Biochimica et Biophysica Acta, 461 (1977) 327–338

BBA 47341

PURIFICATION AND PHOTOCHEMICAL PROPERTIES OF REACTION CENTERS OF *CHROMATIUM VINOSUM*

EVIDENCE FOR THE PHOTOREDUCTION OF A NAPHTHOQUINONE

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(Received January 21st, 1977)

SUMMARY

(1) Reaction center particles were prepared from *Chromatium vinosum* by use of detergent treatment, gradient centrifugation and extraction with 90 % acetone. The purified particles did not contain light-harvesting bacteriochlorophyll and only a small amount of carotenoid (spirilloxanthin). In addition, they contained cytochromes c_{555} and c_{552}. The major part of the cytochromes was removed by an additional purification step by means of hydroxyapatite column chromatography.

(2) Light-induced difference spectra were measured at room temperature and at 100 K. The most prominent bands in the low temperature difference spectrum were the bacteriochlorophyll bands near 905 nm (*P*-870), around 800 nm (*P*-800) and around 600 nm. The band around 600 nm was resolved into two components, presumably reflecting a bleaching of the *P*-870 component, located at 612 nm, and a shift of the *P*-800 component, located at 588 nm, respectively.

(3) Absorbance difference spectra showed that in reaction centers of *C. vinosum* no photoreduction of ubiquinone occurred, either at room temperature or at 100 K. From the measurement of light-induced absorbance changes in the presence of an external electron donor (*N*-methylphenazonium methosulphate plus ascorbate) evidence was obtained that a naphthoquinone was photoreduced to the semiquinone anion. These results give support to the hypothesis that vitamin K_2 (menaquinone) may act as the primary electron acceptor (X) in *C. vinosum*. The reduction of X was accompanied by an absorbance change due to bacteriopheophytin.

Abbreviations: *B*-800, *B*-820, bacteriochlorophyll types absorbing near 800 and 820 nm, respectively; *P*-800, *P*-870, reaction center bacteriochlorophylls absorbing near 800 nm and 870 nm, respectively; PMS, *N*-methylphenazonium methosulphate; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylene diamine; DAD, 2,3,5,6-tetramethyl-*p*-phenylene diamine; LDAO, lauryl dimethylamine oxide; AUT-particle: obtained by treatment with an alkaline solution urea and Triton X-100 [5, 18].

INTRODUCTION

Isolation of reaction center particles from photosynthetic bacteria by use of a variety of detergents was reported for several species of Athiorhodaceae [1–5]. Especially for *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum*, the properties and components of the reaction center now seem to be well characterized (for a review: see ref. 6). Several lines of evidence support the hypothesis that ubiquinone, possibly in a quinone-iron protein complex is the primary acceptor (X) in reaction centers of these species [6–12].

In contrast, relatively little is known about the reaction center components of other species of photosynthetic bacteria. Measurement of light-induced absorbance changes [13] and extraction experiments [14, 15] with preparations from the Thiorhodacea *Chromatium vinosum* indicated that in this species ubiquinone is not involved in the primary reaction.

In this paper we describe the light-induced absorbance changes of various components of reaction center particles of *C. vinosum*, both at room and at low temperature (100 K). Evidence will be given that a naphthoquinone, presumably menaquinone (vitamin K_2), which compound was reported to be present in photoactive reaction centers of *C. vinosum* from which ubiquinone had been removed [15], is photoreduced to the semiquinone anion. This compound may act as electron acceptor in *C. vinosum* in a similar way as ubiquinone in other species of purple bacteria.

METHODS

Chromatium vinosum was grown at 30 °C in a medium after Hendley [16]. After 5 days of growth, the cells were harvested by centrifugation at $5000 \times g$ and washed once in 0.05 M Tris buffer (pH 8.0). Chromatophores were obtained by sonication with a Branson type S125 sonifier for 10 min at 8 A followed by centrifugation at 4 °C for 30 min at $20\,000 \times g$ in a Sorvall RC-5 centrifuge to remove cell debris. The chromatophore fraction was precipitated by centrifugation for 1 h at $144\,000 \times g$ in a Beckman Model L5-65 ultracentrifuge.

"AUT-particles" and reaction center preparations were obtained as described elsewhere [17] by treatment with an alkaline solution of urea and Triton X-100 [5, 18]. The AUT-particles contained, in addition to the reaction center particles, a variable but small amount (5–8 molecules per reaction center) of bulk bacteriochlorophyll, which could be extracted with cold, 90 % acetone. A similar extraction method was used by Kennel and Kamen [19], and, more recently, by Tiede et al. [20]. In the preparation thus obtained, which will be referred to as "type I-reaction centers", a substantial amount of cytochromes was present, part of which could be removed by chromatography on a hydroxyapatite column, as will be described in Results. The resulting preparation will be referred to as "type II-reaction centers".

