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# Isolation and spectroscopic properties of photochemical reaction centers from *Rhodobacter capsulatus*

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***Rhodobacter capsulatus*** **is an excellent organism for using site-directed mutagenesis to address the goal of a precise correlation between amino acid sequence and protein structure and function. In this paper we show that photochemical reaction centers may be purified by a single DEAE chromatography step from a strain of the organism that lacks the light-harvesting complex known as B800–850 or LH II. These reaction centers show many similarities to the well-studied preparations from *Rhodobacter sphaeroides* and *Rhodopseudomonas viridis,* and appear ideal for many forms of spectroscopy.**

## Introduction

The primary photochemical reactions of bacterial photosynthesis take place within a membrane-bound protein complex known as the photochemical reaction center. This protein complex has been isolated from many bacterial species, and has become one of the most studied intrinsic membrane components. In most species of purple bacteria (both 'sulfur' and 'non-sulfur'), the reaction center consists of three dissimilar peptides, together containing four molecules of bacteriochlorophyll, two of bacteriopheophytin, an iron atom and one or two quinones (see Refs. 1–4). The primary amino acid sequence is known for at least some of the protein subunits in several species [5–8], and the crystal structure of the complex from *Rhodopseudomonas viridis* has now been determined to a resolution of 0.3 nm [9]. The precise correlation between amino acid sequence and protein structure and function is thus a realistic goal for the near future, and experiments using site-directed mutagenesis to alter the amino acid sequence are in hand [10,11].

*Rhodobacter capsulatus* is the only organism where the complete nucleic acid sequence has been determined for all the subunits of the reaction center [5], and the light-harvesting antenna [12]. However, the reaction center has not been thoroughly studied by physical techniques because there has been no easy procedure for reproducibly isolating stable reaction center preparations from this organism. In this paper we describe a remarkably simple procedure for isolating reaction centers from *Rb. capsulatus,* using a strain that lacks one of the light-harvesting antenna complexes which otherwise is rather hard to separate from the solubilized reaction centers. We also

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Abbreviation: LDAO, lauryldimethylamine *N*-oxide.

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present an optical and electron paramagnetic resonance characterization of the reaction centers, and compare them with the much studied preparation from *Rhodobacter sphaeroides.*

## Materials and Methods

*Rhodobacter capsulatus* (known as *Rhodopseudomonas capsulata* before the new taxonomy of Imhoff et al. [13]) strain U34 was grown photoheterotrophically as previously described [10]. This strain, derived from SB1003, has a 3350-bp spectinomycin interposon in the *Nru*I site of the B800–850 (otherwise known as light-harvesting complex II) *Beta* structural gene [10], and no B800–850 is detected in the membranes. This strain shows essentially no reversion to wild type under photosynthetic conditions [10].

Stationary phase cells from 6 l were harvested in a GS3 rotor at 8000 r.p.m. for 30 min, washed and suspended in 10 mM potassium phosphate (pH 7.4) (buffer A), and passed through a French Press at 138 Mpa. Debris was removed by centrifugation in an SS34 rotor at 11 000 r.p.m. for 1 h.

Crude chromatophores were pelleted from the supernatant by ultracentrifugation in a 60 Ti rotor at 45 000 r.p.m. for 3 h. The supernatant was discarded, and the pellet was gently washed with buffer A in situ so as to remove the upper part of the biphasic pellet. The denser part of the pellet was homogenized in buffer A containing 0.05% lauryldimethylamine *N*-oxide (LDAO), henceforth called buffer B. Chromatophores were then pelleted by a second ultracentrifugation.

Chromatophores were solubilized by first homogenizing in 40 ml buffer A, and then adding LDAO to 1.5% and heating at 37°C for 10 min; insoluble material was removed by ultracentrifugation for 1 h. A 30 ml DEAE column (Biogel-A, 100–200 mesh) was packed in buffer B containing 300 mM KCl (buffer C) and washed with buffer A containing 0.6% LDAO (buffer D). The supernatant was loaded onto the column and then 200 ml of buffer D was used to wash all but the reaction centers from the column (about 3 h). Greenish-grey reaction centers are observed to be bound to the top half of the column, while the remainder is pinkish-orange, probably due to bound carotenoid. The column is washed with 50 ml of buffer B, and then increasing steps of KCl in buffer B (10–60 mM) are used to wash the column. The reaction centers are then eluted in buffer B containing 150 mM KCl. Typically 4 ml of peak fractions and 6 ml of side fractions are collected, totalling 30 mg (90 A · ml (absorbance units × ml) at 800 nm) of reaction centers. Note that the KCl concentrations may vary slightly for different preparations. Reaction centers are dialyzed against buffer B overnight at 4°C, and frozen until required.

