PRELIMINARY NOTE

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Isolation of the reaction center of *Rhodopseudomonas viridis*

REED AND CLAYTON[1] and GINGRAS AND JOLCHINE[2] have recently separated photochemical reaction centers from the bulk light-harvesting chlorophylls in carotenoidless mutants of *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum* respectively, using the nonionic detergent Triton X-100. By using the anionic detergent, sodium dodecyl sulfate, we have isolated a reaction center fraction from wild type *Rps. viridis* (NTHC 133), a purple bacterium which contains bacteriochlorophyll *b*, neurosporene, lycopene, and a minor less colored carotenoid (ref. 3 and K. EIMHJELLEN, personal communication). The use of Triton X-100 does not enable a reaction center fraction to be obtained from this organism (R. K. CLAYTON, personal communication; see also ref. 4).

HOLT AND CLAYTON[5] showed that the reaction center in broken cells of *Rhodopseudomonas viridis* consists of a photobleachable pigment, P985 (P960 in dried films of chromatophores), and a pigment P830, which shifts to the blue upon illumination. This reaction center is of particular interest, since, compared to those of bacteriochlorophyll *a*-containing organisms, there is a greater wavelength separation between P985 and P830 than between P890 (or P870) and P800. This may permit eventual circular dichroism spectra, and consequently the molecular organization in the reaction center to be more easily interpreted (*cf.* ref. 6).

The organism was grown photoheterotrophically[7], and harvested either by centrifugation or by precipitation with alum. Packed cells were suspended in an equal volume of 50 mM Tris–HCl (pH 8.0), and broken by sonication in a Raytheon 10 kcycles/sec oscillator (1.2 A for 10 min). The absorbance of the sonicate at the far red wavelength maximum (approx. 1010 nm) was determined, and used to estimate the concentration of bacteriochlorophyll *b* ($\varepsilon_M = 10^5$)[8].

The sonicated material was centrifuged at 15000 × *g* for 30 min. If alum was originally added to aggregate the cells, some chromatophores were present in the supernatant, but 90% or more of the bacteriochlorophyll *b* remained in the precipitate; in the absence of alum, high yields of chromatophores were obtained in the supernatant. The reaction center fraction could be prepared in either case from the major chlorophyll-containing fraction, by homogenizing that fraction with 1.0% sodium dodecyl sulfate in 50 mM Tris (pH 8.0); the final ratio of sodium dodecyl sulfate to bacteriochlorophyll *b* was 12 g/mmole. This ratio of sodium dodecyl sulfate to bacteriochlorophyll *b* solubilized the light-harvesting bacteriochlorophyll in the cells or chromatophores as judged by the disappearance of the major peak of bacteriochlorophyll *b in vivo* (approx. 1010 nm) and the appearance of a major peak at 810 nm in the homogenate; a minor and broad absorption band at 960 nm remained. Addition of excess sodium dodecyl sulfate destroyed this 960-nm band.

The sodium dodecyl sulfate homogenate was centrifuged at 15000 × *g* for 30 min, and the green supernatant was run into a column of hydroxylapatite[9,10] (3.5 cm × 8 cm), equilibrated in 10 mM sodium phosphate–0.2 M NaCl (pH 7.0), and the extract was washed with 100 ml of the buffer–salt solution. Elution with 0.2 M sodium phosphate–0.2 M NaCl (pH 7.0), removed a small fraction (approx. 8 %) of the total bacteriochlorophyll *b* on the column. This fraction was rechromatographed on hydroxylapatite after it had been precipitated with ammonium sulfate and redissolved in a minimal volume of 10 mM sodium phosphate–0.2 M NaCl (pH 7.0). The absorption spectrum of the subsequent eluate (solid curve in Fig. 1a) showed absorption bands at 830 nm and 960 nm, the expected characteristics of reaction centers from *Rps. viridis*[5,11]. The band at 685 nm was thought to represent some oxidized bacteriochlorophyll *b*. The majority of the sodium dodecyl sulfate-solubilized bacteriochlorophyll *b* (810-nm form) was retained by the hydroxylapatite.

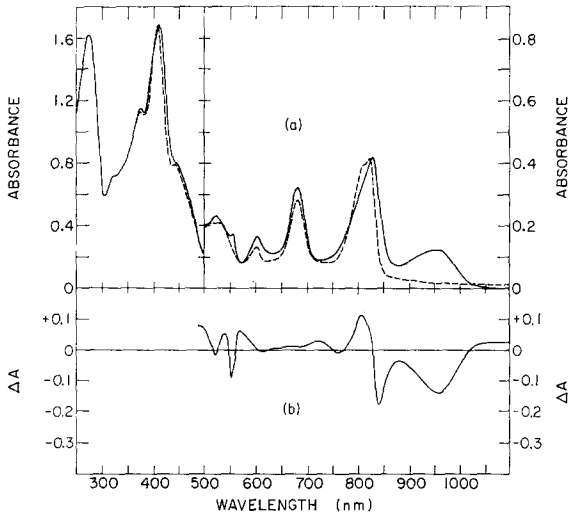

Fig. 1. a. Absorption spectra of the reaction center fraction, recorded in the IR 1 (———) and the IR 2 (– – – –) modes of a Cary 14R spectrophotometer. In the IR 1 mode the sample was illuminated by a weak beam of monochromatic light, while in the IR 2 mode, the sample was illuminated by a strong beam of white light. The sample contained 1 μM of P960. b. The oxidized-*vs.*-reduced difference spectrum of the reaction center preparation. Potassium ferricyanide was used as the oxidizing agent, and sodium dithionite was used as the reductant.