Absorbance spectra, either at room temperature or at 100 K, were recorded on a Cary model 14 R spectrophotometer. Light-induced absorbance changes were measured with a single-beam or with a split-beam apparatus as described earlier [9]. Measurements at 100 K below 320 nm were performed with a split-beam apparatus equipped with a Dewar vessel provided with quartz windows. In order to obtain clear samples upon cooling, the reaction centers were mixed with sucrose (1.0 M) and

glycerol (55 %, v/v). The actinic light for absorbance difference measurements was provided by either a Xenon flash lamp (duration of the flash 8 μs at one-third of the peak) or a quartz-iodine lamp. Suitable interference and absorbance filters were used to select the wavelength of the actinic light and to protect the photomultiplier from stray actinic light. In some experiments a signal averager (Nuclear Chicago, Model 7100 Data Retrieval Computer) was use to improve the signal-to-noise ratio.

RESULTS

Purification and spectral properties of reaction centers

Chemically induced absorbance difference spectra showed that both the high potential cytochrome c_{555} [21] and the low potential cytochrome c_{552} [21] were present in type I-reaction centers. The isolation procedure probably yielded a reaction center particle containing the cytochrome complex described by Kennel and Kamen [19]. The major part of the cytochromes could be removed by passing the preparation over a hydroxyapatite column, equilibrated with 0.01 M phosphate buffer (pH 7.2) containing 0.2 M NaCl, and eluting the reaction centers with 0.1 M phosphate buffer (pH 7.2) containing 0.2 M NaCl. However, in all preparations a small amount of cytochrome remained (see below) which could not be removed by differential centrifugation either.

The stability of the preparations appeared to decrease with increasing degree of purification: AUT-particles were stable for several months at 4 °C, but type I and especially type II-reaction centers became inactive within a few days. For this reason, a part of the experiments to be described here were carried out with AUT-particles.

The absorbance spectrum of the type II-reaction centers is shown in Fig. 1. The absorbance band at 756 nm can be ascribed to bacteriopheophytin; bacteriochlorophyll shows maxima at 598, 799 (*P*-800) and 865 nm (*P*-870). The exact position

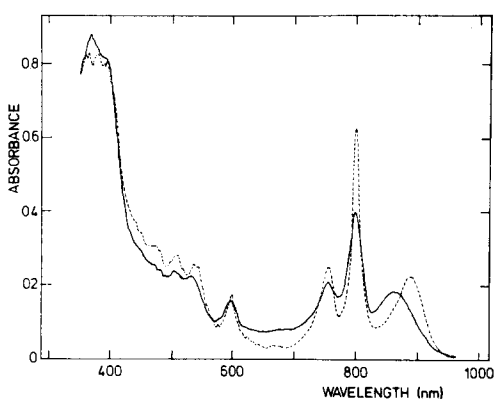

Fig. 1. Absorption spectrum of type II reaction centers (see Methods) of *C. vinosum*. —, room temperature, – – –, 100 K. Optical pathlength: 2 mm. The reaction centers were suspended in a sucrose containing buffer/glycerol mixture. With type I-reaction centers, the spectrum obtained was the same, except for the location of the long wavelength bacteriochlorophyll band. Typically, this band was located at 875 nm for the type I-reaction centers and at 865 nm for the type II-reaction centers at room temperature; at 100 K, the peak wavelengths were 900 and 890 nm respectively.

