $g_{yy} = 2.0051$, and $g_{zz} = 2.0022$ and $\Delta B = 0.7$ mT (*33*). Instrument conditions: microwave power, 2 mW; microwave frequency, 34.087 37 GHz; magnetic field, 1.2083−1.2203 T (a subset of the field range is depicted here); modulation amplitude, 0.2 mT; modulation frequency, 100 kHz; temperature, 100 K.

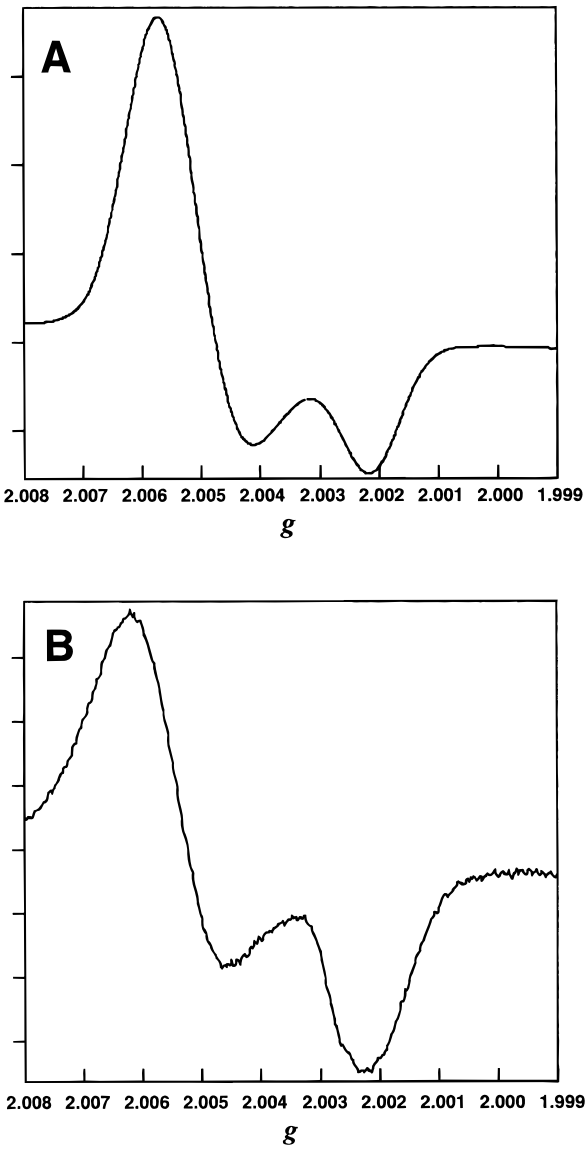

FIGURE 4: Q-band EPR spectrum of (A) reduced vitamin K_1 and (B) photoaccumulated A_1 in cyanobacterial photosystem I. The vitamin K_1 was dissolved in alkaline ethanol at pH 10.0, and the radical was generated by addition of sodium borohydride. The A_1^- radical was generated in *Synechococcus* sp. PCC 7002 reaction centers by reduction in darkness with sodium dithionite at pH 10.0 for 30 min and photoaccumulation at 205 K for 40 min. Instrument conditions for panel A: microwave power, 5.42 mW; microwave frequency, 34.057 02 GHz; magnetic field, 1.2100−1.2130 T (a subset of the field range is depicted here); modulation amplitude, 0.1 mT; modulation frequency, 100 kHz; temperature, 100 K. The spectrum represents an average of four scans. Instrument conditions for panel B: microwave power, 2 mW; microwave frequency, 34.087 37 GHz; magnetic field, 1.2083−1.2203 T; modulation amplitude, 0.2 mT; temperature, 100 K. The spectrum represents an average of eight scans.

