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ISOLATION AND SPECTRAL CHARACTERISTICS OF THE PHOTOCHEMICAL REACTION CENTER OF *RHODOPSEUDOMONAS VIRIDIS*

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SUMMARY

A method is described for isolation of the *Rhodopseudomonas viridis* reaction center complex free of altered, 685 nm absorbing pigment. This improved preparation contains two *c*-type cytochromes in the ratio *P*-960: cytochrome *c*-558 : cytochrome *c*-553 of 1 : 2 : 2 to 3. The near infrared spectral forms of the reduced preparation are located at 790, 832, 846 and 987 nm at 77 K; the oxidized complex absorbs at 790, 808, 829 and approx. 1310 nm. The 790 nm band is attributed to bacteriophaeophytin *b* and the other absorbances to bacteriochlorophyll *b*. The visible absorption bands may be assigned to these pigments and to the cytochromes present and, probably, to a carotenoid. The presence of two bacteriochlorophyll *b* spectral forms in the P^+-830 band suggests that exciton interactions occur among pigments in the oxidized, as well as the reduced, reaction center. Changes in the 790 and 544 nm bands upon illumination of the reaction center preparation at low redox potential may be indicative of a role for bacteriophaeophytin *b* in primary photochemical events.

INTRODUCTION

The bulk of the photosynthetic pigments in an organism function to gather light quanta and feed the absorbed energy to a few specialized chlorophyll molecules located in a reaction center where the primary photochemical event occurs. In recent years a greater understanding of the mechanism of the energy conversion process in the reaction center has been obtained mainly from studies of preparations of the reaction center devoid of antenna pigments and of secondary redox components involved in the reactions prior and subsequent to the primary photochemical event. Such preparations are obtained by fractionation of detergent-solubilized photosynthetic membranes of an organism. To date, all reaction center preparations have

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been isolated from bacteria, and all except one have contained bacteriochlorophyll *a*. The exception is the preparation from the bacteriochlorophyll *b*-containing bacterium, *Rhodopseudomonas viridis*, which was first obtained at the end of the last decade [1, 2]. The complex was found to contain bacteriochlorophyll *b* as *P*-830 and photobleachable *P*-960, and high and low potential cytochromes, cytochrome *c*-558 and cytochrome *c*-553, respectively, both of which could reduce photooxidized P^+-960 [3].

The *R. viridis* reaction center has one particular advantage over all other equivalent preparations in that larger differences in energy levels occur between the different spectral forms of bacteriochlorophyll *b* in the reaction center. Consequently the spectral forms are more easily distinguished in spectra of this reaction center than in those of bacteriocholorophyll *a*-containing bacteria. Studies on the reaction center complex of *R. viridis* are anticipated to supplement considerably an understanding of the primary charge separation in the bacterial reaction center, and to be valuable in the comparative biochemical aspects of photosynthesis. However, further investigations of the biochemical and biophysical properties of the initial preparation [1, 2] were impeded by the presence of significant amounts of a pigment absorbing at 685 nm that is not photochemically active [1]. Due to the renowned lability of bacteriochlorophyll *b* [4, 5] the 685 nm pigment was tentatively identified as an oxidation product of bacteriochlorophyll *b* which appeared as an artifact of the isolation procedure. In subsequent years attempts have been made to eliminate both the 685 nm material and the secondary electron donors (cytochromes *c*-553 and *c*-558) from the preparation so that the properties of this complex can be better studied. We now report an improved preparatory procedure which essentially eliminates the oxidized bacteriochlorophyll *b*, but not the cytochromes, and describe spectral functional and some biochemical characteristics of the reaction center preparation.

METHODS AND MATERIALS

R. viridis was cultured by the method of Eimhjellen et al. [6], except for substitution in the culture medium of ammonium citrate and NaCl for sodium succinate. Cells were harvested from 1- to 2-week-old cultures by centrifugation at 5000 × *g* for 10 min, washed once with 50 mM Tris buffer, pH 8.0, and used directly or stored frozen. Approx. 10 ml packed volume of cells was resuspended in 10 ml of the Tris buffer and passed twice through a French Pressure cell (Aminco) at 10 000–12 000 lb/inch^2. The bacteriochlorophyll *b* content of the broken cell suspension was determined from its absorbance at the near infrared maximum ($\approx$ 1015 nm) using $\varepsilon = 10^5$ $1 \cdot mol^{-1} \cdot cm^{-1}$ [7].