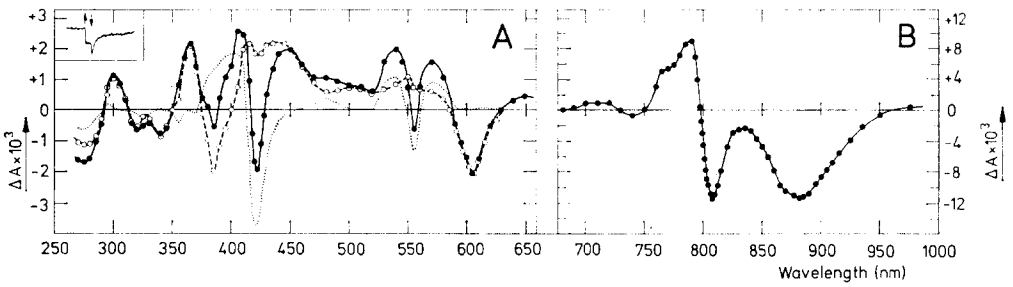

Fig. 2. Spectrum of light-induced absorbance changes (a, ●–●) observed at room temperature with AUT-particles after illumination for 5 s with non-saturating actinic light of 600 nm (intensity: 3.4 mW/cm²) for the regions 270–550 nm and 650–1000 nm and actinic light of 800 nm (intensity 2.8 mW/cm²) for the region 550–650 nm. The spectra were normalized for equal absorption change at 883 nm. The absorption at 883 nm was about 0.1. Optical pathlength: 1 mm. Spectrum of the rapid phase of the light-off kinetics (b, ○–○) (see insert). This spectrum was normalized to spectrum a at 600 nm. Dotted line: spectrum a minus spectrum b. Insert: kinetics of the absorbance change at 420 nm. Actinic light (600 nm) was switched on and off as indicated by the upward and downward pointing arrows. A: spectra in the 250–650 nm range. B: spectra in the 650–1000 nm range.

of the long wavelength band depended upon the preparation and seemed to be correlated with its stability: less purified preparations, in which this band was located at longer wavelengths, appeared to be more stable. The same phenomenon was observed by Lin and Thornber [22] with reaction centers prepared from the same species in a different way.

At low temperature (100 K) the absorbance band of *P*-870 was shifted towards a longer wavelength by about 25 nm and most of the other peaks were sharpened compared with the room temperature spectrum (Fig. 1, dotted line), as was also observed with reaction centers of *Rps. sphaeroides* [9]. The carotenoid content of this preparation is relatively low, as judged from the absorbance in the region 450–550 nm.

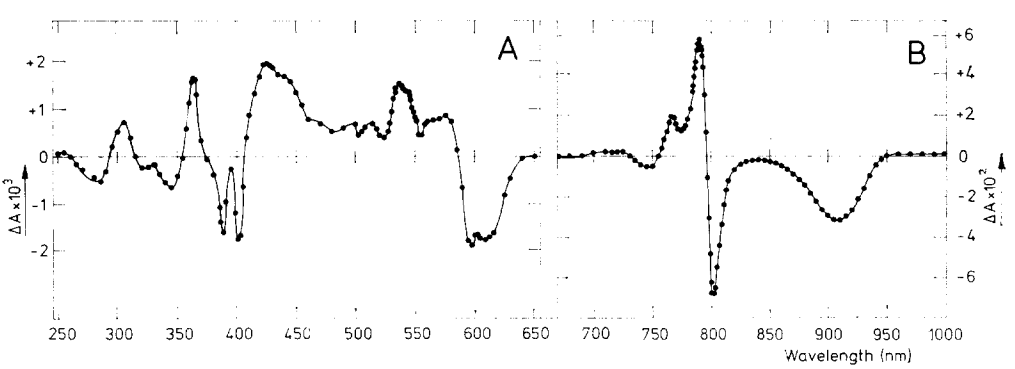

Fig. 3. Light minus dark absorption difference spectrum of AUT-particles at 100 K. Illumination: 5 s with non-saturating actinic light of 880 nm (intensity 2.3 mW/cm²) for the region 250–630 nm and actinic light of 420 nm (intensity 3.7 mW/cm²) for the region 580–1000 nm. Both regions of the spectrum were normalized at 610 nm. The absorbance at 883 nm was about 0.3. Optical pathlength: 1 mm.