The extracted A_1^- spectrum in *C. vibrioforme* can be further compared with the Q-band EPR spectrum of reduced vitamin K_1^- in ethanol (Figure 4A) and photoaccumulated A_1^- in *Synechococcus* sp. PCC 7002 (Figure 4B). We note that the high-field trough in the cyanobacterial A_1 spectrum is also contaminated with a population of reduced A_0. The larger anisotropy of cyanobacterial A_1^- compared with reduced vitamin K_1^- is considered to be derived from stronger mixing of the *n*- and π^* orbitals due to spin−orbit coupling which may, in turn, result from H-bonds to protein or Coulombic interactions with charged amino acid residues (*33*). The g_{xx} and g_{yy} components of the *Chlorobium* A_1^- spectrum (Figure 3B) are also upfield-shifted (the g_{zz} component of the experimental spectrum is considered unreliable) compared with A_1^- in reduced vitamin K_1^-, which may indicate a similar binding environment within the PscA protein. If the proposed phylloquinone binding site in *S. elongatus* includes the R_{694}GYWQELIESIVWAH region on PsaA and the R_{674}GYWQELIETLVWAH region on PsaB (*33*), then the comparable menaquinone-7 binding site would include the region R_{804}GSRLEGADIQTRTI on PsbA. The relatively low similarity between the proposed PsaA/PsaB and PscB quinone binding regions hints that the quinone environment may include other regions of the reaction center protein.

DISCUSSION

In this paper, we have shown that menaquinone-7 is a constituent of membranes and reaction center complexes isolated from *C. vibrioforme*. Previously, photoactive reaction center preparations from green sulfur bacteria have been reported to lack quinones (*23*, *24*). One difference is that in our isolation procedure, we have maintained anaerobic conditions and used the mild detergent *n*-dodecyl-*β*-D-maltoside. We specifically avoided the use of Triton X-100, a detergent that has been shown to remove phylloquinone in photosystem I preparations which lack the iron−sulfur clusters (*34*). The menaquinone-7 content of three different reaction center preparations was very close to two molecules per P840. This result suggests that menaquinone-7 in the green sulfur bacterial reaction center is the counterpart to phylloquinone in photosystem I.

The total amount of menaquinone-7 in the membranes is much larger than the amount bound to the reaction center complex. However, a nonspecific binding or coelution of the two molecules of menaquinone-7 is, in our opinion, highly unlikely, since the detergent should have removed a loosely bound population. Furthermore, chlorobiumquinone, which is similar to menaquinone-7 in structure, was efficiently separated from the reaction center complex during the isolation procedure. The close resemblance in terms of *g* values and line widths to the cyanobacterial A_1^- spectrum is further evidence that the radical represents protein-bound A_1^- and not simply reduced, adventitious menaquinone-7.

The finding that reaction center preparations from *Chlorobium* devoid of quinones have been shown to possess electron transfer to the terminal iron−sulfur clusters may be explained if, as we have suggested (*10*), the reaction center is highly analogous in structure and function with photosystem I. In photosystem I preparations in which the phylloquinone has been chemically extracted, electron transfer occurs at low temperatures, albeit with a decreased quantum yield (*35*, *36*). At room-temperature electron-transfer remains blocked beyond A_0. A similar low-temperature electron transfer may also occur in green sulfur bacterial reaction centers in the absence of quinone. Room-temperature electron transport in a preparation from *C. limicola* devoid of detectable quinones would suggest a situation different from photosystem I (*23*), although it cannot be excluded that the electron transport was due to residual quinone below the detection limit. Unfortunately, it is not

yet possible to assay electron transfer to the iron−sulfur clusters using time-resolved optical techniques because electron transfer from the bound cytochrome to $P840^+$ outcompetes the characteristic back-reaction from the iron−sulfur clusters at room temperature. Alternatively, it is possible that menaquinone-7 is not an obligate electron acceptor in the green sulfur bacterial reaction center, either at low or room temperatures. In *Heliobacterium chlorum*, extraction of menaquinone did not alter electron transport to an appreciable extent (*37*). Further studies are needed to determine if the reaction center complex from *H. chlorum* differs from photosystem I and the green sulfur bacterial reaction center.

