Purification and characterization of *Rhodocyclus gelatinosus* photochemical reaction center

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(Received 1 July 1991)

Key words: Reaction center; Photosynthesis; Ubiquinone; Menaquinone; (*Re. gelatinosus*)

Photoactive *Rhodocyclus gelatinosus* reaction centers were isolated from photoreceptor units by temperature induced phase separation in the presence of octyl β-D-thioglucopyranoside/decyltetraoxethylene mixture (Agalidis, I. and Reiss-Husson, F. (1991) Biochem. Biophys. Res. Commun. 177, 1107-1112) and further purified by ion exchange chromatography. They consisted of three polypeptides L, M, H, with apparent molecular weights of 24, 28 and 34 kDa, respectively. The tetraheine cytochrome c associated to the reaction center (RC) in vivo was removed during purification. Spectral properties of the reaction center indicated a pigment composition similar to that of *Rhodobacter sphaeroides*. The reaction center contained only the primary quinone (QA) which was identified with menaquinone MK₉ (Agalidis, I. et al. (1991) Z. Naturforsch. 46c, 99-105). The absorption spectrum of QA⁺ was typical of a menasemiquinone exhibiting a major peak at 412 nm. Charge recombination time for P + QA⁺ was 35 ms. QB site could be reconstituted with ubiquinones UQ₀, UQ₆, UQ₉ and UQ₁₀ with P + UQ⁻ charge recombination decay within 1-2.3 s. Multiflash-induced binary oscillations of Qᵦ in the presence of UQ₁₀ demonstrated that the secondary acceptor functioned as a two-electron gate. In contrast, MK₉ did not reconstitute QB activity as its midpoint redox potential was probably too low relative to that of QA⁺.

**Introduction**

The primary photochemical reactions of bacterial photosynthesis take place within a membrane-bound pigment-protein complex known as the photochemical reaction center (RC). RCs have been isolated from many species of purple bacteria; the best characterized are those from *Rh. sphaeroides* [1-3] and *Chronothium violaceum* [11] and *teledontium* [8], *Rps. viridis* [5] and *Ectothiorhodospira sp.* [12], a tetraheme cytochrome c is tightly bound to the RC and eventually copurified. On the other hand, it has been reported by two independent groups that RCs isolated from *Re. gelatinatus* with different protocols and detergents contained only L and M subunits [13,14]. Moreover, in the case of LDAO preparation [13], EPR studies [15] demonstrated the absence of the iron atom normally bound to the quinone acceptors QA and QB associated with a metal ion. In several species like *Chromatium vinosum* [11] and *tepida* [8], *Rps. viridis* [5] and *Ectothiorhodospira sp.* [12], a tetraheme cytochrome c is tightly bound to the RC and eventually copurified. On the other hand, it has been reported by two independent groups that RCs isolated from *Re. gelatinatus* with different protocols and detergents contained only L and M subunits [13,14]. Moreover, in the case of LDAO preparation [13], EPR studies [15] demonstrated the absence of the iron atom normally bound to the quinone acceptors QA and QB, a fact which might be related to the absence of the H subunit and to the lability of this preparation.

In two preceding papers [16,17] we have described the properties of a purified photoreceptor unit from *Re. gelatinatus*, which contains the RC associated with 12-15 light-harvesting complexes B875 and with a tetraheine cytochrome c. We then devised a simple method for dissociating the photoreceptor unit into its components [18]. This method consists of incubation of...
a membrane extract with decyltetrathylenoxide (C\textsubscript{10}E\textsubscript{4}), in the cold, followed by temperature-induced phase separation; a crude fraction containing photoactive RC then partitioned in the detergent poor phase. In the present work we describe the properties of this RC preparation after its further purification by ion exchange chromatography. With this isolation procedure \textit{Rc. gelatinosus} RC was separated from the tetraheme cytochrome \textit{c} to which it was strongly associated within the photoreceptor unit; it retained its photochemical activity and still contained the H subunit.