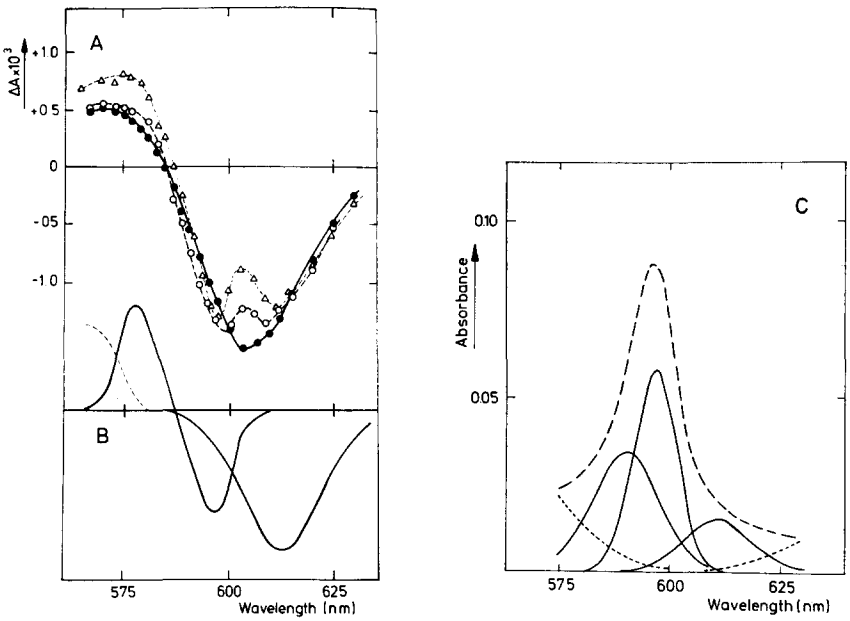

Fig. 4. (A) Light-induced difference spectrum of AUT-particles measured at different temperatures. ●–●: 295 K; ○– – –○: 210 K; △– – –△: 100 K. Further conditions as for Fig. 3. (B) Analysis of the difference spectrum of Fig. 4A measured at 100 K. The spectrum is resolved in a bleaching of a band located at 612 nm and a shift of a second band centered at 588 nm. (C) Computer simulation of the absorption spectrum of type II-reaction centers. The dashed line represents the measured spectrum.

Figs. 2A and 2B (solid line) show the light-induced absorbance difference spectrum of AUT-particles measured at room temperature. Among the most prominent features are the bleaching at 883 nm and at 605 nm and a band shift* centered around 797 nm. This spectrum is similar to the one obtained by Ke et al. [13] with subchromatophore particles from *C. vinosum*, except for the shoulder in our spectrum at 765 nm, which can be attributed to bacteriopheophytin [8, 9, 23]. With more purified reaction centers (type I and type II), we obtained a spectrum very similar to the one of Fig. 2B; the only significant difference was the position of the long wavelength peak due to the bleaching of the *P*-870 band, which was shifted by about 10–15 nm to shorter wavelength in these preparations.

In the difference spectrum measured at 100 K (Figs. 3A and B), the *P*-870 band is shifted to about 905 nm and the peaks of the *P*-800 band are sharpened. The positive band at 765 nm, due to bacteriopheophytin, can be observed more clearly now. The spectrum obtained with type I-reaction centers was identical to that of Fig. 3, except for the position of the *P*-870 band in the infrared. In contrast to the results obtained with reaction centers from *Rps. sphaeroides* [9] the ultraviolet region (250–350 nm) of the difference spectrum (Fig. 3A) shows that absorbance changes due to the reduction of ubiquinone to the semiquinone anion are absent in reaction centers of *C. vinosum*, indicating that ubiquinone is not the primary electron acceptor in this species.

* According to recent results of dichroism studies [23] this "band shift" represents a bleaching of the dimer and a concomitant appearance of a monomer band.

Further differences with the spectrum obtained with *Rps. sphaeroides* reaction centers at low temperature are the presence of double peaks around 600 and 400 nm. To obtain some information about the origin of these peaks the absorbance difference spectrum was measured at different temperatures. Fig. 4A shows the spectrum of AUT-particles in the region 560–630 nm at 100 K, at 210 K and at room temperature. At temperatures below 220 K, the band is resolved in two peaks. Similar difference spectra were obtained with type I-reaction centers and with chromatophores, either with continuous illumination or after a short flash.

It has been proposed earlier that the absorbance band near 600 nm in *Rps. sphaeroides* reaction centers consists of two bands: one due to *P*-870 and one due to *P*-800 [5, 24, 25]. Fig. 4B shows that it is possible to interprete the difference spectrum as a bleaching of a band at 612 nm, which may be attributed to *P*-870, and a blue shift of a band at 588 nm. A band near 612 nm is also obtained when the low temperature absorption spectrum of type II reaction centers is analyzed into Gaussian components (Fig. 4C). By such an analysis, it was also possible to obtain a band near 588 nm; in that case, however, an additional band near 595 nm was generated, which is apparently not affected by illumination. In this connection, it should be noted that the 800 nm band in *Rps. sphaeroides* reaction centers also seems to consist of more than one component [3, 25].

Difference spectra in the region 380–420 nm measured at 100 K, 210 K and room temperature in the presence of 60 μM potassium ferricyanide are shown in Fig. 5. The ferricyanide was added to keep the cytochrome c_{555} in the oxidized state; without its addition, photooxidation of this cytochrome became apparent at temperatures above 160 K. It should be noted that the spectra are not exactly comparable because of the reoxidation of the primary acceptor by ferricyanide at room temperature (see below). Nevertheless, the results show that the band at 402 nm increases strongly at lower temperatures. The origin of this band is not clear yet.