Optical spectra were recorded with a Lambda Diode Array model 3840 spectrometer linked to a Perkin-Elmer model 7300 workstation. Flash induced optical absorbance changes were measured with a rapidly responding double-beam spectrometer constructed by the Bio-Instrumentation Group at the University of Pennsylvania as described previously [14]. Electron spin resonance spectra were recorded on a Varian E109 spectrometer interfaced to an IBM personal computer, equipped with an Air Products flowing helium cryostat [15]. Lauryldimethylamine *N*-oxide (Ammonyx) was obtained from Onyx Chemical Co. Jersey City, NJ. Ubiquinone-10 (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone) was obtained from Calbiochem, Los Angeles, CA, and Ametryne (2-ethylamino-4-isopropylamino-6-methylthio-*s*-triazine) from Chem Service, West Chester, PA.

## Results

Fig. 1 shows the optical absorption spectrum of the isolated reaction centers, and the light-induced difference spectrum. Fig. 2 shows the spectrum at low temperature. These spectra are rather similar to those reported earlier for a reaction center preparation from *Rb. capsulatus* Ala pho$^+$ [16], but indicate that the current preparation is substantially free of solubilized bacteriochlorophylls and bacteriopheophytins that contaminated the earlier preparation. Furthermore, the spectra presented here are from a carotenoid-containing strain, and the features at 438, 470 and 502 nm (443, 471 and 504 nm at low temperature) are due to carotenoid. A small shift of the carotenoid spectrum can be discerned in the light-minus-dark spectrum in Fig. 1. This is due to an electrochro-

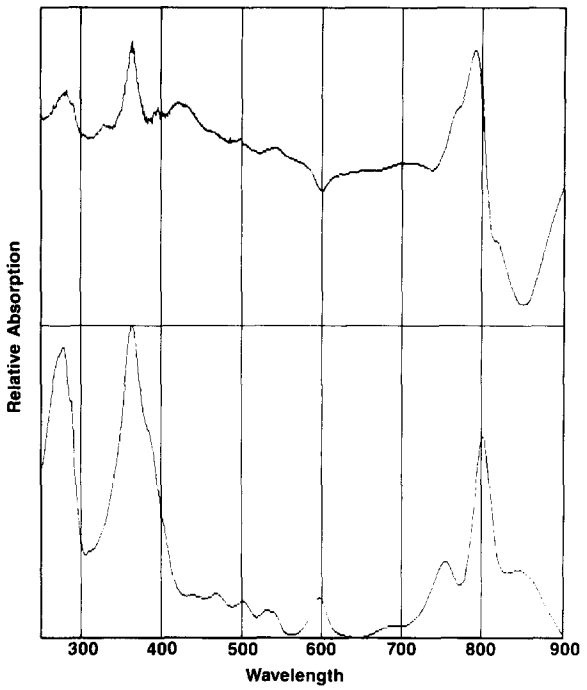


Fig. 1. Isolated reaction centers from *Rb. capsulatus* U34. The lower panel shows the absorption spectrum measured in a diode array spectrometer. The $A_{280}/A_{800}$ ratio is 1.4. The upper panel shows the light-minus-dark difference spectrum of the same sample, multiplied 4 times.

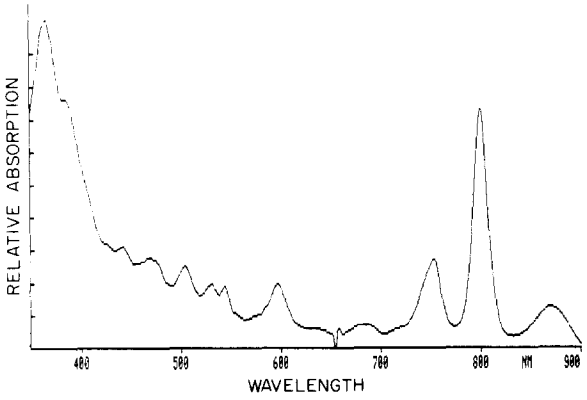


Fig. 2. Low-temperature spectrum of isolated reaction centers from *Rb. capsulatus* U34. Reaction centers were made 50% (v/v) in glycerol and frozen in liquid nitrogen. When the sample was thoroughly cooled, it was placed in the sample holder of the diode array spectrometer, and the average of 16 rapid scans (total time, 1 s) obtained.