Materials and Methods

**Chemicals**

C\textsubscript{10}E\textsubscript{4} was purchased from Bachem (Switzerland) and octyl-\(\beta\)-thioglucopyranoside (OTG) from Fluka. Ubiquinones Q\textsubscript{8} (Pfalz and Bauer), Q\textsubscript{10} and Q\textsubscript{16} (Sigma), Q\textsubscript{9} and menaquinone MK\textsubscript{8} (Hofmann La Roche) were used. Diaminodiurol was obtained from Aldrich. Stock aqueous solutions of Q\textsubscript{9}, Q\textsubscript{10}, MK\textsubscript{8} and MK\textsubscript{9} (5-10 mM) were prepared in presence of 10% Brij 35 (Merck) as already described [17]. Stock solutions of Q\textsubscript{0} were in ethanol.

**RC preparation**

A crude extract containing RC-B875 complexes (\(A_{875nm}=8-12, 20-150 \text{ mg protein}\)) was prepared by incubation of chromatophores with 7-8 mg/ml OTG, 0.2 M NaCl and 1 mM PMSF as previously described [16]. It was diluted with 10 mM Tris-HCl, 1 mM EDTA, 0.2 M NaCl (pH 8) to a final value of \(A_{875nm}=4-5\); C\textsubscript{10}E\textsubscript{4} was added from a concentrated stock solution to a final concentration of 2.5-3 mg/ml. The residual OTG concentration amounted to 3.5-5 mg/ml. The solution was stirred slowly overnight in the dark at 4°C and then heated in a thermostatic bath to 20°C (the cloud point of C\textsubscript{10}E\textsubscript{4} [19]) which resulted in phase separation [18]. After centrifugation for 10 min at 20°C and 2000 \(\times\) g the upper phase was recovered. It contains crude RCs and was largely devoid of B875 antenna. It was then diluted \(v:v\) with a 10 mM Tris-HCl buffer (pH 8.0), containing 1 mM EDTA, 17.4% glycerol, 0.5 mg/ml C\textsubscript{10}E\textsubscript{4} and adsorbed onto a DEAE-Sepharose column (10-20 ml bed volume) equilibrated at 4°C with 10 mM Tris-HCl, 1 mM EDTA, 0.1 M NaCl, 8.7% glycerol, 0.5 mg/ml C\textsubscript{10}E\textsubscript{4}, pH 8. Chromatography was done in the dark at 5°C with a flow rate of 20-30 ml/h. The by-pass contained inactive RCs still associated with small amounts of B875 complex. After washing with the starting buffer, the remainder of the preparation was eluted by stepwise increases of NaCl concentration. Two fractions containing RCs were eluted respectively at 0.15 and 0.2-0.25 M NaCl and their absolute absorption spectra were recorded. Within each fraction only tubes with \(A_{800nm}/A_{900nm} < 1.4\) and \(A_{600nm}/A_{665nm} \geq 2\) were pooled. The purity of these fractions was checked by SDS PAGE according to Fling and Gregerson [20] with a Minigel apparatus. Protein was measured by the method of Peterson [21].

**Stability in presence of various detergents**

To samples of purified RC (0.7 \(\mu\)M) in 10 mM Tris buffer, pH 8, containing 0.03% C\textsubscript{10}E\textsubscript{4}, 5.8% glycerol each of the following detergents was added: octylpentaoxyethylene (Bachem) (6 mg/ml); Brij 35 (2 mg/ml); OTG (3 mg/ml); sodium cholate (Merck) (1.5 mg/ml); dodecyl dimethylammonio-3-propane sulfonate (sulfobetaine SB12, Serva) (2 mg/ml); 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonate (CHAPS, Fluka) (1 mg/ml); sodium deoxycholate (Merck) (1 mg/ml) plus sodium cholate (1 mg/ml); dodecyl-\(\beta\)-imidiodisodium propionate (Deriphat 160, Henkel) (1 mg/ml); LDAO (Fluka) (1 mg/ml). The final concentration of these detergents (in brackets) was not far from their critical micellar concentration. RC stability was tested immediately after detergent addition and once more after overnight incubation at 4°C in the dark.