Reaction center particles were isolated from broken cells by modifications of the original method [1, 2] as follows (Fig. 1): To the suspension in the dark at room temperature were added 70 mg sodium dodecyl sulfate/μmol bacteriochlorophyll *b*, and solid $Na_2S_2O_4$ to a concentration of 0.01 M. The volume was adjusted with Tris buffer so that the final concentration of SDS was less than 1.5 %, and the suspension was stirred for 15 min in the dark. The near infrared spectrum was checked to ascertain that the antenna pigment absorption maximum had shifted from 1015 to 810 nm (cf. ref. 2). The following procedure was then applied directly to a suspension containing not more than 10 μmol of bacteriochlorophyll *b*. The suspension, which

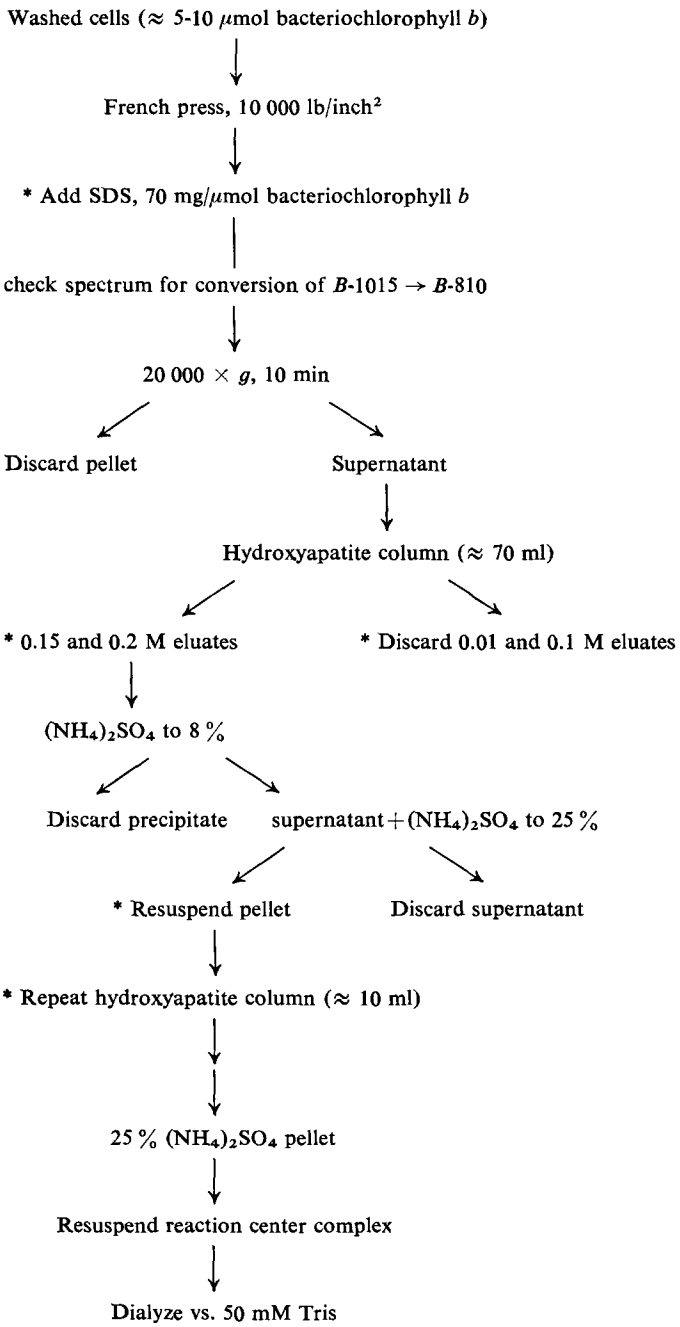

Fig. 1. Protocol for preparation of the *R. viridis* reaction center. Steps marked * were performed with buffers containing 0.01 M $Na_2S_2O_4$. See Methods and Materials for details.

was much clarified, was centrifuged at 20 000 × *g* for 20 min to remove cell wall debris and unsolubilized membrane material. The carefully decanted supernatant was then applied to a hydroxyapatite column, approx. 70 ml packed volume in a 100 ml syringe, which had been equilibrated with 0.01 M sodium phosphate buffer, pH 7.0, containing 0.2 M NaCl and 0.01 M $Na_2S_2O_4$. When all the bacteriochlorophyll *b*-containing material had been adsorbed to the column, it was washed with approx. 50 ml of the equilibration buffer, followed by 50 ml of 0.1 M phosphate buffer containing the same added salts. At this stage the column appeared green throughout most of its length with a brownish band at the front. A reaction center-enriched fraction (the brownish band) was eluted from the column with about 100–150 ml of 0.15 M phosphate buffer. If the brownish band did not elute from the column during this step, 0.2 M buffer was then used. These buffers also contained 0.2 M NaCl and 0.01 M $Na_2S_2O_4$. Column flow rate was maintained at 2–3 ml/min with a peristaltic pump. Presence of reaction center (*P*-960) in the eluates was ascertained from absorption spectra.

