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Redox properties of an H-subunit-depleted photosynthetic reaction center from *Rhodopseudomonas viridis*

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Abstract

Recently, we reported that a H-subunit-depleted photosynthetic reaction center (RC-H) was purified from purple nonsulfer photosynthetic bacterium *Rhodopseudomonas viridis* (*Rps. viridis*) using a strong detergent sodium alkyl ether sulfate. We compared the redox properties of a native photosynthetic reaction center (RC) and RC-H of *Rps. viridis*. In RC-H prepared by our method, secondary quinone (Q_B) was removed while primary quinone (Q_A) was retained. Absorption spectrum of RC-H was similar to that of RC. After reconstitution of ubiquinone 10 into Q_B sites, RC-H showed electron transfer activity that was the same as that for native RC. This is the first report about the redox properties of RC-H of *Rps. viridis*. © 1998 Elsevier Science B.V.

Keywords: Photosynthetic reaction center; H Subunit; Redox property; (*Rhodopseudomonas viridis*)

1. Introduction

We prepared an H-subunit-depleted photosynthetic reaction center (RC-H) from the chromatophore membrane of *Rhodopseudomonas viridis* (*Rps. viridis*) using polyacrylamide gel electrophoresis in the presence of a detergent of sodium POE(3) alkyl ether sulfate (AES) [1,2]. The absorption spectrum of RC-H was similar to that of the native photosynthetic reaction center (RC), although the bacteriochlorophyll dimer (Bch2) absorption peak at 960 nm was slightly lower and the bound cytochrome peak at 550–560 nm was slightly higher.

The role of the H-subunit in bacterial RC from the photosynthetic bacterium *Rhodobacter sphaeroides* (*Rb. sphaeroides*) was not clear but was thought to stabilize the protein structure to ensure electron transfer from bacteriochlorophyll dimer (Bch2) to primary quinone (Q_A) and then to secondary quinone (Q_B). The H-subunit-depleted reaction center (LM complex) from *Rb. sphaeroides* was well analyzed by Debus et al. [3]. Although LM complex has slightly different properties from RC, RC-H from *Rps. viridis* has not been well analyzed yet.

In this study, we therefore characterized the redox properties and electron transfer activities of RC-H by

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redox titration with a platinum electrode and the flash-photolysis measurement. This study is the first reported analysis of the redox properties of RC-H from *Rps. viridis*.

2. Materials and methods

2.1. Preparation of RC-H

Photosynthetic bacterium *Rps. viridis* (ATCC19567) was cultured semianaerobically under illumination using succinate as a carbon source [4]. The chromatophore membrane was prepared as reported previously by Jacob and Miller [5]. The RC was purified by gel filtration in the presence of lauryldimethylamine N-oxide (LDAO) using a procedure previously reported by Hara et al. [6]. The RC-H was purified by polyacrylamide gel electrophoresis. A total of 5 ml of chro-matophore membrane (A_{1020} = 100) was mixed with 5 ml of 20% (w/v) AES (normal ether sulfate, Nikko Chemicals, Tokyo) and 1 ml glycerol. After solubilization for 30 min at 25°C, this mixture was centrifuged for 1 h at 130 000 $\times g$. The supernatant was electrophoresed on 12% (w/v) polyacrylamide gel according to Laemmli's method except that the gel and electrode solution contained 0.1% (w/v) AES instead of 0.1% (w/v) SDS [7]. A main brown band containing the RC-H was sectioned out. The RC-H was extracted from the gel using a buffer containing 10 mM Tris–HCl (pH 8.2), 0.5% (w/v), and *n*-octyl-β-D-glucoside (β-OG) (Dojindo Laboratories, Japan). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) for analysis of protein subunits was also done by Laemmli's method [7].

2.2. Estimation of molar extinction coefficient

The RC molar extinction coefficient is 300 mM^{-1} cm^{-1} [8]. We estimated the RC-H molar extinction coefficient at 830 nm by comparing the absorbance of RC and RC-H, and also by measuring the amount of protein, bacteriochlorophylls, irons, and leucine, which is an amino acid after hydrolysis of both proteins. Protein concentration was determined by different methods as by measuring absorbance at 280