**Spectrophotometric measurements**

The amount of photochemically active RC was estimated in various preparations by absorbance changes induced at 600 nm by continuous saturating red light, assuming \(\Delta \epsilon 600 \text{ nm} = 20 \text{ M}^{-1} \text{ cm}^{-1}\) [22]. Alternatively, activity was estimated by the extent of bleaching of the absorption band at 865 nm in the presence of Q\textsubscript{0} induced by blue continuous saturating light. Flash-induced absorbance changes in near IR and visible region and analyses of kinetics were done as in Ref. 17.

**Results**

**Reaction center isolation and stability**

We have already shown [18] that a micellar solution of C\textsubscript{10}E\textsubscript{4} and OTG was able to split off the RC from RC-B875-cytochrome \textit{c} photoreceptor units. A fraction containing RCs, depleted from antenna, could then be isolated by temperature-induced phase separation at 20°C (C\textsubscript{10}E\textsubscript{4} cloud point [19]) (see Materials and Methods). This crude RC fraction was still photochemically active [18]. It was further purified by ion exchange chromatography on DEAE-Sepharose in two fractions eluted by 0.15 M and 0.2 M NaCl, respectively, the last one being the most enriched in RC (see below).

A typical absolute absorption spectrum of purified RC is illustrated in Fig. 1. The positions and the relative amplitudes of the bands attributed to the Q\textsubscript{y} transitions of the Bchls and Bpheos are the same as...
those observed for *Rb. sphaeroides*. The $Q_y$ band of the Bchl dimer peaked at 865 nm, whereas in the preparation of Clayton and Clayton [13] (which was actually a LM unit) it was shifted to 850 nm. These spectral features indicate that *Rc. gelatinosus* RC has the same pigment composition as other purple bacteria: 4 Bchls $a$ and 2 Bpheos $a$.

In the visible range, presence of a bound carotenoid is indicated by the absorption bands at 496.5 and 464 nm, the third vibrational component likely being masked by the strong absorption of the Soret band. Thus, the visible absorption bands of the carotenoid in *Rc. gelatinosus* RC are blue shifted by about 7 nm relative to the spheroidene bound to *Rb. sphaeroides* RE [23].

The Soret region of the spectrum is dominated by the strong contributions of the Bchls and Bpheos; the weak absorption in the 420 nm range suggested that a major part of the tetraheme cytochrome $c$ initially bound to RC in the photoreceptor units [16] had been lost during the preparation. Indeed, 'dithionite-reduced minus oxidized' difference absorption spectra measured in the $a'$ band region of the cytochrome (540–565 nm range) indicated the presence of only 0.1 heme per RC (not shown).

In the electrophoretic pattern of the most purified RC fraction, three dominant polypeptides were observed corresponding to apparent molecular masses of 24, 28 and 34 kDa (see Fig. 2, lanes 2 and 5). They were already present in the photoreceptor unit [16] and probably correspond to the L, M, and H subunits, respectively. Significant differences in electrophoretic mobilities were observed for each of these subunits when compared to the corresponding ones in *Rb. sphaeroides* RC (Fig. 2, lanes 2, 3). Furthermore, we noticed that the staining of the L subunits was much weaker when the samples were heated at 100°C for 1 min in the presence of SDS and DTE than when they were incubated at room temperature. Sometimes the M band was double (compare lanes 2 and 5 in Fig. 2). Absence of antenna was demonstrated by the lack of polypeptides of low (< 10 kDa) molecular masses. Nevertheless RC purification was far from complete, as shown by the presence of several weaker bands corresponding to relative molecular masses in the range 40–90 kDa, which likely belong to extraneous proteins of the crude extract (see lanes 2, 5). These contaminants were not removed by a gel filtration step (not shown). Partial purification of the RC was otherwise shown by the ratio of absorbance at 280 nm relative to 800 nm, which was never lower than 1.30. Removal of cytochrome $c$ was confirmed by the weak staining in the region corresponding to 43 kDa polypeptides, as compared to electrophoretic pattern of photoreceptor units (see Ref. 16).