Appropriate eluates were pooled, and solid $(NH_4)_2SO_4$ added to a concentration of 8 % (w/v). The precipitate removed by centrifugation was enriched in 810 nm-absorbing material. The supernatant was made up to 25 % (w/v) $(NH_4)_2SO_4$ by addition of more solid salt, and a reaction center-enriched fraction recovered by centrifugation. This precipitate was solubilized in 50 mM Tris, pH 8.0, containing 0.01 M $Na_2S_2O_4$, and if necessary applied to a 10 ml hydroxyapatite column in a 30 ml syringe for further purification. The same elution procedure was used, with buffer volumes scaled down proportionately. The reaction center fraction was again obtained from column eluates by differential $(NH_4)_2SO_4$ precipitation, and the final pellet resuspended in a small volume of 50 mM Tris, pH 8.0, without reducing agent. The preparation can be stored in closed containers at 4 °C in the dark for several weeks. It should be dialyzed in the dark vs. the same buffer to remove any residual reducing agent before photooxidation is attempted.

Spectra were recorded on a Cary 14R operated in the IR1 or IR2 modes, or on an Aminco DW2 using the split beam mode, and in some instances, the low temperature attachment. Kinetic studies were performed with the Aminco DW2 in the dual beam mode.

Hydroxyapatite was prepared by the method of Siegelman et al. [8]. Other chemicals were of reagent grade. Sodium dodecyl sulfate was obtained from Matheson, Coleman and Bell.

Cytochrome concentrations were calculated from $\Delta A5$ measured from peak to trough at the α bands in chemical difference spectra, using $\Delta\varepsilon = 20\,000\ 1 \cdot mol^{-1} \cdot cm^{-1}$. $\varepsilon_{960\,nm}$ was taken to be $10^5\ 1 \cdot mol^{-1} \cdot cm^{-1}$ [7].

RESULTS

The isolation procedure

A method by which a spectrally pure reaction center preparation of *R. viridis* can be obtained is described in detail in Methods and Materials and summarized in Fig. 1. It is estimated that about one-half of the *P*-960 applied to the first hydroxyapatite column is recovered in the final reaction center preparation. It was shown previously [2] and also observed here that treatment of the photosynthetic membranes

of this bacterium with sodium dodecyl sulfate causes the near infrared maximum of the antenna bacteriochlorophyll *b* to shift to shorter wavelengths in discrete spectral steps (cf. Fig. 1 in ref. 2). It was found to be important for obtaining a spectrally pure reaction center that all the antenna pigment was converted to the 810 nm spectral form before chromatography of the sodium dodecyl sulfate extract on hydroxyapatite. Most of the 810 nm form remains adhered to the column after elution of the reaction center, but a spectrally pure fraction of the antenna pigment-protein can be obtained by elution with higher concentrations (greater than 0.25 M) of sodium phosphate. A small portion of the 810 nm form frequently co-elutes with the reaction center,and can usually be removed by ammonium sulfate fractionation and/or rechromatography of the eluate.

The presence of a reducing agent during the time the *R. viridis* membranes are in contact with sodium dodecyl sulfate is required to prevent formation of the 685 nm pigment. If the 685 nm pigment is formed, its removal from the reaction center preparation is most difficult. Only after the reaction center is obtained in a spectrally pure form can the reducing agent be omitted from buffers with impunity. $Na_2S_2O_4$ was found to be the most satisfactory reducing agent investigated; sodium ascorbate was not adequate.

The ratio of detergent to bacteriochlorophyll *b* was higher than that used in the earlier preparations [1, 2]; the higher ratio enhanced chromatographic separation of the 810 nm form of the light-harvesting pigment from the reaction center complex. However, the solubilized membranes must not remain in the presence of the high concentration of sodium dodecyl sulfate any longer than necessary, and the material should be kept in the dark until it is adsorbed onto the first column and the excess detergent has been washed through.

Differential ammonium sulfate precipitation also improved the original purification procedure, but resulted in reduced recovery of the desired material. If maximum yield is important, the first 8 % (w/v) $(NH_4)_2SO_4$ precipitate can be applied to another hydroxyapatite column to recover additional reaction center complex.