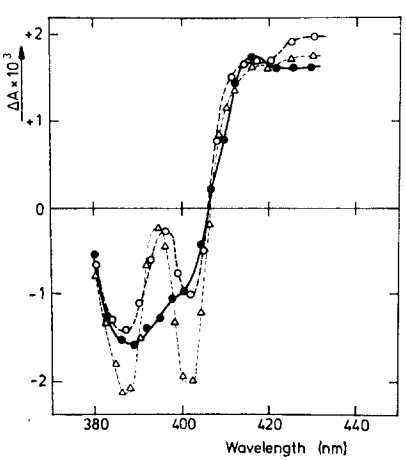

Fig. 5. Light-induced differences spectrum of AUT-particles measured at different temperatures. ●–●: 295 K; ○– – –○: 210 K; △– – –△: 100 K. Addition: 60 μM potassium ferricyanide. Further conditions as for Fig. 3.

Photooxidation of cytochromes

The bands at 422 and 555 nm in the difference spectrum of Fig. 2 indicate the photooxidation of cytochrome c_{555} in AUT-particles. The amount of cytochrome oxidized was approximately equal to that of P^+-870. The kinetics of the decay of the absorbance changes in the region 270–580 nm (insert Fig. 2A) were clearly biphasic. In the spectrum of the rapid phase of the decay (Fig. 2A, dashed line), no absorbance changes of the cytochrome were observed; apparently this spectrum reflects the back reaction of oxidized *P*-870 with reduced acceptor mainly. The difference between the two spectra clearly shows the α and γ bands of the cytochrome (Fig. 2A, dotted line). At temperatures below 160 K (Fig. 3), no photooxidation of cytochrome c_{555} occurred. Similar results were obtained with type I-reaction centers, but in type II-reaction centers, absorbance changes due to cytochromes were nearly absent also at room temperature.

In the presence of 1 mM ascorbate, which reduced the low potential cytochrome c_{552}, reversible light-induced absorbance changes due to the photooxidation of this cytochrome were observed. Fig. 6 shows the difference spectrum. In the 360–650 nm region, the spectrum is similar to that reported by Tiede et al. [20], but the ratio $\Delta A_{423}/\Delta A_{552}$ is considerably higher, presumably due to better spectral resolution. The maximal amount of cytochrome that was oxidized varied between 1 and 2 molecules per reaction center. The cytochrome was also oxidized at 100 K. The amount was

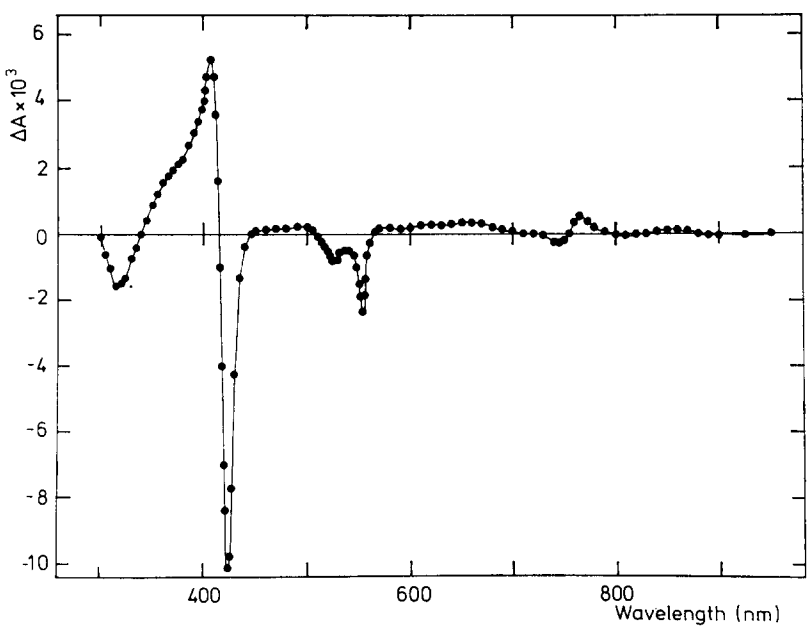

Fig. 6. Absorbance difference spectrum measured at room temperature with AUT-particles in the presence of 1 mM ascorbate. Illumination: 5 s with infrared actinic light (Schott RG 780 and Kodak IR 455-filters; intensity 2.3 mW/cm^2) for the ultraviolet and the visible part of the spectrum and with actinic light of 595 nm (Schott AL 595 interference filter; intensity 3.4 mW/cm^2) for the infrared part of the spectrum. Both parts of the spectrum were matched at 422 nm. Dark time between the illuminations: 2 min. *P*-870 concentration: 1.0 μM. Optical pathlength 1 mm.

only 0.8 molecule per reaction center at this temperature and the absorbance changes were irreversible.

