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# PHOTOREDUCTION OF MENAQUINONE IN THE REACTION CENTER OF THE GREEN PHOTOSYNTHETIC BACTERIUM *CHLOROFLEXUS AURANTIACUS*

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**Photochemically active reaction centers were isolated from the facultatively aerobic gliding green bacterium *Chloroflexus aurantiacus*. The absorption difference spectrum, obtained after a flash, reflected the oxidation of P-865, the primary donor, and agreed with that observed in a purified membrane preparation from the same organism (Bruce, B.D., Fuller, R.C. and Blankenship, R.E. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 6532–6536). By analysis of the kinetics in the presence of reduced *N*-methylphenazonium methosulfate to prevent accumulation of oxidized P-865, the absorption difference spectrum of an electron acceptor was obtained. The electron acceptor was identified as menaquinone (vitamin K-2), which is reduced to the semiquinone anion in a stoichiometry of approximately one molecule per reaction center. Reduction of menaquinone was accompanied by changes in pigment absorption in the infrared region. Our results indicate that the electron-acceptor chain of *C. aurantiacus* is very similar to that of purple bacteria.**

## Introduction

There are indications that the gliding green bacteria (Chloroflexaceae) occupy an intermediate position between the 'classical' green bacteria (Chlorobiaceae) and the purple photosynthetic bacteria. The main light-harvesting pigment of Chloroflexaceae is BChl *c*, which is contained in chlorosomes as in Chlorobiaceae [1–4]. On the other hand, the spectral properties of the reaction center and of the primary electron donor are clearly reminiscent of those of purple bacteria [1,5,6].

A major difference between Chlorobiaceae and purple bacteria is in the organization of the electron-acceptor chain. In the first group of organisms this chain contains iron-sulfur centers as electron acceptors [7], whereas in purple bacteria the secondary electron acceptors are ubi- and menaquinones (for a review, see Ref. 8). In the present paper we present a study of the secondary electron acceptor of *Chloroflexus aurantiacus*. By means of absorption difference spectroscopy it will be shown that this acceptor is menaquinone (vitamin K-2). This strongly supports the notion that the photosynthetic electron-transport chain of *Chloroflexus* resembles that of purple bacteria.

## Materials and Methods

*C. aurantiacus*, strain J-10-fl, was grown anaerobically at 50°C and 2000 lx in a modified medium of Pierson and Castenholz as described in Ref. 3. Cultures were continuously flushed with $N_2$. Purified reaction centers were prepared by a modification of a method developed by Pierson and Thornber (personal communication). Broken cells were prepared by sonication in Tris buffer (10 mM, pH 8.0) and centrifuged to remove intact

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; P-865, primary electron donor.

cells and larger fragments. The supernatant was incubated with 3% (v/v) lauryldimethylamine *N*-oxide for 60 min at room temperature and afterwards dialyzed overnight against Tris buffer, containing 0.1% (v/v) lauryldimethylamine *N*-oxide. Centrifugation during 16 h at 200000 × *g* on a 0.1–1.0 M sucrose gradient yielded a fraction enriched in reaction center in a band around 0.3 M sucrose. This fraction was applied to a DEAE-52 column, washed with Tris-lauryldimethylamine *N*-oxide buffer (10 mM Tris, pH 8.0, 0.025% (v/v) lauryldimethylamine *N*-oxide) and the reaction center was eluted with a linear gradient of 0–0.3 M NaCl in Tris-lauryldimethylamine *N*-oxide buffer. If necessary the column step was repeated. The final product was dialyzed against Tris-lauryldimethylamine *N*-oxide buffer to remove NaCl.

Light-induced absorption changes were measured as described in Refs. 9 and 10. Xenon flashes (duration 13 μs at half maximum intensity) were used as actinic illumination. Infrared light was provided by a Schott RG 645, 2 mm filter for measurements in the region 230–600 nm and by a combination of RG 715, 2 mm and RG 780, 2 mm for the region 600–700 nm. Illumination with blue light, filtered by a BG 38, 4 mm and a Calflex C was used for the spectral region 700–930 nm. Suitable interference and absorption filters were used to prevent actinic light from reaching the photomultiplier.