Room temperature absorption spectra

The improved reaction center preparation has negligible absorption at 685 nm, but otherwise room temperature absorption spectra of reduced and oxidized samples (Fig. 2) appear similar to those reported earlier [1, 2]. In the near infrared the reduced material exhibits absorption maxima at 960 and 830 nm with a shoulder at approx. 790 nm. In the best preparations the ratio of $A_{830\,nm}/A_{960\,nm} = 2.5$. Photo- and chemical oxidation (Figs. 2 and 3a) cause similar changes in the spectral forms; the absorbance of *P*-960 virtually disappears, a new infrared band located at 1310 nm appears, *P*-830 shifts to shorter wavelength, and a shoulder at approx. 810 nm appears.

The Q_x band of bacteriochlorophyll *b* absorbs at 605 nm in the visible region of the spectrum (Fig. 2) and the major Soret band maximum of this pigment is at 375 nm (Fig. 6). There is no obvious band which may be attributed to carotenoid absorption, (however, see Discussion). Chemical analysis to confirm the presence of carotenoids in the improved preparation will be reported in the future. Cytochrome α and β bands are distinct in the wave-length range 510–560 nm (Fig. 2). Difference spectra (Fig. 3b) reveal the presence of two cytochromes (cytochromes *c*-553 and

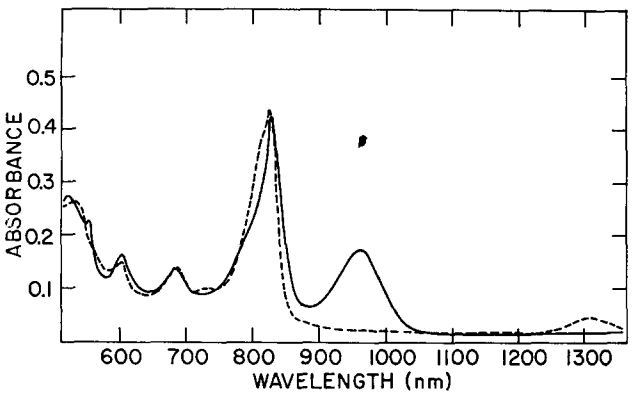

Fig. 2. Room temperature absorption spectra of the isolated (—) and photooxidized (- - -) *R. viridis* reaction center preparation in 50 mM Tris buffer, pH 8.0; Cary 14R spectrophotometer in IR 1 and IR 2 modes, respectively.

c-558); the cytochrome Soret bands are a composite with a maximum at approx. 415 nm (Fig. 6). Absorbance in the region of 540 nm may also be contributed by bacteriophaeophytin *b* (see Discussion).

The relative amounts of *P*-960 and the high (cytochrome *c*-558) and low (cytochrome *c*-553) potential cytochromes were calculated as described in Methods and Materials. A ratio for *P*-960: cytochrome *c*-558: cytochrome *c*-553 of 1 : 2 : 2 to 3 was found for various samples.

Low temperature absorbance spectra

At liquid nitrogen temperature *P*-960 is shifted to 987 nm (its position in the intact cells [9]) and the other near infrared bands are better resolved (Fig. 4). In the reduced state, *P*-830 is seen to consist of a major band at 832 nm and a shoulder at approx. 846 nm. When the complex is oxidized chemically, or photooxidized at room temperature and frozen immediately, the 987 nm band is bleached, *P*-830 shifts to

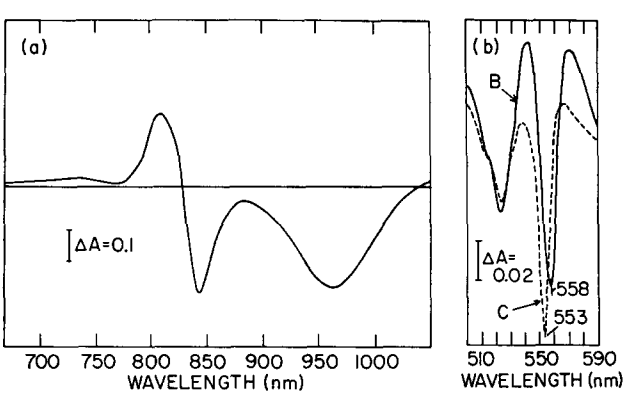

Fig. 3. Room temperature difference spectra of *R. viridis* reaction center preparation in 50 mM Tris buffer, pH 8.0. (a) Potassium ferricyanide vs. sodium ascorbate, (b) Ferricyanide vs. ascorbate (—) and ascorbate vs. sodium dithionite (- - - - -).