Purified RC preparations were stable in absorption and photochemical activity for one week when kept at 4°C in presence of 5–8% glycerol and C$_{10}$E$_4$. Later peculiar spectral modifications were observed; the amplitude of P865 donor band began to increase progressively relative to that of the 800 nm absorbance band; it also shifted slightly towards the blue region of the spectrum. In absence of glycerol, this modification was accelerated. In contrast, the $Q_y$ band of Bpheos and the 800 nm band (assigned to the two 'voyeur' Bchls) remained unchanged. Therefore, the modification of the donor $Q_y$ band might result from a detergent effect on the dimer or on its binding site. At the same time these spectral changes were assisted by a decrease of RC activity. Prolonged illumination, even of fresh RC preparations, led to a decrease of flash-induced signals without a modification of the absorption spectrum.

Fig. 2. SDS-PAGE electrophoresis with a discontinuous gel system of various samples. Acrylamide concentration: 12.5%. Lane 1: crude membrane extract before phase separation. Lanes 2 and 5: purified *Rc. gelatinosus* RCs fractions after DEAE-Sepharose chromatography; samples (10 and 25 μg protein) incubated at room temperature (see text). Lane 3: pure *Rb. sphaeroides* Y RC. Lanes 4 and 6: protein markers; from top to bottom: 94, 67, 43, 30, 20.1 and 14.4 kDa.
Because of this relative fragility, we checked a number of non-ionic detergents as possible substitutes for C₁₀E₄ (see Materials and Methods). We looked for alterations in the absolute absorption spectrum and then measured the magnitude of light-induced absorption bleaching at 600 nm. From these tests altogether it turned out that *Rc. gelatinosus* RC was stable only in presence of Brij 35, cholate and CHAPS. In presence of OTG and Deriphat 160 activity was impaired even if the absorption spectrum of the RC remained unchanged; we did not check if the primary quinone was removed. Among the most harmful detergents were sulfobetaine SB12 and LDAO, which led to pheophytinisation of Bchl.

**Characterisation of the primary quinone acceptor**

Previous studies of the photoreceptor unit led us to conclude that the first quinone acceptor in *Rc. gelatinosus* RC is MK₅ [16]. Its identification was further supported by quinone analysis and by flash experiments on intact as well as on Q₅b partially depleted preparations [17]. A flash-induced absorbance change observed on a purified *Rc. gelatinosus* RC fraction is shown in Fig. 3 (lower trace). The signal decayed in the dark as a monoeXponential with a mean time of 35 ms; addition of herbicide did not modify this kinetics (not shown). This result implies that Q₅b has been removed during the RC preparation and therefore the decay is exclusively due to P¹Q₅ charge recombination. This

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**Fig. 3.** Exponential decay kinetics of flash-induced absorbance change measured at 780 nm (1 μM RC suspended in the same buffer as in Fig. 1). Lower trace: without addition; mean time (t = 1/e decay time) t = 0.036 s. Upper trace: addition of 48 μM UQ₁₀, 1 mg/ml Brij 35. Inset: decay kinetics in presence of UQ₁₀ recorded on a longer time scale; 80% of the signal relaxed with t = 2.2 s.

**Fig. 4.** Flash-induced difference spectra of the primary quinone acceptor in *Rc. gelatinosus* RC, in UV-visible (A) and near infrared (B) ranges. Absorbance signals were measured 35 ms after the flash (see text). Spectral band pass: 4 nm. RCs (3 μM) were suspended in presence of 500 μM DAD in the same buffer as in Fig. 1. The continuous curve was calculated by interpolation with a program (Kaleidagraph) using cubic spline method on a MacIntosh computer.
reaction was however slower than in photoreceptor units, where the rate was determined to be about 10 ms [16,17]. Presumably, this modification of the recombination rate is the result of the treatment with detergents during the purification steps.