## Results and Interpretations

The absorption spectrum of a purified reaction center preparation of *C. aurantiacus* is shown in Fig. 1. The spectrum is virtually identical to that of a preparation obtained by Pierson and Thornber [5] using a different isolation procedure. The bands at 865, 812 and 603 nm are due to BChl *a*; those at 756 and 532 nm are due to BPh *a*. The strong absorption in the Soret region is due to both pigments. The origin of the band at 680 nm is not clear; similar bands have been observed in reaction center preparations of purple bacteria [11,12]. The spectrum shows no evidence for the presence of carotenoid; the weak band at 495 nm may be largely due to BPh *a*.

The room-temperature absorption difference

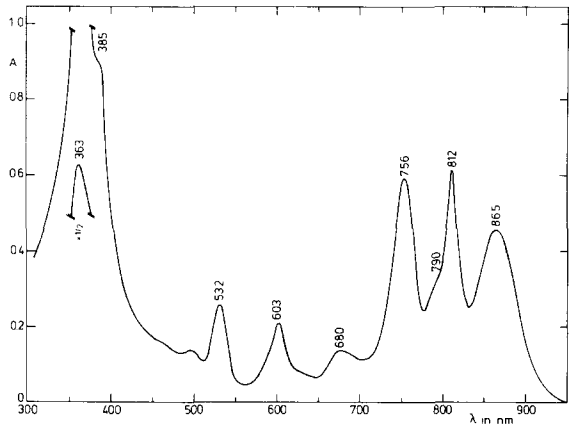


Fig. 1. Room-temperature absorption spectrum of a reaction center preparation of *C. aurantiacus* in Tris-lauryldimethylamine *N*-oxide buffer (10 mM Tris, pH 8.0, 0.025% (v/v) lauryldimethylamine *N*-oxide).

spectrum obtained 10 ms after a saturating xenon flash is shown in Fig. 2. Identical absorption changes were observed upon continuous illumination with saturating light. The spectrum is essentially identical to that obtained with a purified membrane fraction of *C. aurantiacus* [6] after a xenon flash, indicating that the isolation procedure yielded an intact reaction center. The spectrum also agrees with the chemically induced difference spectrum reported by Pierson and Thornber [5] for a reaction center preparation

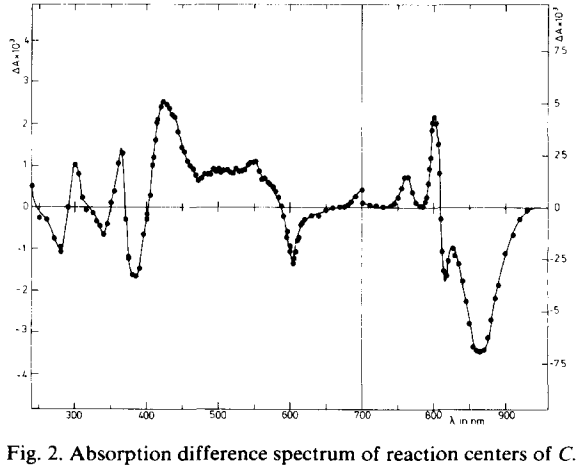


Fig. 2. Absorption difference spectrum of reaction centers of *C. aurantiacus* obtained 10 ms after a saturating xenon flash. The absorbance was $9 \cdot 10^{-3}$ at 865 nm. $T = 293$ K. Sample in Tris-lauryldimethylamine *N*-oxide buffer.

similar to ours. As noted earlier, it resembles that found for several purple bacteria [13]. The light-induced absorption changes are mainly caused by the oxidation of the primary donor P-865. The most prominent features are the bleaching of the long-wavelength BChl *a* band at 865 nm, an apparent band shift of the pigments absorbing at 812 nm and a slight increase in the amplitude of the BPh *a* band at 756 nm. Under saturating illumination approx. 75% of P-865 was reversibly photooxidized as indicated by the extent of the bleaching at 865 nm. No absorption changes due to cytochrome oxidation were detected.