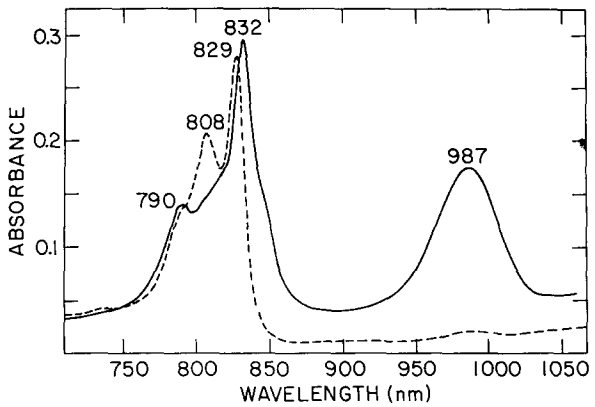

Fig. 4. 77 °K near infrared absorption spectrum of the reduced (—) and photooxidized (– – – –) *R. viridis* reaction center preparation in 55 % glycerol/22.5 mM Tris buffer, pH 8.0, Aminco DW-2, 0.2 cm path length. Oxidized sample was obtained by illuminating the preparation immediately prior to freezing it.

829 nm, the 846 nm absorbance is lost, and a new band appears at 808 nm (Fig. 4). The 77 °K difference spectrum (Fig. 5) shows the changes in *P*-830 more clearly: Both 846 and 837 nm absorbances are bleached, and two distinct bands appear at lower wavelengths, 828 and 808 nm. The distinct 790 nm shoulder is not significantly changed by oxidation.

In the visible region (Fig. 6) the major absorbance of the isolated complex when reduced with $Na_2S_2O_4$ is located at 420 nm at 77 °K. If the complex is photooxidized prior to freezing, this absorbance is shifted to 413 nm. The 607 nm band becomes partially bleached and skewed upon oxidation. Low temperature clearly resolves other features of these spectra in the region of carotenoid and cytochrome α and β bands. The absorbances and their probable assignments are listed in Table I and discussed below. The spectrum obtained when the sample containing excess

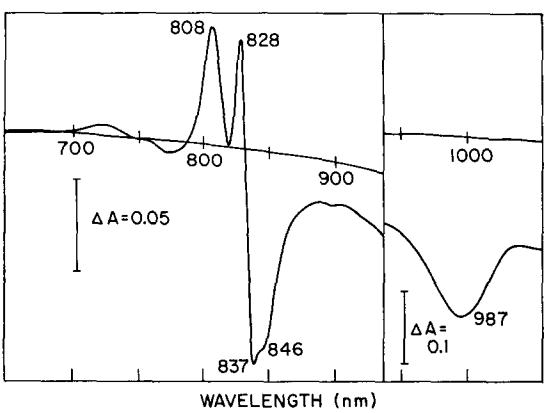

Fig. 5. 77 °K near infrared difference spectrum of photooxidized vs. reduced *R. viridis* reaction center preparation in 55 % glycerol/22.5 mM Tris buffer, pH 8.0; Aminco DW-2, 0.2 cm path length.

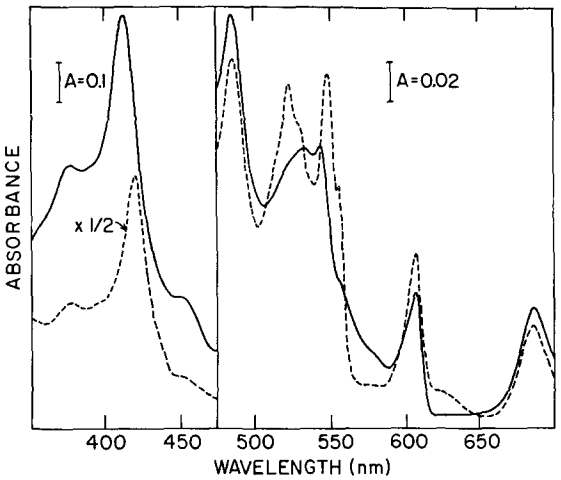

Fig. 6. 77 °K visible absorption spectrum of photooxidized (—) and $Na_2S_2O_4$-reduced (– – – –) *R. viridis* reaction centers in 55 % glycerol/22.5 mM Tris buffer, pH 8.0.

$Na_2S_2O_4$ (dashed trace in Fig. 6) is illuminated immediately before freezing is considered in the next section.