In the presence of diaminodurol which is a rapid electron donor to flash-activated P+, the lifetime of photoreduced Q$_A$ could be extended to several seconds. Therefore, the differential absorption spectrum (Q$_A^-$ minus Q$_A$) could be obtained by measuring the amplitude of the flash-induced signal as a function of wavelength (Fig. 4). This amplitude was measured 35-50 ms after the flash, when P$^+$ was already rereduced by DAD and thus not interfering with Q$_A^-$ signal. Close examination of this difference absorption spectrum between 380-500 nm allows one to distinguish three peaks and a shoulder in the range 400-450 nm with a maximum at 412 nm (Fig. 4A). These spectral features are roughly similar to those already detected in Rps. viridis RC [24] and attributed to the radical anion of menaquinone. The characteristic flash-induced absorption changes measured in the infrared region (Fig. 4B) are ascribed to the electrochromic shift of the Q$_y$ absorption band of the Bpheo located on the pigment A branch; this shift is induced by the negative charge of the neighbouring menasemiquinone.

Reconstitution of Q$_B$ activity

In order to check if Q$_B$ could be reconstituted, we added an excess of various ubiquinones to Rc. gelatinosus RCs (1-3 $\mu$M) (see Table I). Reconstitution was tested by the rate of the decay of a flash-activated P$^+$ signal, measured in near IR or visible region of the absorption spectrum. In Table I are given the mean times of back reactions measured in presence of Q$_0$, Q$_6$, Q$_9$, Q$_{10}$ and MK$_8$. The large slowing down of P$^+$ rereduction rate indicates that all ubiquinons became functional at the QB site. When long chain prenylogs were bound to the QB site, the back reaction had a major exponential phase with a mean time of 1-2.3 s (see Table I and Fig. 3 inset); moreover, under these conditions full reconstitution of Q$_B$ took place, as no fast phase due to P$^+$Q$_A^-$ decay could be observed (see Fig. 5, upper trace). When Q$_0$ played the role of Q$_B$, the overall rate of the back reaction was several hundred of milliseconds (see Table I and Fig. 5). In addition, the kinetics was biexponential with a slow phase relaxing on a time scale of seconds. In the particular case of Fig. 5, only 75% of Q$_B$ sites were reconstituted with Q$_0$ as illustrated by the weight of the remaining fast phase of 20 ms.

When terbutryn was added after the restoration of Q$_B$ site with Q$_{10}$, P$^+$ relaxation became again very fast; the herbicide thus blocked the electron transfer from Q$_T^-$ to Q$_B$ (not shown).

Secondary activity was otherwise demonstrated by binary oscillations of stable absorbance changes mea-

**Table I**

<table>
<thead>
<tr>
<th>UQ</th>
<th>$\mu$M</th>
<th>N</th>
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<th>$\tau_2$(s)</th>
<th>d</th>
<th>f</th>
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</thead>
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<td>Q$_0$</td>
<td>140-200</td>
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<td>0.29±0.13</td>
<td>63±13</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Q$_6$</td>
<td>48-60</td>
<td>6</td>
<td>1.37±0.3</td>
<td>75±12</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>Q$_9$</td>
<td>13</td>
<td>2</td>
<td>1.0</td>
<td>50</td>
<td>4.3</td>
<td>50</td>
</tr>
<tr>
<td>Q$_{10}$</td>
<td>50-60</td>
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<td>2.3±0.73</td>
<td>81±9.4</td>
<td>0.7</td>
<td>6.6</td>
</tr>
<tr>
<td>MK$_8$</td>
<td>50-60</td>
<td>2</td>
<td>(44-48)×10$^{-3}$</td>
<td>95</td>
<td>-</td>
<td></td>
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</tbody>
</table>

Fig. 5. Decay kinetics of flash-induced absorbance change measured at 785 nm on a RC sample (1 $\mu$M). Lower trace: without addition; $\tau_1$ = 0.036 s. Upper trace: addition of 140 $\mu$M UQ$_{10}$; $\tau_1$ = 0.023 s (32%), $\tau_2$ = 0.43 s (68%); other conditions as in Fig. 1.