The kinetics corresponding to the absorption changes of Fig. 2 are shown in Fig. 3A at two different wavelengths. The kinetics showed an exponential decay, with a half-time of 60 ms, comparable to that observed in a purified membrane preparation [6]. In order to obtain information about the possible contribution by an electron acceptor to the absorbance changes, ascorbate and increasing amounts of *N*-methylphenazonium methosulfate were added to the sample. In the presence of 5 mM ascorbate and 25 $\mu$M *N*-methylphenazonium methosulfate, $P^+$-865 was rereduced within 1 ms after a flash (Fig. 3, recording C, 600 nm). As illustrated by the kinetics at 420 nm (C), the remaining absorption changes can then be attributed to the reduction of the electron acceptor. Intermediate *N*-methylphenazonium methosulfate concentrations (Fig. 3B) resulted in biphasic kinetics at 420 nm.

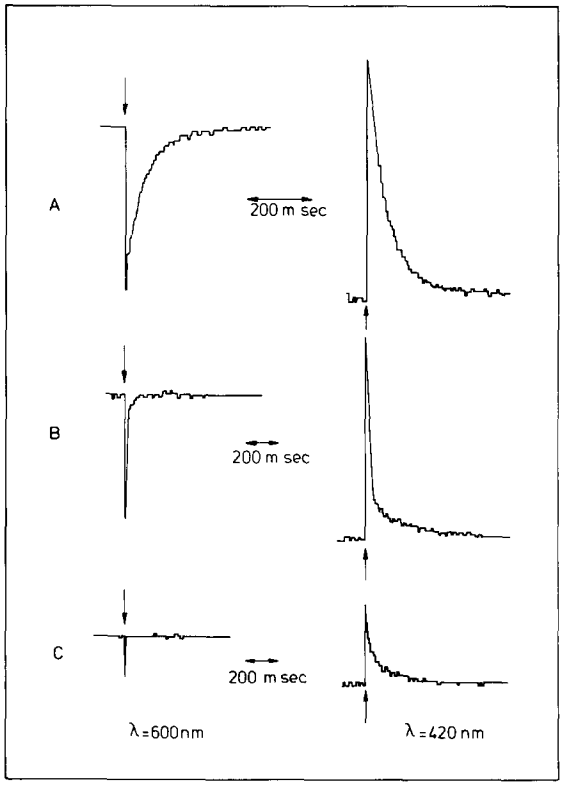


Fig. 3. Decay kinetics at 420 and 600 nm after a xenon flash. Conditions as for Fig. 2, except for the presence of: (A) 5 mM ascorbate (the kinetics were identical in the absence of ascorbate), (B) 5 mM ascorbate and 5 $\mu$M *N*-methylphenazonium methosulfate, (C) 5 mM ascorbate and 25 $\mu$M *N*-methylphenazonium methosulfate.

Fig. 4 (solid line) shows the spectrum of the absorption changes remaining after the rapid rereduction of $P^+$-865 by *N*-methylphenazonium methosulfate, obtained 10 ms after a xenon flash. We attribute this spectrum to the reduction of an electron acceptor, possibly accompanied by electrochromic shifts of neighboring pigments. The dashed line in Fig. 4 represents the in vitro difference spectrum of the reduction of vitamin K-1 ($Q^- - Q$) obtained by pulse radiolysis (Land, E.J., personal communication). Apart from a red shift of the in vivo spectrum, which was also observed

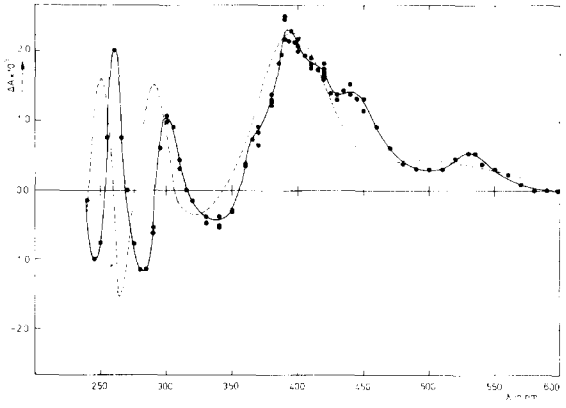


Fig. 4. Solid line: absorption difference spectrum, measured 10 ms after a flash. Sample in Tris-lauryldimethylamine *N*-oxide buffer, in the presence of 25 $\mu$M *N*-methylphenazonium methosulfate and 5 mM ascorbate. $A_{865} = 4.5 \cdot 10^{-2}$. Dashed line: in vitro absorption difference spectrum of vitamin K-1 ($Q^- - Q$) in methanol, obtained by pulse radiolysis (Land, E.J., personal communication). The two spectra were normalized at 395 nm.

in a similar preparation from the purple bacterium *Chromatium vinosum* [14], the two spectra are clearly similar.