Photoreduction of the "intermediary" and "primary" electron acceptors

The infrared region of the spectrum in Fig. 6 shows that the absorbance changes around 765 nm, ascribed to bacteriopheophytin, can also be observed in the presence of ascorbate. Figs. 7 and 8 show the results obtained with a higher time resolution and higher intensities of illumination. The kinetics in the near infrared region (Fig. 7A) suggested that two different components accounted for the absorbance changes observed. One of these components (a) was characterized by a very rapid change at the onset of actinic light and by a slow decay after the light was turned off. The rise kinetics

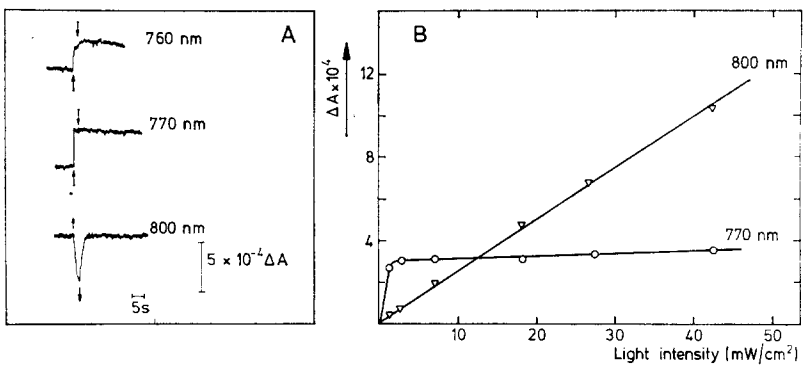

Fig. 7. (A) Kinetics of light-induced absorbance changes at 760, 770 and 800 nm of type I-reaction centers in the presence of 5 mM ascorbate upon illumination with blue actinic light (Corning CS 4-96, CS 4-97 and a Balzers Calflex filter; intensity 18 mW/cm²). Illumination time: 2 s. P 870-concentration 1 μM. (B) Amplitude of the absorbance changes at 770 and 800 nm as a function of the intensity of actinic light. Further conditions as for Fig. 7A.

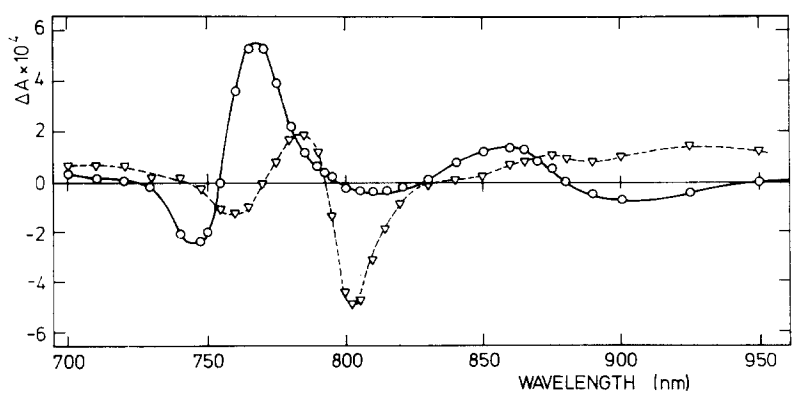

Fig. 8. Absorbance difference spectra of the two components observed in type I-reaction centers upon illumination in the presence of 5 mM ascorbate. (a) Solid line: difference spectrum measured 2 s after the actinic light was switched off. (b) Dashed line: difference spectrum of the rapidly decaying signal upon darkening. Conditions as for Fig. 7A.