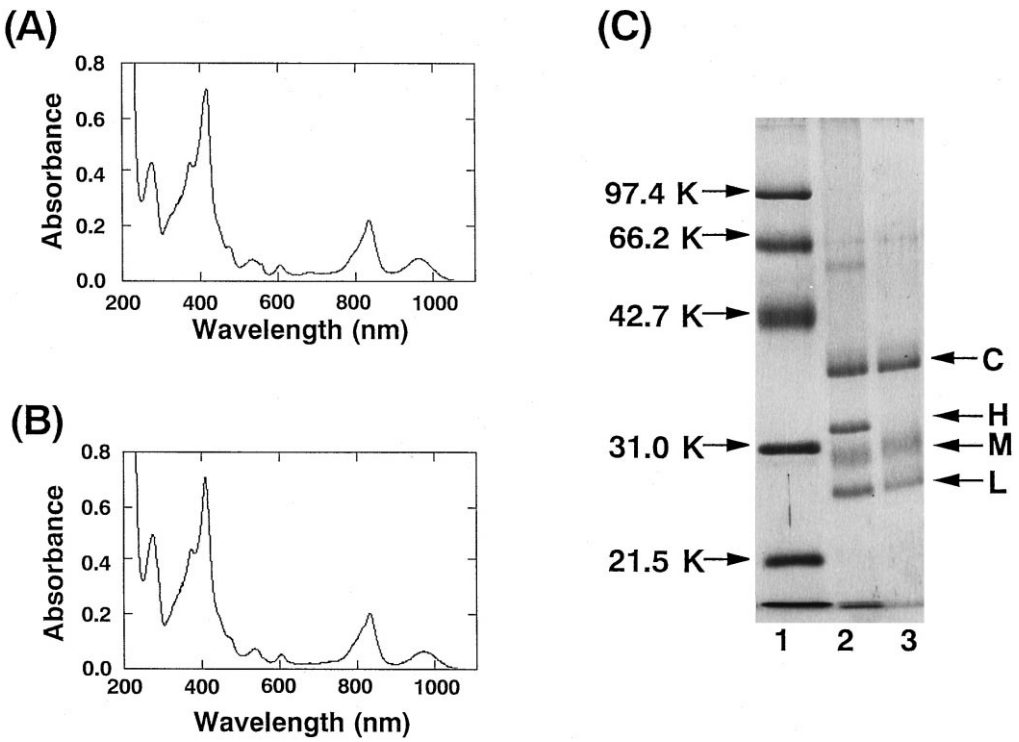


Fig. 1. Absorption spectrum of (A) RC-H and (B) RC and (C) SDS-PAGE of (lane 1) molecular weight markers for RC (lane 2) and RC-H (lane 3) on 12% (w/v) polyacrylamide gel. Spectra were measured at room temperature in 10 mM Tris–HCl (pH 8.2) and 0.1% (w/v) LDAO. Arrows in (C) indicate C-(bound cytochrome), H-, M-, and L-subunit.

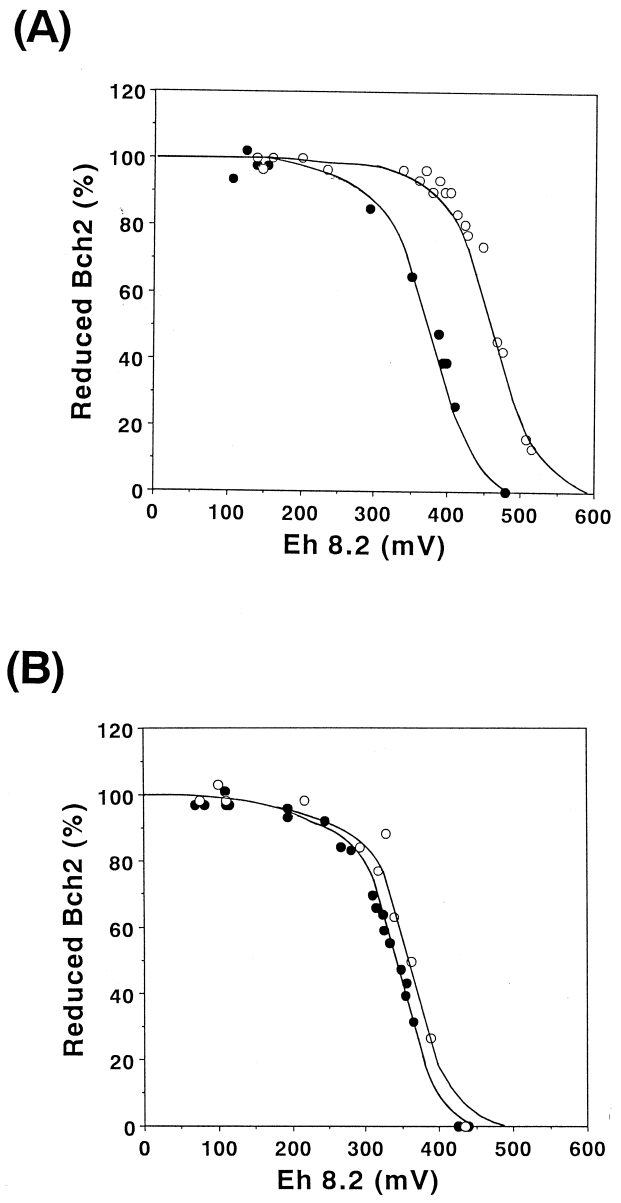


Fig. 2. Bch2 redox titration (A) before dialysis for RC-H (closed circles, $Em_{8.2} = 376$ mV, $R^2 = 0.978$) and for RC (open circles, $Em_{8.2} = 462$ mV, $R^2 = 0.990$) and (B) after dialysis for RC-H (closed circles, $Em_{8.2} = 342$ mV, $R^2 = 0.991$) and RC (open circles, $Em_{8.2} = 364$ mV, $R^2 = 0.976$). Absorbance at 960 nm was measured to estimate Bch2 reduction at different ambient potentials. In (A), the RC solution contained 50 mM Tris–HCl (pH 8.2), 1 μM RC, 0.0038% (w/v) LDAO, an aliquot of K-ferricyanide, and Na-ascorbate; and the RC-H solution contained 1 μM RC-H in 50 mM Tris–HCl (pH 8.2), 0.026% (w/v) β-octyl-D-glucoside, an aliquot of K-ferricyanide, and Na-ascorbate. In (B), the solutions contained 1 μM RC-H or 1 μM RC, and 10 mM Tris–HCl (pH 8.2), 0.1 mM EDTA, 0.1% (w/v) Na-deoxycholate, 0.2% (w/v) Na-deoxycholate, an aliquot of K-ferricyanide, and Na-ascorbate.