It has been recently found (Hale, M.B., Blankenship, R.E. and Fuller, R.C., personal communication) that the only quinone present in *C. aurantiacus* is menaquinone (vitamin K-2). In view of the similarity of the absorption spectra of menaquinone and vitamin K-1 [15] and because of the similarity between the present spectrum and that obtained in *Chromatium* we conclude that menaquinone functions as an electron acceptor in *C. aurantiacus*.

If differential specific extinction coefficients of 10.2 $mM^{-1} \cdot cm^{-1}$ at 395 nm (Land, E.J., personal communication) and 128 $mM^{-1} \cdot cm^{-1}$ at 865 nm (as for *Rhodopseudomonas sphaeroides*, see Ref. 16) are assumed for menaquinone reduction and P-865 oxidation, respectively, a stoichiometry of 0.83 reduced menaquinone per oxidized P-865 was calculated. Taking into account the uncertainties in the extinction coefficients, this seems to be convincing evidence that one molecule of menaquinone is reduced per oxidized P-865. The nature of the shoulder at 450 nm in the difference spectrum is not clear. It was also observed in the difference spectrum of *Chromatium*, and more recently, in reaction centers of *Rhodopseudomonas viridis* [17], even after complete extraction of all ubiquinone, which in *Rps. viridis* acts as a secondary quinone acceptor. The shoulder was also observed in reaction centers of *Rps. sphaeroides* when ubiquinone had been replaced by menaquinone.

*o*-Phenanthroline (2 mM), either in the presence or in the absence of *N*-methylphenazonium methosulfate, did not affect the extent of the absorbance changes. In purple bacteria *o*-phenanthroline is known to inhibit electron transport between the first and the second quinone acceptor [8,18,19]. Thus, we conclude that a secondary quinone acceptor, which may be present in the membrane, is removed during the preparation of the reaction centers. The rate of reoxidation of menaquinone was somewhat enhanced in the presence of inhibitor. This suggests that the *o*-phenanthroline-binding site was still present.

The presence of a negative charge on the menasemiquinone anion also resulted in absorption changes around 760 nm, that can be attributed to BPh *a*, and smaller changes above 800 nm (Fig. 5). Again, these changes were only observed if P-865 oxidation was eliminated by the addition of *N*-methylphenazonium methosulfate. Under these conditions the kinetics of the absorption changes at 395 and 760 nm were identical. Changes in pigment absorption accompanying the reduction of electron acceptors have been observed in a variety of preparations, including chloroplasts [20] and purple bacteria [19]. Interestingly, the spectrum of Fig. 5 resembles more that obtained upon reduction of the second quinone acceptor in *Rps. sphaeroides* than of the first ubiquinone or menaquinone acceptor in several purple bacteria [14,19].

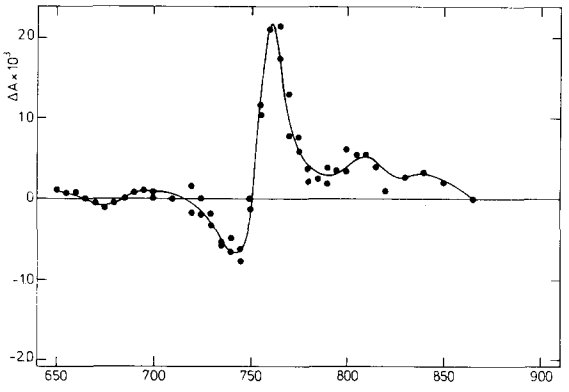


Fig. 5. Near-infrared absorption changes obtained in the same way and under the same conditions as for Fig. 4.

Summarizing, we conclude that the first stable electron acceptor, at least on a millisecond time scale, in *C. aurantiacus* is a bound menaquinone molecule which is converted to the menasemiquinone anion upon reduction. This is clear support for the hypothesis that electron transport in the reaction center of Chloroflexaceae resembles that of purple bacteria, where ubiquinones and menaquinones are known to act as electron acceptor.

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