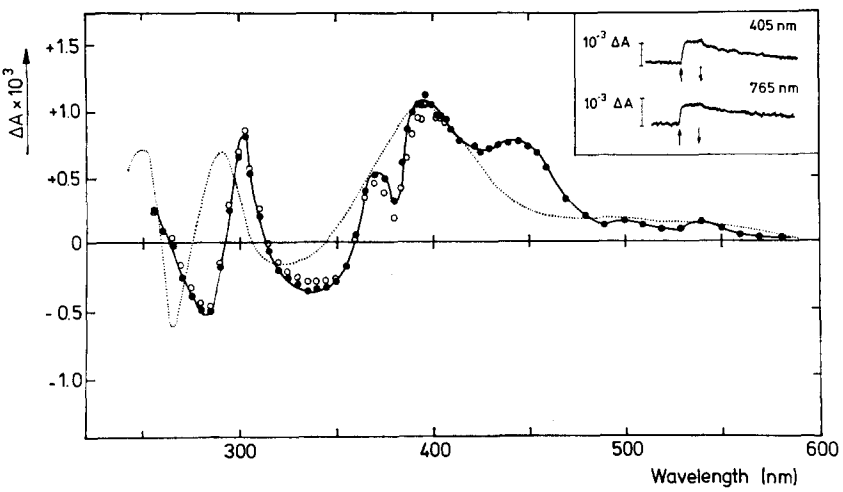

Fig. 9. ●–●, Light-induced difference spectrum of type I-reaction centers in the presence of 150 μM PMS and 1 mM ascorbate. Illumination: 5 s with infrared actinic light of 880 nm (intensity: 2.4 mW/cm^2). Dark time between the illuminations was 60 s. The open symbols refer to the values after correction for absorbance changes due to the oxidation of the added electron donors. This correction was obtained empirically by measurement of absorbance changes induced by the addition of small amounts H_2O_2 to a solution of PMS and ascorbate in Tris buffer (pH 8.0). The spectrum measured in the near infrared region in these conditions was identical to the spectrum of Fig. 8 (solid line). The insert shows the kinetics of the absorbance changes at 400 nm and 765 nm upon illumination for 5 s. *P*-870 concentration: 1.0 μM. Optical pathlength: 1 mm. ....., in vitro difference spectrum for the reduction of vitamin K_1 to the semiquinone anion in methanol (as measured by Dr. E. J. Land, private communication), calculated for a concentration equal to that of *P*-870.

of the other component (b) were significantly slower, but the decay was complete within 2 s. Component a was saturated at relatively low intensity (like the oxidation of cytochrome c_{552}), whereas component b could not be saturated (Fig. 7B). The spectrum of component a (Fig. 8, solid line) shows the absorbance changes around 765 nm, ascribed to bacteriopheophytin, and, in addition, a band shift centered at 880 nm, which probably represents an electrochromic shift of the *P*-870 band. The spectrum of component b is quite similar to the spectrum obtained with preparations in which the primary acceptor (X) had been reduced chemically before illumination [20, 26, 27]; this spectrum has been ascribed to the photoreduction of the long-wavelength bacteriopheophytin, acting as the "intermediary" acceptor (I).

Absorbance changes of the primary acceptor (X) may be expected to occur also in the presence of ascorbate, but the relatively large cytochrome changes will interfere with the detection of this compound. Therefore a more efficient donor system, PMS plus ascorbate, was used. The difference spectrum obtained with reaction centers in the presence of 150 μM PMS and 1 mM ascorbate is shown in Fig. 9 (solid line). Apart from a shift by about 15 nm to longer wavelengths the spectrum is quite similar to the in vitro difference spectrum for the reduction of vitamin K_1 to the semiquinone anion (Fig. 9, dotted line), measured by Land (E. J. Land, private communication). This indicates that the primary acceptor in *C. vinosum* is a naphthoquinone, presumably a vitamin K. The same results were obtained if TMPD (0.2 mM) or DAD

(0.4 mM) was used as an electron donor instead of PMS. These compounds, however, turned out to be less efficient donors: upon illumination, the cytochrome was oxidized first and its rereduction occurred in the light with a half time of approximately 1 s. In addition to the reduction of vitamin K, we observed absorbance changes of bacteriopheophytin in the infrared region with a spectrum identical to that of Fig. 8 (solid line). The trough at 380 nm might also be due to bacteriopheophytin. The origin of the absorbance increase around 450 nm is unclear yet. With PMS and ascorbate, the kinetics of the absorbance changes were the same throughout the whole spectrum; Fig. 9 (insert) shows that the kinetics for the reduction of the acceptor (measured at 400 nm) and for the absorbance change of the bacteriopheophytin (measured at 770 nm) were also identical. The dependence on the intensity of actinic illumination was likewise the same for the absorbance changes of both the acceptor and bacteriopheophytin.

The difference spectrum of the primary acceptor in *Rps. sphaeroides* was obtained by Slooten [7] by comparing the absorbance changes in the presence and absence of ferricyanide. With preparations of *C. vinosum*, this method usually fails because the difference between the absorbance difference spectra measured in the absence and presence of ferricyanide (which presumably reoxidizes the primary acceptor rapidly) reflects also the (relatively large) absorbance changes due to cytochrome in addition to the difference spectrum of the primary electron acceptor. However, in type II-reaction centers, cytochromes were nearly absent and their contribution to the difference spectrum was consequently much lower. After correction for the residual cytochrome changes (representing the oxidation of about 1 cytochrome molecule per 15 reaction centers) subtraction of the difference spectra measured in the region 380–620 nm with these particles gave a spectrum which was similar to that of Fig. 9, and likewise showed a maximum near 400 nm.