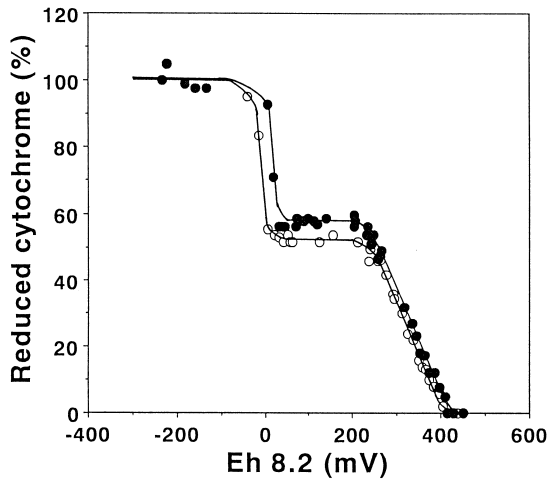


Fig. 3. Redox titration of hemes in bound cytochrome in RC (open circles) and in RC-H (closed circles). Absorbance at 558 and 552 nm was measured to estimate reduced hemes. The RC solution contained 1 μM RC, 0.0024% (w/v) LDAO, 100 mM Tris–HCl (pH 8.2), 1 mM K-ferricyanide, an aliquot of Na-ascorbate, 0.1 mM 2,3,5,6-tetramethylphenylene diamine, 0.1 mM phenazone methosulfate, 0.1 mM 2-hydroxy1,4-naphthoquinone, and 0.1 mM tetranitrotetrazolium blue. The RC-H solution contained the same components as in the RC solution except 1 μM RC-H replaced the RC, and 0.033% (w/v) β-octyl-D-glucoside replaced the LDAO. Apparent $Em_{8.2}$ of high- and low-potential hemes in RC was 320 mV ($R^2 = 0.993$) and 14 mV ($R^2 = 0.991$), respectively.

nm (UV method), and by the biuret method [9], Lowry method [10], and Bradford method with Coomassie brilliant blue G-250 [11]. Bovine serum albumin was used as the standard in the protein assays. We always measured both of RC and RC-H and calculated the coefficient of RC-H as a relative value with the coefficient of RC as 300 mM^{-1} cm^{-1}.

Bacteriochlorophylls (Bch) were extracted from the RC and RC-H as follows. First, a 0.1-ml sample of RC or RC-H was mixed with 2 ml of an acetone–methanol mixture (7:2), incubated for 2 min at room temperature, and centrifuged for 2 min at $8000 \times g$. The supernatant was collected (first extract), and the pellet was reextracted with 1 ml of acetone–methanol (second extract) and added to the first extract. The absorption spectrum of the combined extract was measured by a spectrophotometer. A molar extinction coefficient of 1.22×10^5 M^{-1} cm^{-1} at 790 nm was used to estimate the Bch concentration [12]. The RC-H molar extinction coefficient was estimated by