Light-induced absorbance changes

Kinetics of photooxidation. When the improved reaction center preparation is exposed to light at room temperature, after being dialyzed vs. 50 mM Tris buffer in the dark, *P*-960 and cytochrome *c*-558 become oxidized and *P*-830 shifts to shorter wavelengths. Photobleaching of the cytochrome *c*-558 α band and of *P*-960 occur with time constants too rapid to be measured with the Aminco DW-2 spectrophotometer. Recovery of *P*-960 in the dark is very slow; 30 min or more are required for full

TABLE I

PROBABLE ASSIGNMENTS OF COMPONENTS IN *R. VIRIDIS* REACTION CENTER PREPARATION TO SPECTRAL FORMS OBSERVED IN OXIDIZED AND REDUCED 77 °K SPECTRA

Wavelength (nm)		Probable assignment
Reduced	Oxidized	
375	375	bacteriochlorophyll *b* Soret
420	413	cytochrome Soret and bacteriochlorophyll *b* Soret
452(s)	452(s)	carotenoid?
485	485	carotenoid?
514(s)	–	carotenoid?
522	520(s)	cytochrome β band
530(s)	532	cytochrome β band?
544(s)	544	bacteriophaeophytin *b*
548	–	cytochrome α band
555	555(s)	cytochrome α band
607	607	bacteriochlorophyll *b* Q_x transition

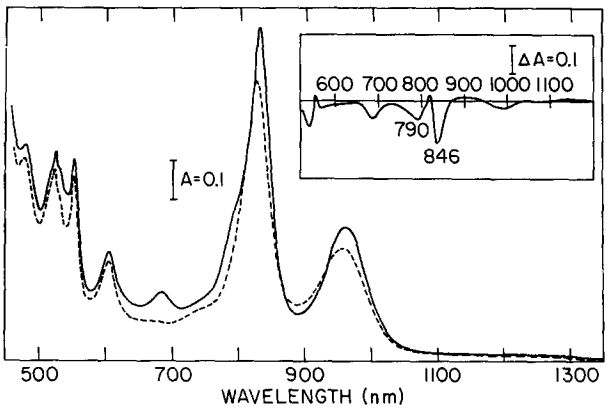

Fig. 7. Room temperature absorption spectra of *R. viridis* reaction center preparation in 50 mM Tris buffer, pH 8.0, reduced with excess $Na_2S_2O_4$ in the dark (—) and after 60 s exposure to strong white light (– – – –); Cary 14R. Inset: Difference spectrum after 10 s exposure to light.

recovery. The time required for recovery can be shortened to approx. 30 s by addition of redox components, *N*-methylphenazonium methyosulfate and $K_4(Fe(CN)_6)$. In dialyzed preparations *P*-960 also undergoes reversible photooxidation at 77 °K (Malkin, R., personal communication).

Effect of excess $Na_2S_2O_4$. In the presence of excess dithionite, $E_m \approx -0.6$ V, the primary electron acceptor, X, of the reaction center, $E_m = -0.06$ to -0.16 V [10], is reduced. Under these conditions, the spectrum of the preparation obtained in the IR2 mode of the Cary 14R spectrophotometer, i.e. upon strong illumination, is different from both the normal reduced and the photooxidized spectra (cp. Figs. 2 and 7). *P*-830 and *P*-960 are partially bleached and shifted to shorter wavelengths. The 1310 nm band of P^+-960 does not appear. After a 10 s exposure to strong white light the complex shows bleachings centered at 790, 685 and approx. 540 nm (inset, Fig. 7). These bleachings, relatived to the total absorption at those wavelengths, are greater than in other regions of the spectrum. Further illumination appears to superimpose additional bleachings of the *P*-960, *P*-830 and Q_x bacteriochlorophyll *b* bands upon the other absorbance losses. At liquid nitrogen temperature, the 540 nm bleaching is seen to be centered at 544 nm, and a significant bleaching is also observed in two Soret bands (Figs. 6 and 8).

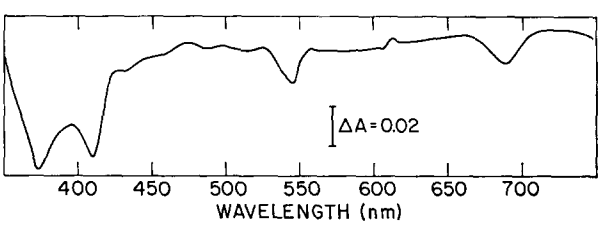

Fig. 8. 77 °K visible difference spectrum: $Na_2S_2O_4$-reduced *R. viridis* reaction centers exposed to strong white light before freezing vs. $Na_2S_2O_4$-reduced reaction centers; samples in 55 % glycerol/ 22.5 mM Tris buffer, pH 8.0; Aminco DW-2, 0.2 cm path length.