mic response to the dipole set up between oxidized primary donor and reduced quinone [17], and is quite distinct from, and much smaller than, the response of the carotenoid pigments in the light-harvesting II complex to delocalized potentials across the chromatophore membrane [18,19]. Note that at low temperature the feature near 534 nm at room temperature, attributable to bacteriopheophytin [1–4], resolves into two sharp features at 531 and 546 nm. It is the pheophytin absorbing at the longer wavelength that functions in electron transfer between the primary donor and the quinone [1–4]. The spectra of Figs. 1 and 2 are very similar to those obtained from *Rb. sphaeroides*, although the light-induced changes in the infra-red are somewhat broader in *Rb. capsulatus* (see Refs. 1–4, and 16).

Fig. 3 shows an SDS-polyacrylamide gel of the isolated reaction centers; it shows the three subunits expected for the reaction center, with only very minor additional bands that could be aggregates of the individual subunits.

Fig. 4 examines the kinetics of light-induced reactions within the reaction center at room temperature. The figure shows the very rapid oxidation of the 'primary donor' by a single-turnover flash, and its subsequent re-reduction as the system relaxes. As prepared, this re-reduction has a half-time of approx. 80 ms. Upon the addition of ubiquinone-10 this slows dramatically, to about 1.3 s. The subsequent addition of Ametryne, an Atrazine-type herbicide, restores the initial, more rapid, kinetics. We interpret these findings as indicating that the reaction centers, as prepared, contain only the initial quinone acceptor, $Q_A$. Photochemistry thus results in electron transfer only as far as $Q_A$, and this electron can return to the

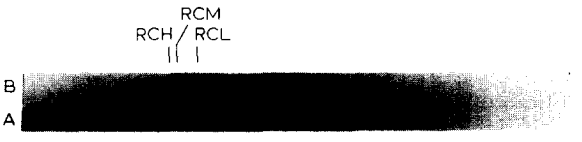


Fig. 3. SDS-polyacrylamide gel of reaction centers from *Rb. capsulatus*. Lane A: Wild-type chromatophore membranes from strain U15g(pU21) [10], a plasmid complemented deletion strain having wild-type reaction centers and light-harvesting proteins. Lane B: reaction centers from strain U34 prepared by a single DEAE column chromatography. 15% Isocratic gel, 0.1% SDS [28] stained with Coomassie blue. The top of gel is at the left side.

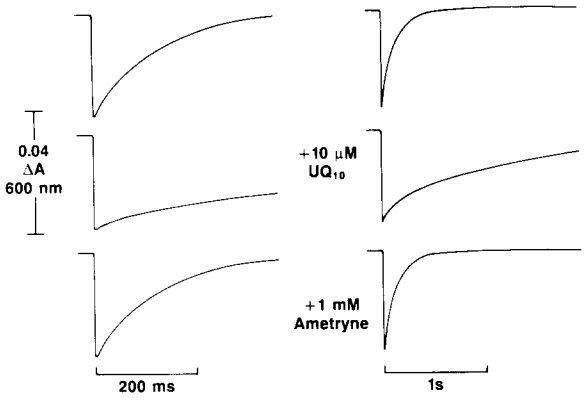


Fig. 4. Flash-induced kinetics of the isolated reaction centers from *Rb. capsulatus* U34. Reaction centers ($A_{801} = 0.801$) in 20 mM *N*-morpholinopropanesulfonate/100 mM KCl/0.3% LDAO were illuminated with a brief flash of light (20 μs at half height) and the absorption of the primary donor followed at 600 nm. Ubiquinone and Ametryne were added as indicated.