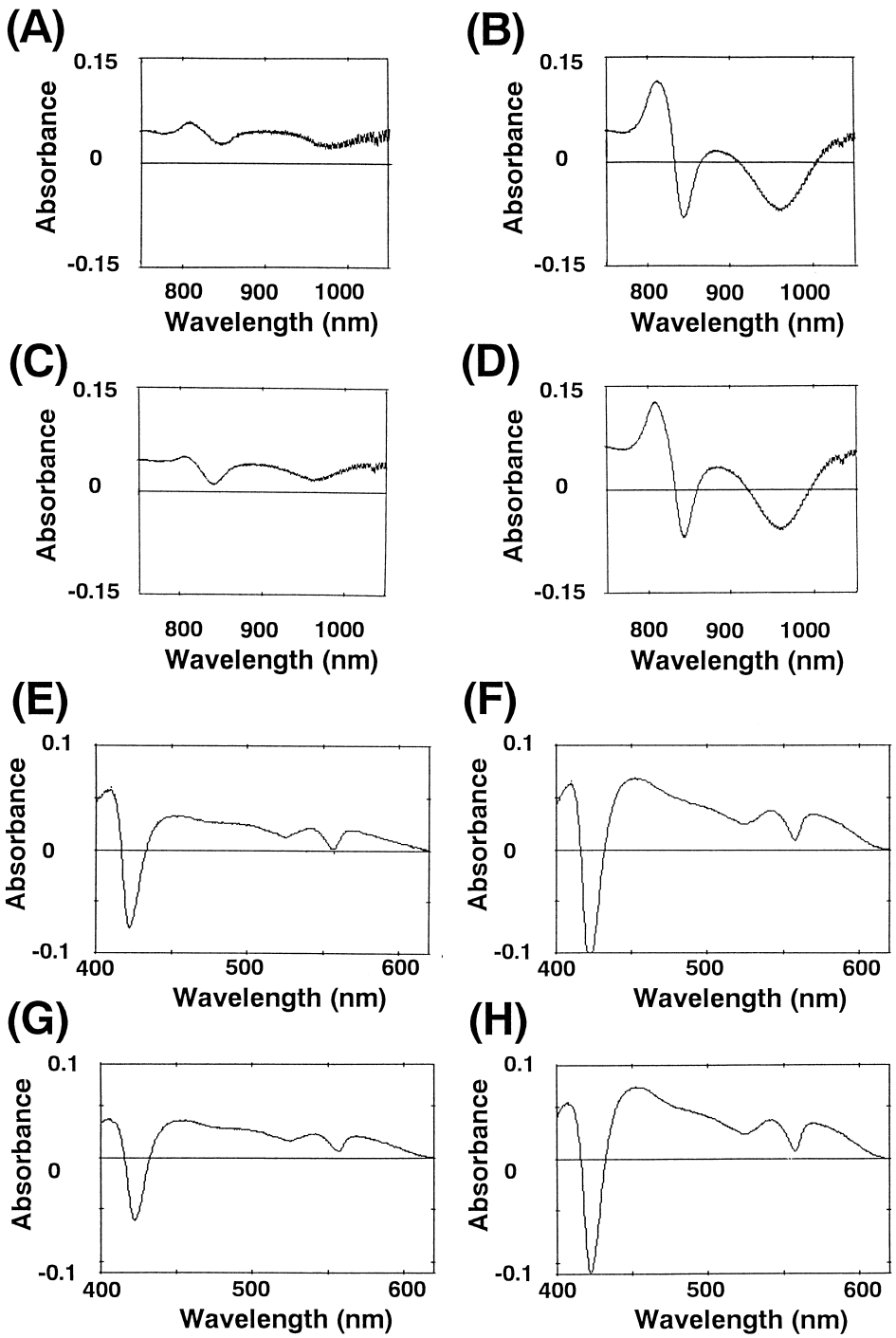


Fig. 4. Effect of reconstitution of UQ_{10} into RC and RC-H. Continuous light-induced spectra of RC (A), (B), (E), (F) and that of RC-H (C), (D), (G), (H) in the near infrared regin (A)–(D) or the visible region (E)–(H). A total of 2 μM of either RC-H or RC in 10 mM Tris–HCl (pH 8.2), 0.1 mM EDTA, 0.1% (w/v) Na-deoxycholate, 0.2% (w/v) Na-deoxycholate, an aliquot of K-ferricyanide, and Na-ascorbate. A total of 250 μM UQ_{10} was also contained in (B), (F), (D), (H). The ambient redox potential at pH 8.2 was (A), (E) 68 mV; (B), (F) 64 mV; (C), (G) 62 mV; (D), (H) 67 mV.

comparing the amount of extracted Bch to the absorbance at 830 nm in an aqueous solution.

For amino acid analysis, the RC and RC-H were hydrolyzed for 22 h at 110°C in 6 N HCl containing 0.01% (v/v) phenol. Analysis was done using an Hitachi L-8500 analyzer (Japan), which is based on the ninhydrin reaction. The RC and RC-H concentrations were calculated from the leucine content and amino acid sequence data [13–15]. The RC and RC-H were hydrolyzed in the presence of nitric acid and then used to measure iron content with an atomic absorption spectrophotometer (Seiko Instrument SPS1100, Japan).

2.3. Characterization of redox properties

The redox titration of Bch2 and of hemes in the bound cytochrome was done as described by Dutton [16] with the Bch2 and heme molar extinction coefficients at 960 nm (123 mM^{-1} cm^{-1}) and 550–560 nm (20 mM^{-1} cm^{-1}), respectively [8]. The platinum electrode with a reference Ag/AgCl electrode in the redox titration experiments was calibrated with quinhydron solution at pH 7.0 as 297 mV. The calculation of the ambient redox potential at pH 8.2 ($Eh_{8.2}$) included a correction in which 60 mV/pH was subtracted from $Eh_{7.0}$. The Em values were estimated from titration curves by linear least-square fit of the data points falling in the 15–85% reduced state. This fit was used instead of a non-linear fitting program because non linear-fitting program with a Nernst equation was not available in our laboratory. Also calculated was the square of the correlation coefficient (R^2).

Continuous light-induced spectra of RC and RC-H were measured by a conventional spectrophotometer (Shimazu UV-160, Japan) with cross illumination. Actinic light was supplied through from a glass fiber-guided light source (Luminar Ace LA50, Hayashi Watch-Works, Japan). Stray light was reduced by using a blue glass filter that allowed the visible region to pass through (Corning 4-76, USA) and a filter that allowed the near infrared region to pass through (Fuji SC-72, Japan). Measurement of the spectra began 30 s after the start of illumination.

Table 1
Continuous light-induced oxidation of Bch2 in RC or RC-H

Sample	Oxidation of Bch2 at 960 nm	Oxidation of hemes at 556–558 nm
RC	0.090 μM	1.81 μM
RC + UQ_{10}	0.736 μM	2.54 μM
RC-H	0.174 μM	1.37 μM
RC-H + UQ_{10}	0.794 μM	2.86 μM

The solution contained 2 μM RC or RC-H in 10 mM Tris–HCl (pH 8.2), 0.1 mM EDTA, 0.1% (w/v) Na-cholate and 0.2% (w/v) either in the presence or absence of 125 μM ubiquinone 10 as described in the legend of Fig. 4.

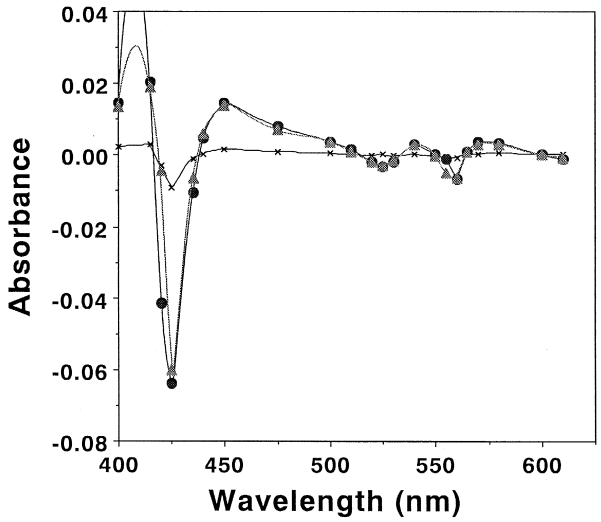


Fig. 5. Flash-induced spectra of RC-H (crosses), RC-H reconstituted with UQ_{10} (closed circles), and RC reconstituted with UQ_{10} (triangles). The solution for reconstituted RC-H contained 1 μM RC-H in 10 mM Tris–HCl (pH 8.2), 0.1 mM EDTA, 0.1% (w/v) Na-cholate, 0.2% (w/v) Na-deoxycholate, an aliquot of K-ferricyanide, and Na-ascorbate. The solution for RC contained the same components except RC replaced RC-H. The solutions for reconstituted RC-H and reconstituted RC also contained 125 μM UQ_{10}. $Eh_{8.2}$ was 210 ± 30 mV in the spectra measurements.