In this paper, we also show that an anisotropic EPR signal appears in green sulfur bacterial reaction centers during photoaccumulation which resembles A_1, the phylloquinone anion radical in photosystem I. This signal appears to be derived from menaquinone-7, the only quinone detected in the isolated *C. vibrioforme* reaction centers. In this context, the disappearance of the EPR signal with prolonged illumination may be explained by its double reduction to dihydromenaquinone-7. The only significant difference between the EPR spectra obtained with *C. vibrioforme* membranes and with isolated reaction centers is the time necessary for disappearance of the EPR signal with prolonged illumination. We have recently shown that a similar disappearance of the photoaccumulated A_1^- signal occurs after a brief illumination period in *Synechococcus* sp. PCC 7002 reaction centers in which the PsaE and PsaF polypeptides have been deleted by mutagenesis (*38*). The explanation offered is that the absence of these peripheral polypeptides opens a solvent channel to the phylloquinone acceptor, allowing A_1^- to be protonated during the first electron reduction step. The second electron reduction step becomes thermodynamically facile because the reduction potential of the semiphylloquinone/phylloquinol couple is higher than that of the phylloquinone/semiphylloquinone couple (*39*), and results in an EPR-silent state. The *C. vibrioforme* reaction center complex contains fewer peripheral polypeptides than photosystem I, and polypeptides homologous to PsaE and PsaF have not been detected. If the PscA homodimer in *Chlorobium* has a three-dimensional structure similar to that of the PsaA/PsaB heterodimer in cyanobacteria, then the solvent channel may become available simply by removing the complex from the membranes using a mild detergent such as *n*-dodecyl-*β*-D-maltoside. Under these conditions, double reduction to diamagnetic A_1^{2-} could be expected to occur, with subsequent appearance of a large paramagnetic A_0^- radical.

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REFERENCES

1. Sakurai, H., Kusumoto, N., and Inoue, K. (1996) *Photochem. Photobiol. 64*, 5−13.
2. Golbeck, J. H. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1642−1646.
3. Feiler, U., and Hauska, G. (1995) in *Anoxygenic Photosynthetic Bacteria* (Blankenship, R. E., Madigan, M. T., and Bauer, C. E., Eds.) pp 665−685, Kluwer Academic Publishers, Dordrecht.
4. Hager-Braun, C., Jarosch, U., Hauska, G., Nitschke, W., and Riedel, A. (1997) *Photosynth. Res. 51*, 127−136.
5. Illinger, N., Xie, D. L., Hauska, G., and Nelson, N. (1993) *Photosynth. Res. 38*, 111−114.
6. Kjær, B., Jung, Y. S., Yu, L. A., Golbeck, J. H., and Scheller, H. V. (1994) *Photosynth. Res. 41*, 105−114.
7. Kusumoto, N., Inoue, K., Nasu, H., and Sakurai, H. (1994) *Plant Cell Physiol. 35*, 17−25.
8. Kusumoto, N., Inoue, K., and Sakurai, H. (1995) *Photosynth. Res. 43*, 107−112.
9. Miller, M., Liu, X. M., Snyder, S. W., Thurnauer, M. C., and Biggins, J. (1992) *Biochemistry 31*, 4354−4363.
10. Scott, M. P., Kjær, B., Scheller, H. V., and Golbeck, J. H. (1997) *Eur. J. Biochem. 244*, 454−461.
11. Nitschke, W., Feiler, U., and Rutherford, A. W. (1990) *Biochemistry 29*, 3834−3842.
12. Nitschke, W., Sétif, P., Liebl, U., Feiler, U., and Rutherford, A. W. (1990) *Biochemistry 29*, 11079−11088.
13. Büttner, M., Xie, D. L., Nelson, H., Pinther, W., Hauska, G., and Nelson, N. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 8135−8139.
14. Liebl, U., Mockensturm-Wilson, M., Trost, J. T., Brune, D. C., Blankenship, R. E., and Vermaas, W. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 7124−7128.
15. van de Meent, E., Kobayashi, M., Erkelens, C., van Veelen, P. A., Otte, S. C. M., Inoue, K., Watanabe, T., and Amesz, J. (1992) *Biochim. Biophys. Acta 1102*, 371−378.
16. Feiler, U., Albouy, D., Pourcet, C., Mattioli, T. A., Lutz, M., and Robert, B. (1994) *Biochemistry 33*, 7594−7599.
17. Golbeck, J. H. (1992) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 43*, 293−324.
18. Malkin, R. (1996) in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yocum, C. F., Eds.) pp 313−332, Kluwer Academic Publishers, Dordrecht.
19. Powls, R., and Redfearn, E. R. (1969) *Biochim. Biophys. Acta 172*, 429−437.
20. Frigaard, N. U., Takaichi, S., Hirota, M., Shimada, K., and Matsuura, K. (1997) *Arch. Microbiol. 167*, 343−349.
21. Klughammer, C., Hager, C., Padan, E., Schutz, M., Schreiber, U., Shahak, Y., and Hauska, G. (1995) *Photosynth. Res. 43*, 27−34.
22. Oh-oka, H., Kakutani, S., Matsubara, H., Malkin, R., and Itoh, S. (1993) *Plant Cell Physiol. 34*, 93−101.
23. Frankenberg, N., Hager-Braun, C., Feiler, U., Fuhrmann, M., Rogl, H., Schneebauer, N., Nelson, N., and Hauska, G. (1996) *Photochem. Photobiol. 64*, 14−19.
24. Muhiuddin, I. P., Rigby, S. E. J., Evans, M. C. W., and Heathcote, P. (1995) in *Photosynthesis: from Light to Biosphere*, Vol. II (Mathis, P., Ed.) pp 159−162, Kluwer Academic Publishers, Dordrecht.
25. Nitschke, W., Feiler, U., Lockau, W., and Hauska, G. (1987) *FEBS Lett. 218*, 283−286.
26. Tsuji, K., Takaichi, S., Matsuura, K., and Shimada, K. (1995) in *Photosynthesis: from Light to Biosphere*, Vol. IV (Mathis, P., Ed.) pp 99−103, Kluwer Academic Publishers, Dordrecht.
27. Wintermans, J. F. G. M., and de Mots, A. (1965) *Biochim. Biophys. Acta 109*, 448.
28. Holo, H., Broch-Due, M., and Ormerod, J. G. (1985) *Arch. Microbiol. 143*, 94−99.
29. Olson, J. M., Giddings, T. H., and Shaw, E. K. (1976) *Biochim. Biophys. Acta 449*, 197−208.