After prolonged exposure of the complex to illumination in the presence of excess $Na_2S_2O_4$, dialysis of the sample overnight in the dark vs. 50 mM Tris buffer restores the original spectral shape, but not the maximum absorbance of the preparation, indicating that a partial loss of active reaction center has occurred. All these absorbance losses, without the appearance of any significant new bands, are not amenable to a straightforward explanation of changes in the reaction center chromophores.

DISCUSSION

The results presented above indicate that sodium dodecyl sulfate treatment solubilizes two populations of bacteriochlorophyll *b*-containing particles, a reaction center complex and an antenna pigment-protein, from the photosynthetic membranes of *R. viridis* without significantly impairing photochemical activity of the former. The spectral shifts of the chromophores in both particles (*P*-985 → *P*-960 and *B*-1015 → *B*-810) resulting from sodium dodecyl sulfate treatment could be owing to replacement of some membrane lipids by detergent molecules or slight alterations in protein conformation, as well as to removal of the complexes from the stacked lamellae in which they are located in the native state. The shift of *P*-960 to *P*-985 at liquid N_2 temperature suggests that the original change may be reversible.

Unpublished observations in our laboratory on the lability of the antenna bacteriochlorophyll as *B*-810 and the relative stability of the reaction center complex, lead to the suggestion that the pigment absorbing at 685 nm arises mainly, and probably entirely, from the light-harvesting component. Pucheu et al. [11] have also equated the 685 nm-absorbing pigment in their green *R. viridis* subchromatophore fraction with light-harvesting bacteriochlorophyll *b*. Successful isolation of uncontaminated *R. viridis* reaction center requires its separation from antenna pigment-protein before chemical alteration (*B*-810 → 685 nm pigment) of the latter has occurred. High SDS concentrations permitting rapid membrane solubilization, and rapid column flow rates, were thus found to be advantageous.

The spectrum of the improved reaction center preparation is similar to that reported earlier [1] with three notable exceptions. The large 685 nm absorbance is almost totally absent from the new complex; the shoulder at approx. 450 nm is considerably attenuated, thus making a small band at 485 nm distinct (Figs. 2 and 6); and no approx. 930 nm component has been detected (cf. ref. 12). The Soret band of the 685 nm pigment probably contributed to absorption at approx. 450 nm in the earlier preparation [11], so that elimination of the altered pigment from the complex results in loss of both 685 and approx. 450 nm bands from the spectrum. It is possible that the approx. 930 nm component reported earlier [12] was an artifact of preparation or storage of the reaction center.

The clearly resolved features of the low temperature absorption and difference spectra of the improved complex make possible tentative assignments of spectral forms to particular chromophores or groups of chromophores. In the near infrared, bacteriochlorophyll *b* and probably bacteriophaeophytin *b* are the pigments responsible for the absorption bands observed. CD studies [12] of the earlier preparation indicated that exciton interactions most probably occur among two or more of the few bacteriochlorophyll *b* molecules in each reaction center. As Sauer [13] has

emphasized, the number of observable bands indicates only the minimum number of chromophores interacting in the exciton state, and it is not proper to assign individual bands to individual molecules because all energy levels are shared by the interacting pigment array. With this precaution in mind, we suggest that at least three bacteriochlorophyll *b* molecules are present in the reduced *R. viridis* reaction center, two or more of these form exciton states, and they contribute the 832, 846 and 987 nm spectral forms. As suggested by Philipson and Sauer [12], we attribute the 790 nm band to bacteriophaeophytin *b* although the presence and stoichiometry of bacteriophaeophytin *b* in the *R. viridis* reaction center has yet to be unequivocally demonstrated. The absorbance of the shoulder between the 790 and 832 nm bands (Fig. 4) could be contributed by another bacteriochlorophyll *b* form, a slight amount of contaminating antenna pigment-protein, a small percentage of oxidized reaction center, and/or a second bacteriophaeophytin *b* energy level. This last possibility would require exciton interaction between reaction center bacteriophaeophytin to cause band splitting, and would be consistent with the lower absorbance at 790 nm compared with the bacteriophaeophytin *a* band in bacteriochlorophyll *a*-containing reaction centers.