Flash-induced oxidation of Bch2 at 450 nm and of cytochrome oxidation at 560 nm was measured by a flash-photolysis apparatus (Photal RA-412HS, Otsuka Electronics, Japan) with a xenon flash of 10 μs duration. Again, two filters were used to reduce the stray light; a blue glass filter as before and a filter that allows the near infrared region to pass through (Toshiba ITF-50S-83RT, Japan). We measured the fast (0–10 ms) and slow (0–1 s) time kinetics of flash-induced change in absorbance at 450 nm. Each data point was an average of data for five flashes, where the interval between each flash was 20 s. The fast kinetic trace between 0.05 and 4 ms and the slow kinetic trace between 5 and 400 ms were fitted by a

Table 2
Kinetic parameters of back electron transfer with quinone-reconstituted samples

Sample	Rate k_1 in 0.05–4 ms (A_{450} CBG)	Rate k_2 in 5–400 ms (A_{450} CBG)	Amplitude of ΔA_{450} ($Eh_{8.2}$ in mV)
1. RC-H	7710 S^{-1} (0.0016)	3.8 S^{-1} (0.0003)	0.0050 (181)
2. RC-H + UQ_0	550 S^{-1} (0.0147)	18 S^{-1} (0.0063)	0.0200 (164)
3. RC-H + UQ_{10}	810 S^{-1} (0.0129)	23 S^{-1} (0.0082)	0.0210 (205)
4. RC-H + K_1	727 S^{-1} (0.0043)	24 S^{-1} (0.0014)	0.0100 (181)
5. RC-H + K_2	950 S^{-1} (0.0040)	77 S^{-1} (0.0021)	0.0095 (196)
6. RC-H + K_3	3810 S^{-1} (0.0046)	14 S^{-1} (0.0025)	0.0075 (167)
7. RC	640 S^{-1} (0.0185)	18 S^{-1} (0.0005)	0.0246 (208)
8. RC + UQ_0	550 S^{-1} (0.0147)	18 S^{-1} (0.0063)	0.0200 (164)
9. RC + UQ_{10}	660 S^{-1} (0.0020)	19 S^{-1} (0.0110)	0.0230 (222)
10. RC + K_1	2230 S^{-1} (0.0013)	76 S^{-1} (0.0018)	0.0040 (193)
11. RC + K_2	850 S^{-1} (0.0089)	27 S^{-1} (0.0022)	0.0160 (204)
12. RC + K_3	880 S^{-1} (0.0123)	19 S^{-1} (0.0055)	0.0180 (184)

curve-fitting program (Igor Pro, Wavemetrics, USA) with a single exponential decay plus a constant background (CBG). The CBG corresponds to a very slow decay component that can not be fitted in the two time scales considered here.

2.4. Reconstitution and extraction of quinones

Reconstitution of quinones into RC and RC-H were done according to the method by Debus et al. [3] in which each sample was dialysed for 15–20 h at 4°C against a buffer 1 containing 10 mM Tris–HCl (pH 8.2), 0.1 mM EDTA, 0.1% (w/v) Na-cholate, and 0.2% (w/v) Na-deoxycholate. Dialysed samples containing 2 μM RC or RC-H were then incubated in buffer 1 for 15–20 h with 250 μM quinones.

Estimation of quinone in RC and RC-H was done according to a slightly modified method by Takamiya and Takamiya [17]. RC and RC-H were dialysed for 10 h at 4°C against buffer 1. Quinone was then extracted for 30 min at 35°C with a 5 ml acetone–methanol mixture (1:1). The modification then involved adding 5 ml of petroleum ether, mixing by agitation, and then collecting the upper petroleum layer after centrifugation. We obtained another extract by repeating the petroleum ether-extraction. The combined extracts were then dried by evaporation. The dried material was redissolved in ethanol to obtain the oxidized form of quinone. An aliquot of NaBH4 was then added to reduce the quinone to quinol.