30. Collins, M. D. (1994) in *Chemical Methods in Prokaryotic Systematics* (Goodfellow, M., and O'Donnell, A. G., Eds.) pp 265−309, John Wiley & Sons, Chichester.
31. Thurnauer, M. C., and Gast, P. (1985) *Photobiochem. Photobiophys. 9*, 29.
32. Heathcote, P., Hanley, J. A., and Evans, M. C. W. (1993) *Biochim. Biophys. Acta 1144*, 54−61.
33. van der Est, A., Prisner, T., Bittl, R., Fromme, P., Lubitz, W., Möbius, K., and Stehlik, D. (1997) *J. Phys. Chem. B. 101*, 1437−1443.
34. Warren, P. V., Golbeck, J. H., and Warden, J. T. (1993) *Biochemistry 32*, 849−857.
35. Sétif, P., Ikegami, I., and Biggins, J. (1987) *Biochim. Biophys. Acta 894*, 146−156.
36. Mansfield, R. W., and Evans, M. C. W. (1987) *FEBS Lett. 220*, 74−78.
37. Kleinherenbrink, F. A. M., Ikegami, I., Hiraishi, A., Otte, S. C. M., and Amesz, J. (1993) *Biochim. Biophys. Acta 1142*, 69−73.
38. Yang, F., Shen, G., Schluchter, W. M., Zybailov, B., Ganago, A., Golbeck, J. H., and Bryant, D. A. (1998) in *The Phototrophic Prokaryotes* (Peschek, G. A., Loeffelhardt, W., and Schmetterer, G., Eds.) Plenum Press, New York, in press.
39. Bottin, H., and Sétif, P. (1991) *Biochim. Biophys. Acta 1057*, 331−336.

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