The fraction from hydroxylapatite was examined in a Cary 14R recording spectrophotometer. The spectrum of the sample illuminated by a weak beam of monochromatic light, and that of the sample illuminated by a strong beam of white light are shown in Fig. 1a; the difference spectrum between an oxidized and a reduced sample is given in Fig. 1b. All the major light-induced absorbance changes observed in broken cells[5] were seen in the fraction. There was a bleaching of the 960-nm peak and a blue shift of the 830-nm peak. Changes in absorbance were also observed at 610 nm (bacteriochlorophyll *b*). If the preparation is kept in darkness for 10 min after exposure to the strong actinic light, there is a reappearance of the 960-nm peak and a return of the 830-nm band to its original location. Thus, the isolated fraction

shows all the light-induced absorbance changes which are characteristic of the reaction center of the organism. In addition to the reaction center chlorophylls, the preparation also contains cytochromes and carotenoids. Difference spectra—oxidized (K_3FeCN_6) or reduced ($Na_2S_2O_4$ or tetramethyl-*p*-phenylenediamine) *vs.* the untreated sample—revealed the presence of two cytochromes, C553 and C558. All the C553 was in the oxidized state, whereas most of the C558 occurred in the reduced state. The carotenoids were extracted from the reaction center preparation with 80 % ethanol, and fractionated from the bacteriochlorophyll *b* by extraction into light petroleum. Thin-layer chromatography revealed the presence of one major carotenoid, lycopene. By assuming $\varepsilon_M = 2 \cdot 10^4$ (ref. 8) for the cytochromes and $E_{1\%} = 3450$ (ref. 12) for the carotenoid, the following molar ratio was obtained, P960:lycopene:C553:C558 = 1:1:5:2.

Upon continued exposure of the reaction center preparation to light, the C558 becomes completely oxidized, and its subsequent reduction in the dark is extremely slow. However, the addition of 10–15 μM phenazine methosulfate permits a rapid reduction of the C558, presumably by the reductant formed in the photoact. The quantum requirement for C558 oxidation was measured in the presence of phenazine methosulfate to be between 4 and 10 quanta per electron with the assumption of $\Delta\varepsilon$ at 424 nm = 100 $mM^{-1} \cdot cm^{-1}$ for C558. This range of values is comparable to the estimate of 5–6 for the quantum requirement of C553 oxidation in intact cells[11].

The purity of the reaction center was examined by ultracentrifugation and by polyacrylamide gel electrophoresis. In the Spinco Model E ultracentrifuge, the reaction center was examined in three solvents: 0.2 M sodium phosphate–0.2 M NaCl (pH 7.0), in 50 mM Tris (pH 8.0), and in 50 mM Tris–0.1 % sodium dodecyl sulfate. In the first solvent a very broad boundary was observed which sedimented with the majority of the color in the solution and at a faster rate than the major component in the other solvent systems. In 50 mM Tris a boundary of 16 S was observed together with some boundaries of low concentration but of higher *s* values (probably aggregates of the 16-S component); no slower sedimenting boundaries were observed. In the presence of sodium dodecyl sulfate, no boundaries of greater than 5 S were seen, and the majority of the color sedimented with this 5-S boundary; it is possible that in this solvent system boundaries of less than 5 S are present. The sample after centrifugation in 50 mM Tris–0.1 % sodium dodecyl sulfate still retained the optical characteristics of the reaction center. Polyacrylamide gel electrophoresis (7 % acrylamide in 50 mM sodium phosphate buffer $\pm$ 0.1 % sodium dodecyl sulfate) showed the same aggregation–dissociation phenomena observed in the ultracentrifuge. Prolonged electrophoresis in the presence of detergent released some of the bacteriochlorophyll *b* from the complex. There was little, if any, contamination by other protein species. Thus the reaction center, as isolated, appears reasonably pure, but the size of the unit is variable. Further work is in progress to determine the size of the minimal unit, which retains reaction center activity.

It appears that sodium dodecyl sulfate dissolves the light-harvesting bacteriochlorophyll *b*, and that part of the lamella which contains the reaction center. The two pigmented fractions are separable by hydroxylapatite chromatography, or by sucrose density centrifugation (*cf.* ref. 1). The isolated reaction center is stable and can be kept at room temperature, or at 4°, in the dark for several days without appreciable loss of reversible photobleaching.

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