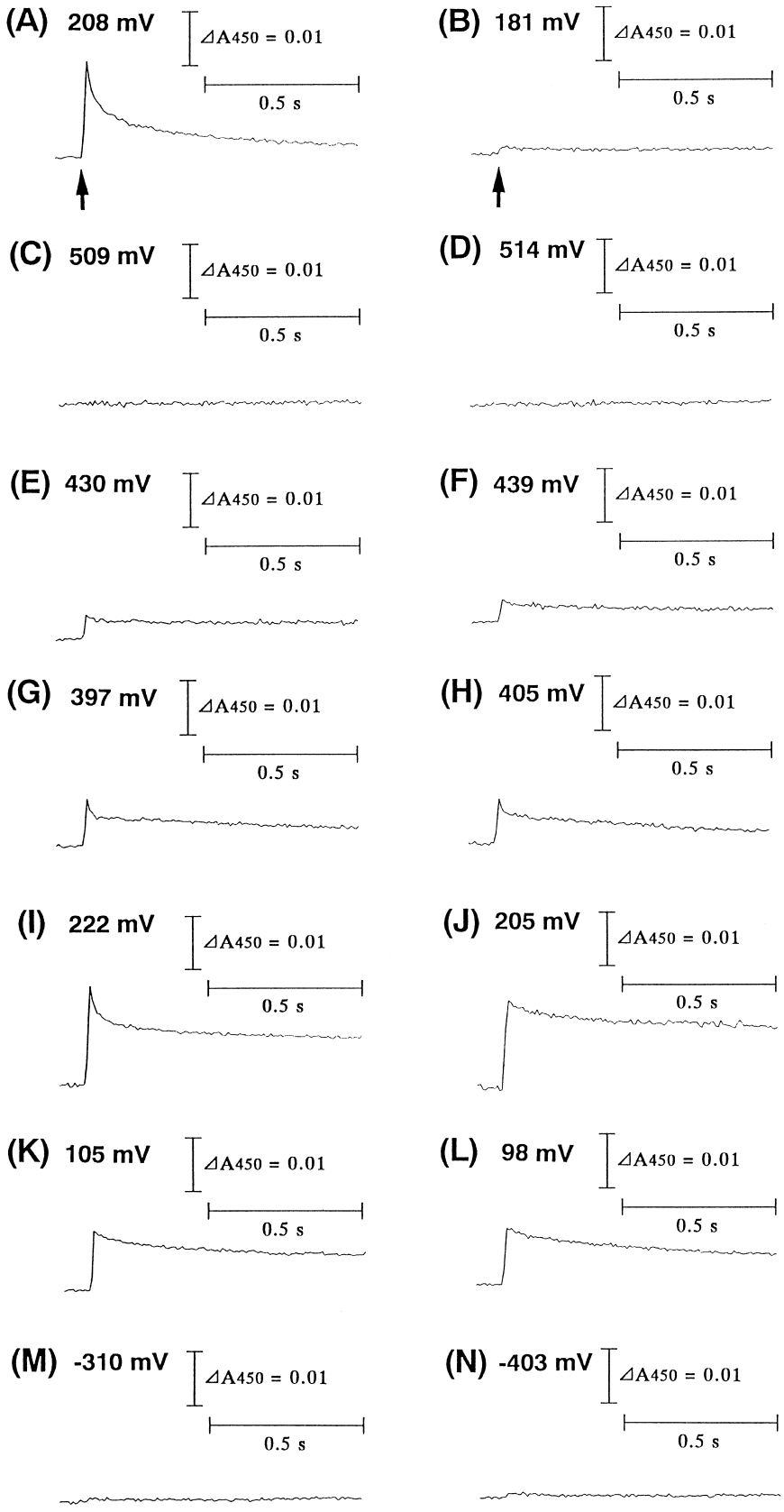
3. Results

3.1. Spectrum of RC-H

The RC-H absorption spectrum resembled that for RC (Fig. 1A). SDS-PAGE showed that H-subunit was contained in RC but not in RC-H (Fig. 1B).

The RC absorption maximum at 830 nm is from bacteriochloro-phyll monomer (Bch) and bacteriopheophytin (Bph). Clayton and Clayton [8] reported the RC molar extinction coefficient at 830 nm to be 300 mM^{-1} cm^{-1}. Molar extinction coefficient for RC-H estimated by (a) extraction of bacteriochlorophyll *b* (302 mM^{-1} cm^{-1}), (b) amino acid analysis based on ninhydrin reaction (415 mM^{-1} cm^{-1}), (c) determination of iron content by atomic absorption (313 mM^{-1} cm^{-1}), (d) protein assay by UV absorption (434 mM^{-1} cm^{-1}), (e) protein assay by the biuret method (334 mM^{-1} cm^{-1}), (f) protein assay by the Lowry method (501 mM^{-1} cm^{-1}), and (g) protein assay by the Bradford method (462 mM^{-1} cm^{-1}) were obtained. The molar extinction coeffi-

Fig. 6. Xenon flash-induced oxidation and re-reduction of Bch2 of RC (A, C, E, G, I, K, M) and that of RC-H (B, D, F, H, J, L, N) at 450 nm. Samples were prepared the same as the samples for Fig. 7. The ambient redox potential at pH 8.2 was (A) 208 mV, (B) 181 mV, (C) 509 mV, (D) 514 mV, (E) 430 mV, (F) 439 mV, (G) 397 mV, (H) 405 mV, (I) 222 mV, (J) 205 mV, (K) 105 mV, (L) 98 mV, (M) −310 mV, and (N) −403 mV. Arrows in (A) and (B) show the time that the flash occurred.


(A) 208 mV
ΔA450 = 0.01
0.5 s
(B) 181 mV
ΔA450 = 0.01
0.5 s
(C) 509 mV
ΔA450 = 0.01
0.5 s
(D) 514 mV
ΔA450 = 0.01
0.5 s
(E) 430 mV
ΔA450 = 0.01
0.5 s
(F) 439 mV
ΔA450 = 0.01
0.5 s
(G) 397 mV
ΔA450 = 0.01
0.5 s
(H) 405 mV
ΔA450 = 0.01
0.5 s
(I) 222 mV
ΔA450 = 0.01
0.5 s
(J) 205 mV
ΔA450 = 0.01
0.5 s
(K) 105 mV
ΔA450 = 0.01
0.5 s
(L) 98 mV
ΔA450 = 0.01
0.5 s
(M) -310 mV
ΔA450 = 0.01
0.5 s
(N) -403 mV
ΔA450 = 0.01
0.5 s

cient for RC-H was larger than that for RC although bacteriochlorophyll determination showed the values to be similar.

3.2. Redox titration

The mid-point potential of Bch2 ($Em_{8.2}$ = 462 mV) in the RC-H was significantly shifted ($Em_{8.2}$ = 376 mV) compared with that in RC (Fig. 2A). A relatively large Em shift (40–90 mV) occurred when we diluted RC and RC-H directly with 10 mM Tris–HCl (pH 8.2). However, when we dialysed both samples against buffer 1, the Em shift was only 20 mV (Fig. 2B), compare with an Em shift of 86 mV when it was measured before dialysis. This decrease shows that the apparent Em shift in RC-H was mainly from residual detergent in the sample.