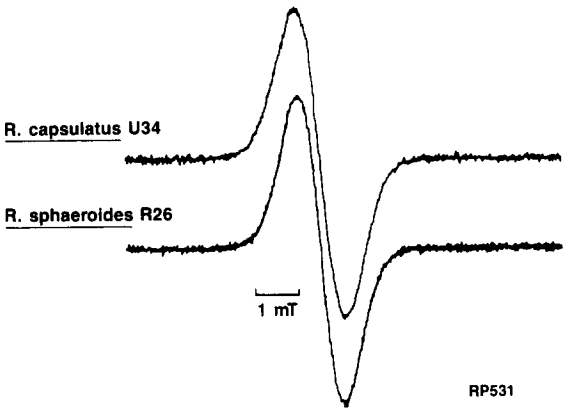


Fig. 5. Electron spin resonance spectra of the photo-oxidized primary donor in isolated reaction centers. The reaction centers ($A_{801} = 80$) were examined at 6 K with 1 mW of applied power and a modulation of 0.25 mT. Reaction centers from *Rb. sphaeroides* are shown for comparison. These are light-minus-dark difference spectra.

oxidized primary donor with a half-time of about 80 ms. The addition of ubiquinone-10 reconstitutes secondary quinone ($Q_B$) activity, so that $Q_A^-$ can now reduce $Q_B$. The return of an electron from $Q_B^-$ to the oxidized primary donor is slower, with a half-time of 1.3 s. The addition of Ametryne displaces $Q_B$ from its binding site, thereby preventing the electron leaving $Q_A^-$ for $Q_B$, and so the more rapid decay is restored. Very similar behavior is seen in *Rb. sphaeroides* (e.g., Ref. 2).

Fig. 5 shows the ESR spectrum of the oxidized 'primary donor' or 'special pair' of the *Rb. capsulatus* reaction center, and compares it with that found in *Rb. sphaeroides.* The signal in the *Rb. capsulatus* reaction centers is slightly broader (1.09 mT) than that seen in *Rb. sphaeroides* (1.02 mT) under the conditions used here, but the signal is otherwise very similar, and is centered at the same *g*-value (close to $g = 2.0025$, see Refs. 1–4). At low temperatures the back reaction from $Q_A^-$ to the oxidized primary donor discussed above speeds up: at 14 K the half-time is approx. 20 ms in both *Rb. capsulatus* and *Rb. sphaeroides,* as measured by the decay of the flash-induced ESR signal at $g = 2$ (experiments in collaboration with M.R. Gunner and D.E. Robertson).

Fig. 6 shows the ESR spectrum of the reduced primary quinone acceptor, which shows prominent features at $g = 1.82$ and $g = 1.69$. Again this is very similar to the signal seen in *Rb. sphaeroides,* where it has been interpreted as the spectrum of a quinone radical in close association with the high-spin ferrous iron atom of the reaction center (see Refs. 1–4).

Fig. 7 shows the ESR spectrum of the spin-polarized triplet state of the primary donor. This signal is seen when reaction centers with reduced

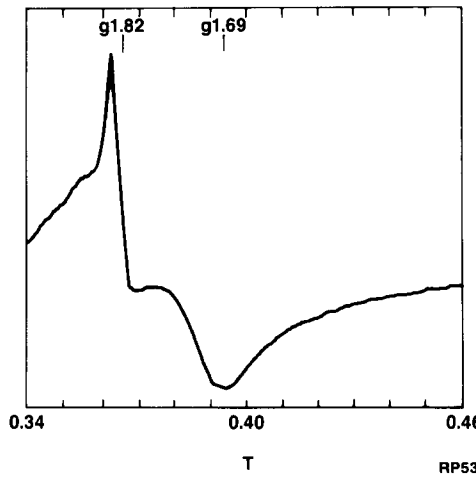


Fig. 6. Electron spin resonance spectrum of the reduced primary quinone in reaction centers from *Rb. capsulatus* U34. Reaction centers ($A_{801} = 80$) were reduced with a slight excess of sodium dithionite, and examined at 5 K with 20 mW of applied power and a modulation of 2.0 mT.