Fig. 6. Absorbance changes measured at 450 nm after a series of saturating white flashes on a 3 $\mu$M RC sample in presence of 500 $\mu$M DAD, 60 $\mu$M UQ$_{10}$, 1.3 mg/ml Brij 35; other conditions as in Fig. 1.
sured at 450 nm, elicited by a series of saturating flashes in the presence of an excess of Q\textsubscript{B} and DAD (see Fig. 6). These oscillations were attributed to the alternative formation of semiquinone Q\textsubscript{B} and quinol Q\textsubscript{B}$^2$\textsuperscript{-} [25,26]. It is of note that menaquinone MK\textsubscript{s} could not function as a secondary acceptor, the rate of the recombination reaction after the flash being in its presence close to that measured in Q\textsubscript{B}$^2$-less RC (see Table I). These experiments would suggest that in \textit{Re. gelatinosus}, as in \textit{Rps. viridis} [24], the secondary quinone site of the RC binds preferentially ubiquinone prenylogs when the Q\textsubscript{A} site is occupied by the native menaquinone species.

Discussion

RC constituents

The major difficulty for isolating an intact RC from \textit{Re. gelatinosus} results from its lability when interacting with most usual non-ionic or zwitterionic surfactants (see above). In contrast to RCs from other purple bacteria which have been solubilized in the presence of LDAO, \textit{Re. gelatinosus} RC is rapidly denatured by this detergent. In the present work the RC was isolated from \textit{Re. gelatinosus} chromatophores by the combined action of OTG and C\textsubscript{10}E\textsubscript{4}, followed by a temperature-induced phase separation. Further purification was done by ion-exchange chromatography.

The RC isolated with this method still contained the three polypeptides L, M and H besides minor contaminants; this is in contrast to two previous preparations which yielded LM units instead [13,14]. The electrophoretic mobilities of the L, M and H polypeptides would at first view indicate a higher molecular mass relative to the corresponding one of \textit{Rb. sphaeroides} RC (see Fig. 2, lane 2,3). This comparison is, however, of little value, as it has been observed that hydrophobicity of these polypeptides results in anomalous migration in SDS gels, the apparent molecular weights differing from the real ones deduced from sequence analysis in an unexpected manner. The tetraheme cytochrome $c$ initially associated with the RC was removed by the detergent treatment, as seen from spectral analysis as well as from electrophoresis. As in the case of \textit{Chromatium} RC [27] the link between the cytochrome and the RC seems less strong than in the case of \textit{Rps. viridis} where cytochrome $c$ is an integral part of the whole RC structure [3]. It remains to be seen if this weaker binding results from a different molecular structure of either the cytochrome (e.g. absence of lipophilic modification of the N terminal [28]) or of the M subunit C terminal extension which both participate to the tight binding of \textit{Rps. viridis} cytochrome $c$ [3].

Although we did not directly determine the RC Bchl and Bpheo contents, it is clear from the absolute absorption spectrum that they are identical with \textit{Rb. sphaeroides} RC as already observed [13]. The absorption spectrum moreover shows the presence of a bound carotenoid. The vibrational bands of the B1 $\leftrightarrow$ A1 electronic transitions of this pigment are blue-shifted when compared to those observed in pure B875 antenna complex (unpublished observations) or in photoreceptor units, where these bands are much better resolved [16]. This observation is in favor of a cis conformation imposed to the carotenoid by its binding site in the RC [23,29]. Sphaeroidene and hydroxysphaeroidene have been identified as major carotenoids in \textit{Re. gelatinosus} cells [30,31] and one could thus expect the carotenoid bound to the RC to be also of sphaeroidene type. In \textit{Rb. sphaeroides} RC, sphaeroidene is bound to the M subunit as a 15,15' mono cis-isomer [32]. Spectral properties of the carotenoid bound to \textit{Re. gelatinosus} RC are not identical, however, to that of \textit{Rb. sphaeroides}. Thus the RC carotenoid in \textit{Re. gelatinosus} either is not of sphaeroidene type, or is another sphaeroidene conformer. Further experiments are in progress to confirm one of these hypotheses.