Fig. 3 shows redox titration of hemes in the bound cytochrome in RC and in RC-H. Reduction of high-potential hemes in RC and RC-H saturated at 53% and 58% of the total hemes, respectively. RC and RC-H both had two high-potential hemes and two low-potential hemes. For the high potential hemes, the apparent Em was 320 mV for RC and 324 mV for RC-H, and for the low potential hemes, it was 13 mV for RC, and 14 mV for RC-H. The similarity in the values show that Em of hemes was not changed significantly (within experimental error) in RC-H. However, an Em change may have gone undetected because we did not estimate individual Em values for the four hemes as reported by Nitschke and Dracheva [18].

3.3. Continuous light-induced spectra

Fig. 4A,B,E, and F show continuous light-induced spectra of RC, and Fig. 4C,D,G, and H show those of RC-H. Typical light-induced spectra in the near infrared region occurred in RC and RC-H (Fig. 4A,B,C, and D). Absorbance at 960 nm decreased due to Bch2

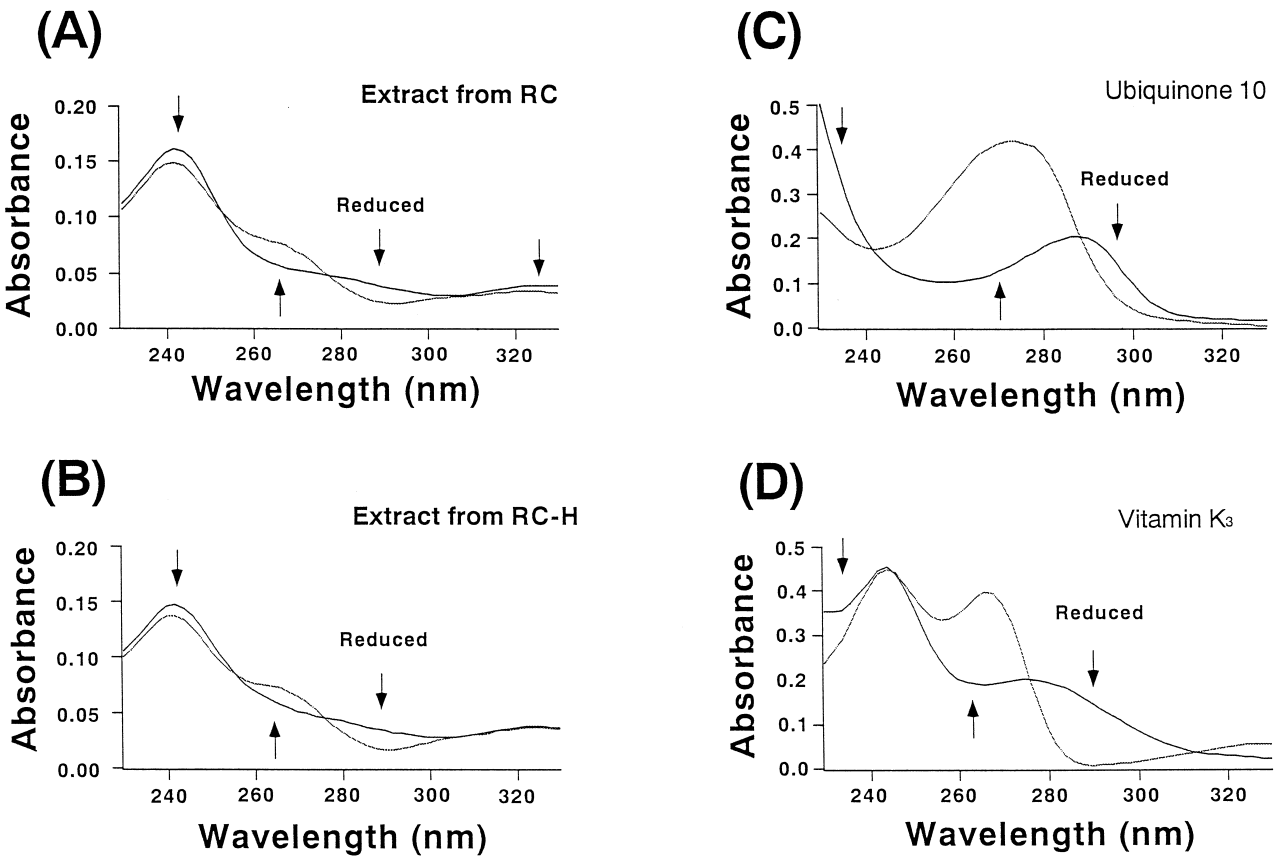


Fig. 7. Absorption spectra of quinones extracted from (A) RC, (B) RC-H, (C) vitamin K_3, and (D) UQ_{10} in oxidized and reduced states. Arrows indicate spectra for the reduced states.

oxidation. The decrease at 844 nm and increase at 810 nm were from the blue stark shift in the absorption maximum of both Bch and Bph at 830 nm. Light-induced oxidation of bound cytochrome at 558 nm occurred in the visible region (Fig. 4E,F,G, and H). Table 1 summarizes the results for the continuous light-induced oxidation of Bch2 and that of bound cytochrome. No substantial difference was found in the continuous light-induced spectra between RC and RC-H after reconstitution of UQ_{10}. The xenon flash-induced spectra of RC and RC-H (Fig. 5) were similar to the continuous light-induced spectra (Fig. 4). Again, as in the closed circles and triangles in Fig. 5, reconstitution of UQ_{10} restored the photochemical activity of RC-H to the level seen for RC.