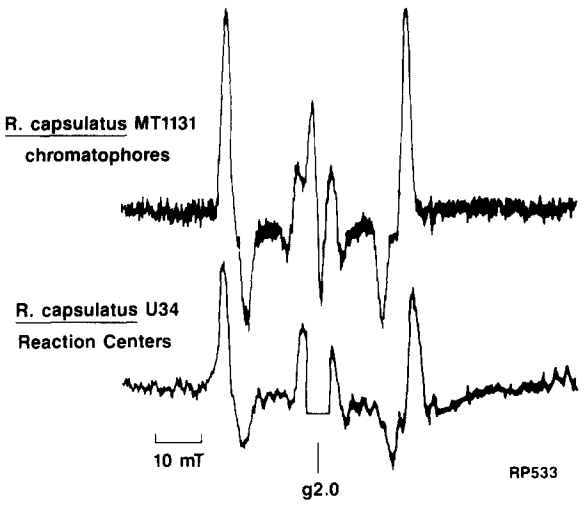


Fig. 7. Electron spin resonance spectra of the spin-polarized triplet of the primary donor in *Rb. capsulatus*. Membranes of strain MT1131 (1.5 mM bacteriochlorophyll) or reaction centers from strain U34 ($A_{801} = 80$) were reduced with a slight excess of sodium dithionite and examined at 6 K with 1 mW of applied power and a modulation of 2.5 mT. These are light-minus-dark difference spectra.

primary quinone are illuminated at low temperatures, and arises as the photo-electron returns from the intermediary bacteriopheophytin acceptor to the oxidized primary donor. The upper spectrum is the triplet seen in chromatophore membranes of a green, but otherwise wild-type strain of *Rb. capsulatus*, which has $D = 186 \cdot 10^{-4}$ $cm^{-1}$ and $E = 31 \cdot 10^{-4}$ $cm^{-1}$, in excellent agreement with earlier findings [20]. The isolated reaction centers show a slightly different signal (lower spectrum), with $D = 195 \cdot 10^{-4}$ $cm^{-1}$ and $E = 38 \cdot 10^{-4}$ $cm^{-1}$. Similar subtle changes are not usually seen in *Rb. sphaeroides* [20], but were seen [21] in *Rhodocyclus gelatinosus* (previously *Rhodopseudomonas gelatinosa*, see Ref. 13). The changes are rather small, in energy, and we are uncertain of their significance.

## Discussion

The photosynthetic apparatus of *Rb. capsulatus* and *Rb. sphaeroides* consists of two distinct light-harvesting complexes, known as B875 or LH I and B800–850 or LH II, and the photochemical reaction center. The two antenna complexes together channel photons to the reaction center (see Refs. 22 and 23 for recent reviews). B875 I is present at an apparently constant concentration, relative to reaction centers, while the amount of B800–850 seems to vary with growth conditions such as the oxygen tension and light intensity. B800–850 is solubilized by 1.5% LDAO, and indeed can be purified by the procedure outlined here from strains that contain this complex, but lack the reaction center, such as Y142 [24] (Robles and Youvan, unpublished observations). In contrast, B875 seems to denature in 1.5% LDAO, and its bacteriochlorophyll then absorbs at 760 nm. This is readily washed from the reaction centers while the latter are bound to a column. The most studied reaction recenter preparations have come from 'blue-green' mutants that lack colored carotenoids (see, e.g., Ref. 25). The dramatic differences in color of such mutants from the wild-type has perhaps obscured the fact that in concert with lacking colored carotenoids, these strains also lack B800–850. The ratio of antenna pigments and protein to reaction centers is thus reduced, and since B875 is denatured by LDAO, purification of the reaction center is relatively simple [25] *. Unfortunately, reaction centers prepared from blue green strains of *Rb. capsulatus* (e.g., Ref. 16) have not proven very stable, so we have chosen to isolate reaction centers from a carotenoid containing strain that lacks B800–850. We have used a strain that has a spectinomycin interposon inserted into the B800–850 *Beta* gene [10], because this strain exhibits a negligible reversion frequency to wild-type under photosynthetic growth conditions [10].

The preparation procedure outlined here is simple and rapid, and clearly results in the isolation of reaction centers that are very similar to the much studied preparation from *Rb. sphaeroides* R26. They are thus amenable to the plethora of

* This simple picture has recently been complicated by the finding that the most studied reaction center preparations, those from *Rb. sphaeroides* R26, come from a strain that over the years since its isolation [26] has regained the B800–850 peptides, but in a deranged form that only bind the 850 nm absorbing bacteriochlorophyll [27]. This latter strain has been designated R26.1 by Davidson and Cogdell [27].

biophysical techniques and spectroscopies that have provided our understanding of the primary events of bacterial photosynthesis. Major advances in this understanding can be expected as the techniques of site-directed mutagenesis are applied to the system, and *Rb. capsulatus* is an ideal vehicle for such studies. With this demonstration of the properties of the wild-type reaction center in this species, the way is clear for a combined biophysical and genetic approach toward understanding the molecular basis for the reaction center's remarkable activities.

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