DISCUSSION

Several characteristics of the purified reaction centers of the Thiorhodacea *Chromatium vinosum* are similar to those of preparations of Athiorhodaceae. The compositions of reaction center pigments (bacteriochlorophyll and bacteriopheophytin) are much alike and the preparations are essentially devoid of light-harvesting pigments, except for a small amount of carotenoid. The absorbance bands at 471, 502 and 534 nm indicate that spirilloxanthin [28] might be the main carotenoid present in our preparation. In chromatophores, the major carotenoid peaks are found at 460, 485 and 515 nm, indicating the presence of rhodopin [28]. If we assume that the extinction coefficients of the carotenoid at 502 nm and of *P*-870 at the near-infrared maximum are 140 [28] and 130 $mM^{-1} \cdot cm^{-1}$ [29], respectively, the preparation shown in Fig. 1 contained about 0.3 molecule of carotenoid per reaction center.

Type I-reaction centers, like the preparation obtained by Tiede et al. [20] contained both the high potential cytochrome c_{555} and the low potential cytochrome c_{552}. The temperature dependence of the cytochrome reactions is in agreement with data reported previously for whole cells [30]: at 100 K, cytochrome c_{552} is photooxidized irreversibly, whereas the oxidation of cytochrome c_{555} occurs only at temperatures above 160 K. This indicates that in this preparation the interaction between the *c*-type cytochrome and the reaction center is still largely intact. The major part of

the cytochromes could be removed, however, by an additional purification step using hydroxyapatite.

The reduction of the primary acceptor (X) is accompanied by infrared absorbance changes with a maximum at 765–770 nm, ascribed to bacteriopheophytin [8, 9, 23], irrespective whether the positive charge resides on *P*-870 (Figs. 2 and 3), on cytochrome c_{552} (Fig. 6) or on an external electron donor like PMS. The maximal amplitude of the absorbance change appeared to be the same in the last two cases and the dependency on the intensity of actinic light was identical for the absorbance changes of bacteriopheophytin and the primary acceptor. These correlations suggest that the absorbance changes reflect an electrostatic interaction with the reduced acceptor (X).

In contrast to the results obtained with reaction centers from *Rps. sphaeroides* [5, 9] and *Rhodospirillum rubrum* [24], the date presented in this paper clearly show that photoreduction of ubiquinone to the semiquinone anion does not occur in reaction centers of *C. vinosum*, neither at low nor at room temperature. The same conclusion was obtained by Ke et al. [13] from measurements with subchromatophore particles from the same species. It has been proposed that menaquinone (vitamin K_2), which compound was reported to be present in photoactive reaction centers of *C. vinosum* from which ubiquinone had been extracted [15], may act as the primary acceptor in this bacterium. Direct support for this hypothesis is given by the difference spectrum measured in the presence of external electron donors (Fig. 9). There is a good resemblance of this spectrum with the in vitro spectrum for the reduction of a naphthoquinone (vitamin K_1) to the semiquinone anion. Since the spectra of the oxidized forms of vitamin K_1 and vitamin K_2 are almost identical [31], it may be assumed that the spectra for the semiquinones are similar also. The major differences between the spectrum measured with reaction centers and the in vitro spectrum are a shift of the first one to a somewhat longer wavelength and the presence of an additional band around 450 nm. The shift of the semiquinone spectrum seems to be a common phenomenon: it was also observed for the acceptor in *Rps. sphaeroides* [7] and for the acceptor of Photosystem II in chloroplasts [32] and may be due to the presence of detergent [33]. The origin of the absorbance increase around 450 nm has not yet been elucidated. The band cannot be due to the oxidation of PMS. Moreover, essentially the same spectrum was obtained with other electron donors (TMPD or DAD instead of PMS). The extinction coefficient that was calculated for the measured absorbance change at 400 nm (10.8 $mM^{-1} \cdot cm^{-1}$, if it is assumed that 1 molecule of semiquinone is formed per reaction center) agrees well with the extinction coefficient for the formation of the vitamin K_1 semiquinone anion (10.2 $mM^{-1} \cdot cm^{-1}$, ref. 34 and E. J. Land, private communication).

ACKNOWLEDGEMENTS

We are very grateful to Dr. E. J. Land (Manchester) for providing us with the in vitro spectrum for the reduction of vitamin K_1 to the semiquinone anion prior to publication. Thanks are due to Mrs. M. F. Onderwater for skilled technical assistance and to Mr. D. Los for carrying out the computer calculations. DAD was a kind gift of Dr. A. Trebst (Bochum). This investigation was supported by the Netherlands Foundation for Chemical Research (SON), financed by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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