3.4. Flash-photolysis measurement

Fig. 6 shows the time course for the xenon flash-photolysis. Oxidation of Bch2 in RC-H (Fig. 6B) without reconstitution of quinones was less than that in RC measured at 450 nm (Fig. 6A). After reconstitution of quinones, both RC and RC-H showed the same kinetics of decay in flash-oxidized Bch2 oxidation, which depended on the ambient redox potential (Fig. 6C–N). Table 2 summarizes the kinetics parameters, showing that both fast- and slow-decay components were evident in the re-reduction of Bch2. When we reconstituted ubiquinones, the fast-decay component rate was 500–800 s^{-1} and the slow one was around 20 s^{-1}. Ubiquinones (UQ_0 and UQ_{10}) were reconstituted better than menaquinones (K_1, K_2 and K_3) in this experiment about amplitude of initial flash-induced change in A_{450}. Quinones extracted from both well-dialysed RC and RC-H have the same absorption spectra (Fig. 7A and B, respectively). These spectra have a closer resemblance to the spectrum of vitamin K_3, a menaquinone, than to the spectrum of UQ_{10} (Fig. 6C and D, respectively). This resemblance indicates that most of the menaquinone in the Q_A site was retained in RC-H, whereas UQ_{10} in the Q_B site was lost during the purification of RC-H.

4. Discussion

The H-subunit is the most easily removable subunit in the *Rb. sphaeroides* RC. Debus et al. [3] used a caotropic ion, 1 M $LiClO_4$, in the presence of 0.1% (w/v) LDAO to prepare the H-subunit and used 0.75 M $LiClO_4$ in the presence of 0.1% (w/v) sodium cholate and 10% (v/v) ethanol to prepare the LM-complex. Although the H-subunit in the *Rps. viridis* RC is more difficult to remove in the presence of caotropic ions, we removed it from other subunits in the presence of 4 M NaSCN and 0.1% (w/v) LDAO (data not shown). We are currently studying the reconstitution of H-subunit.

The measured molar extinction coefficient of RC-H was slightly higher than that of RC. One reason for this difference may be a possible distortion of the protein structure around Bch and Bph, thereby altering the properties of these pigments. Also, the reason that Em values of Bch2 differed from that of RC depending on the difference in detergent (Fig. 2A) may be the result of changes in the Em values of chromophores caused by changes in the micro environment around the chromophore. Replacement of amino acid in the vicinity of Bch2 RC by site-directed mutagenesis significantly changed the Em value in *Rb. sphaeroides* [19] and in *Rps. viridis* [20]. Em values of hemes in the bound cytochromes of *Rps. viridis* RC can be affected by species of detergent [21]. Moreover, Em values of hemes in the bound cytochrome can be controlled electrostatically by amino acid residues around the hemes [22].

We compared our kinetic parameters (Table 2) with those for *Rps. viridis* RC reported by other researchers. Our results for the rate constant of back electron transfer from quinones to $Bch2^+$ in RC or that in RC-H reconstituted with ubiquinones (k_1 = 500–800 ms^{-1}) in a relatively fast time scale (0.05–4 ms) were similar to the those reported by Baciou et al. [23] and Shops and Wraight [24] for the charge recombination reaction from Q_A^- to $Bch2^+$. This similarity means that k_1 represents the charge recombination reaction from Q_A^- to $Bch2^+$. We fitted our results for the kinetic data trace with a single exponential decay equation, and did not distinguish the direct recombination reaction from Q_A^- to $Bch2^+$ from the indirect recombination reaction via the thermally activateable intermediate Bph^-. The latter reaction is dominant at room temperature [23,24]. The rate constant of back electron transfer from quinones to $Bch2^+$ in RC or that in RC-H reconstituted with ubiquinones (k_2 = 18–23 ms^{-1}) in a relatively slow

time scale (5–400 ms) seemed to be the recombination reaction from Q_B^- to $Bch2^+$. Note that our values are slightly higher than those reported by Shops and Wraight [24].

Although the function of H-subunit is still not clear, M- and L-subunits scaffold many redox centers and are directly involved in photochemical reaction in RC. Debus et al. [3] reported that LM-complex (H-subunit-depleted RC) from *Rb. sphaeroides* retained photochemical activity but also found (a) reduction in the rate of electron transfer from Q_A^- to Q_B, (b) reduction in the stability of the semiquinone anions Q_A^- and Q_B^-, (c) elimination of the oscillations of the absorption of semiquinone in response to successive flashes, (d) reduction in the affinity of UQ_{10} for the Q_B site, reduction in sensitivity to inhibitors, and (e) reduction in stability to denaturation by detergents. Schelvis et al. [25] reported that forward electron transfer from bacteriopheophytin to Q_A in iron-depleted RC strongly depended on the length of quinone tall, whereas no such dependence was seen for RC or for iron-depleted LM. Those two reports show that H-subunit ensures effective electron transfer from Q_A^- to Q_B. Comparison of other redox properties of *Rps. viridis* RC and RC-H (e.g., rate of electron transfer from Q_A^- to Q_B) needs to be done, similar to that reported for *Rb. sphaeroides* RC and LM-complex [25]. For the conditions that we studied here, RC and RC-H have similar properties after reconstitution of UQ_{10} in the presence of Na-cholate when prepared using the method of Debus et al. [3].

As far as we know, this study is the first reported analysis of the redox properties of the H-subunit-depleted RC from *Rps. viridis*.

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