$Q\textsubscript{A}$ activity

We have shown that in \textit{Re. gelatinosus} RC Q\textsubscript{A} is a menaquinone and Q\textsubscript{B} an ubiquinone [16,17]. Reaction centers of other purple photosynthetic bacteria like \textit{Chlamydomonas reinhardtii}, \textit{Chromatium tepidum} and \textit{Rps. viridis} also possess this mixed system of electron acceptors [8,11,24]. As shown by the decay kinetics of P$^+$ after a flash the RC was isolated with only Q\textsubscript{A} present; the primary quinone was reoxidized by P$^+$ in 30 ms. As a matter of fact, for RCs in which Q\textsubscript{A} is a menaquinone the rate of the recombination reaction between P$^+$ and Q\textsubscript{A} is several times more rapid than in \textit{Rb. sphaeroides} RC where Q\textsubscript{A} is an ubiquinone [33]. This fact is due to the lower redox potential of a naphthoquinone relative to ubiquinone. We could measure the absorption spectrum of flash-induced Q\textsubscript{A} when P$^+$ was reduced in a few milliseconds by an external electron donor (see Results). The spectrum has an absorption maximum at 412 nm which actually corresponds to the 395 nm semimenaquinone anion absorption band observed in vitro [34]. Therefore this band is red shifted when MK is bound to the Q\textsubscript{A} site. In addition, shoulders at 400, 425 and 450 nm (see Fig. 4A) may arise from electrochromic shifts of the pigments' absorption bands. This rather complex spectral structure of Q\textsubscript{A} in \textit{Re. gelatinosus} RC is very similar to that of light-induced semimenaquinones measured in \textit{Chlamydomonas reinhardtii} RC (for MK\textsubscript{s} [11], in \textit{Rps. viridis} RC (for MK\textsubscript{s} [24], in \textit{Chloroflexus aurantiacus} RC (for MK\textsubscript{10} [35]) and also in \textit{Rb. sphaeroides} RC when Q\textsubscript{A} was substituted with MK\textsubscript{s} [24]. Therefore, the spectral properties of Q\textsubscript{A} in \textit{Re. gelatinosus} RC are an additional support for assigning the primary acceptor to MK\textsubscript{s}. The elec-
trochromic shift of Bpheo Q\textsubscript{A} band induced by a negative charge on Q\textsubscript{A} (see Fig. 4B) is similar to that induced in Rb. sphaeroides RC where Q\textsubscript{A} is UQ\textsubscript{10} [36]. Thus this absorbance change does not depend on the nature of the semiquinone.

\textit{Q\textsubscript{B} activity}

In a previous work [17] we carried out reconstitution experiments of Q\textsubscript{B} site with \textit{Rc. gelatinosus} photoreceptor units in which Q\textsubscript{B} was partially removed by treatment with high concentrations of detergent and orthophenanthroline. The long chain prenylogs of ubiquinone type could reconstitute Q\textsubscript{B} activity. After purification of the RC as described in the present paper, native Q\textsubscript{B} site properties were preserved, as illustrated by flash experiments carried out in presence of ubiquinones. UQ\textsubscript{6}, UQ\textsubscript{9} and UQ\textsubscript{10} were shown to play the role of Q\textsubscript{B} in RC, if MK were bound to the Qt\textsubscript{3} site. In contrast menaquinones could not function as Qt\textsubscript{3} acceptor quinone complex is similar to that of \textit{Rps. viridis} RC whereas the acceptor quinone complex is similar to that of \textit{Rps. viridis} RC. Thus we intend to study in more detail the electron transfer and compare it with the other two well known RCs; work is also in progress to improve its purification.

\textbf{Acknowledgements}

We want to thank Mrs. M.C. Gonnet for her expert assistance in growing bacteria and chromatophores preparation. We are grateful to J.C. Barbet and J.P. Roux for their help in spectrophotometric measurement programming.

\textbf{References}