The 790 nm band is essentially unchanged upon oxidation, and three bacteriochlorophyll *b* bands continue to be present. P^+-960 absorbs at 1310 nm, and both *P*-830 bands are shifted to shorter wavelengths (Fig. 5). Because *P*-830 in oxidized preparations consists of two bands in addition to the 790 nm shoulder, the two or more bacteriochlorophyll *b* molecules responsible for *P*-830 may possibly continue to form an exciton state when the *R. viridis* reaction center is oxidized. This deduction contrasts with those based on data for bacteriochlorophyll *a*-containing complexes, where two absorption bands in *P*-800 have only been demonstrated in the reduced state [12, 14, 15], and the 'simple' CD and liquid N_2 spectra of oxidized samples in the 800 nm region have been attributed to fewer strongly interacting chromophores [13]. However, the zero field splitting parameters of several illuminated bacteriochlorophyll *a*-containing bacteria [10, 16] are consistent with paired chromophores in the oxidized material. The absorption band at 1310 nm attributed to P^+-960 is the lowest energy form reported for a biologically active chlorophyll. The equivalent band in bacteriochlorophyll *a*-containing reaction centers at 1250 nm has been attributed to oxidized bacteriochlorophyll *a* dimers on the basis of EPR line widths and electron spin density distribution [17] and comparisons with in vitro EPR data [18]; some EPR results are compatible with an oligomer with as many as four or five interacting chromophores [19]. In the oxidized *R. viridis* complex P^+-960 may also be a dimer or oligomer; however, the EPR linewidth data are inconclusive [10]. Confirming evidence of near infrared CD studies extending to 1310 nm or absorption spectra of the model system (bacteriochlorophyll $b)_2^+$ in that wavelength region is not yet available.

Assignments of the major bands in the visible region of the low temperature absorption spectrum (Table I) are based on the known properties of bacteriochlorophyll *b* and the cytochromes [1, 3, 6]. Other features in the 450–550 nm region may be owing to carotenoid and bacteriophaeophytin *b*. The former was identified as a component of the earlier reaction center preparation [1]; the presence of bacteriophaeophytin *b* is suggested by the present results. The pigment responsible for the 530 nm absorption, which becomes more prominent upon reaction center oxidation, is most uncertain. For example, oxidized bacteriochlorophyll *b* [20] or a component

of a split Q_x transition of bacteriophaeophytin *b* (in analogy with bacteriophaeophytin *a* in vivo [22]), could be responsible for this band.

The same two cytochromes present in the original preparation of the *R. viridis* reaction center [1, 2] are present in the improved preparation. However, the excess of low potential cytochrome has been reduced to 2-fold in the latter, so that the two cytochromes are present in nearly equal amounts. After dialysis of the preparation the high potential cytochrome, cytochrome *c*-558, is reduced but the low potential cytochrome, cytochrome *c*-553, is oxidized. Thus only cytochrome *c*-558 is oxidized upon photooxidation of the reaction center (cf. ref. 3).

The very slow reduction of P^+-960 after photooxidation of the reaction center preparation in the absence of any added reductant is most probably due to a break in the normal route of cyclic electron flow from X^- to P^+-960 observed in vivo (cf. ref. 10). Addition of *N*-methylphenazonium methyosulfate accelerates the recovery of P^+-960 by bypassing the gaps in the pathway of cyclic electron flow [3].

Illumination of the complex at room temperature in the presence of excess $Na_2S_2O_4$ which reduces the primary electron acceptor, X, may reveal spectral properties of an intermediate stage in the formation of (P^+X^-), e.g. state P^F proposed by Parson et al. [23]. However one must also consider that an abnormal state of the reaction center, such as state P^R [23], could be generated in this situation. *R. viridis* reaction centers treated with dithionite in this way lose their 544 and 790 nm absorption bands, which are not so altered by normal photo- or chemical oxidation or by addition of $Na_2S_2O_4$ in the dark (Fig. 7). Since the 544 nm absorption is likely to be due to bacteriophaeophytin *b*, the observed changes would imply that (i) 790 nm may also be a bacteriophaeophytin *b* band and (ii) bacteriophaeophytin *b* in the reaction center is altered upon formation of the intermediate or abnormal state. $(\text{Bacteriophaeophytin } a)^-$ has been proposed as an intermediate during reaction center oxidation in other photosynthetic bacteria [16, 21, 24], but more evidence is required before the analogous assignment can be extended to the *R. viridis* reaction center. Nonetheless, it is apparent from data now available that this bacteriochlorophyll *b*-containing organism has a reaction center complex similar to those of other photosynthetic bacteria.

ADDENDUM

Recently Pucheu et al. ((1976) Arch. Microbiol. 109, 301–305) have reported isolation of *R. viridis* reaction centers free of 685 nm-absorbing pigment by a method employing the detergent lauryl dimethyl amine oxide.

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