The triplet state of the primary donor of the green photosynthetic bacterium *Chloroflexus aurantiacus*

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Received 12 July 1983

The technique of absorbance-detected electron spin resonance in zero magnetic field (ADMR) was applied to investigate the structure of the reaction center of the facultatively aerobic green bacterium *Chloroflexus aurantiacus*. The triplet-minus-singlet absorbance difference spectrum thus obtained at 1.2 K shows a clear resemblance to those earlier reported for *Rhodopseudomonas viridis* and *Rps. sphaeroides* R-26. The most prominent features are the bleaching of the Q_y band of the primary electron donor at 887 nm and the appearance of a narrow band at 807 nm upon triplet formation. We conclude that the primary electron donor P-865 of *Chloroflexus aurantiacus* is a BChl *a* dimer with Q_y and Q_x absorbance bands at 887 and 606 nm, respectively, at 1.2 K; apparently the triplet state is localized on an optical time scale on one of the constituent pigments of the dimer. The zero field splitting parameters $|D|$ and $|E|$ of P-865 are 197.7 ($\pm$ 0.7) $\times 10^{-4}$ cm^{-1} and 47.3 ($\pm$ 0.7) $\times 10^{-4}$ cm^{-1}, respectively. Decay rates of 12 660 ($\pm$ 750) s^{-1}, 14 290 ($\pm$ 800) s^{-1} and 1690 ($\pm$ 50) s^{-1} were observed for the x, y and z triplet sublevels, respectively.

Triplet state *ADMR* *Electron spin resonance* *Primary donor* Chloroflexus aurantiacus

1. INTRODUCTION

The recently isolated reaction center of the gliding green bacterium *Chloroflexus aurantiacus* possesses a primary photochemistry that resembles that of purple bacteria as indicated by its absorbance spectrum and by the absorbance difference spectrum for the oxidation of the primary donor P-865 [1]. As in purple bacteria, a quinone (menaquinone) functions as secondary electron acceptor [1] and there is evidence that bacteriopheophytin *a* serves as the primary electron acceptor [R.E. Blankenship et al., personal communication; H. Vasmel, unpublished data]. In contrast to the situation encountered for purple bacteria, the reaction center of *C. aurantiacus* appears to contain 3, rather than 2 molecules of BPheo *a* [2].

The width of the ESR line of the cation radical suggested that P-865 is a BChl *a* dimer [3]. Upon oxidation of P-865 bleachings occur of absorbance bands at 865 and 792 nm shifting to 887 and 787 nm, respectively, at low temperature, accompanied by an apparent band shift centered around 810 nm [1]. By means of linear and circular dichroism measurements we recently obtained evidence that the 792 nm band, which has been attributed to the high-energy exciton component of P-865 [2], and the 813 nm absorbance band are due to the interaction of two additional BChl *a* pigments [4]. These studies further indicated interaction between these pigments and P-865.

A sensitive method to study the structure of the reaction center, and in particular that of the primary donor is the triplet-minus-singlet absorbance difference spectrum, obtained by ADMR [5, 6]. We have shown for the purple bacteria *Rhodopseudomonas sphaeroides* R-26 and *Rps.*

Abbreviations: LDAO, lauryl dimethylamine *N*-oxide; BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; P-865, primary electron donor; ADMR, absorbance-detected electron spin resonance in zero magnetic field; T–S spectrum, triplet-minus-singlet absorbance difference spectrum

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001457593/82/0000—0000/$2.75

viridis [6] that the triplet state of the primary donor is a less perturbing probe than the cation of the primary donor and that comparison of the triplet-minus-singlet absorbance difference spectrum (T − S spectrum) with the light-induced absorbance difference spectrum of oxidized–reduced P-865, aids in the interpretation of the latter. Both types of spectra will exhibit identical bleaching of bands due to the disappearance of the reduced form of P-865, but they will differ in band shifts exhibited by neighbouring pigments and in the presence of absorbance bands of the triplet state or of the cation, respectively. The ADMR method also permits the accurate determination of the zero field splitting parameters of the triplet state and the decay rates of the triplet sublevels [7]. These parameters which have previously been obtained for a variety of other photosynthetic bacteria, are thought to reflect the macromolecular arrangement of the primary electron donor and thus allow a comparison between *C. aurantiacus* and the other species [8].

It will be shown that the transition to the triplet state of the primary donor in *C. aurantiacus* reaction centers results in a bleaching of the long-wavelength absorbance band at 887 nm, the appearance of a monomer absorbance band at 807 nm accompanied by a band shift of the pigment absorbing at 813 nm, while no bleaching of the 787 nm band was observed under those conditions. The characteristics of the T–S spectrum are very similar to those of the T–S spectrum of reaction centers of *Rps. sphaeroides* R-26 and *Rps. viridis* [6], and strongly suggest that P-865 consists of a BChl *a* dimer.

The zero field splitting parameters of *C. aurantiacus* lie midway between those of the purple bacteria and those of the green sulfur bacterium *Prosthecochloris aestuarii*; the decay rates are much closer to those measured in purple bacteria [8, 9] than to those of *P. aestuarii* [10].

2. MATERIALS AND METHODS

Reaction centers of *C. aurantiacus*, strain J-10-fl were isolated as in [1]. Samples in 10 mM Tris buffer (pH 8.0) containing 0.025% LDAO, were diluted to 65% (v/v) ethylene glycol and poised at low redox potential by addition of sodium dithionite (10 mM final concentration). The ADMR experiments were performed at 1.2 K as described in [5]. The T–S spectra were recorded as in [6]. The kinetics of the absorbance changes were measured by the pulse method [11, 7].

3. RESULTS AND DISCUSSION

Surveying scans with various monitoring microwave frequencies within the zero field resonance lines revealed two intense absorbance difference bands of the triplet–singlet spectrum, located at 807 and 887 nm. The peak position of the long-wavelength band around 887 nm varied considerably with the monitoring microwave frequency, while the position of the 807 nm band was much less dependent on this frequency. Conversely, the shape of the ADMR spectrum was dependent on the wavelength of the monitoring light. Similar effects have been observed for the triplet ESR spectrum of the purple bacteria *Rps. sphaeroides* R-26 and *Rps. viridis* [12, 13], and are caused by a heterogeneity of the reaction centers. By monitoring the microwave-induced absorbance changes at the 807 nm band, which peak wavelength is relatively insensitive to the microwave frequency, all reaction centers will contribute approximately equally to the triplet ESR spectrum and a correct, non-optically selected ADMR line is obtained. Fig. 1 shows such an ADMR triplet ESR spectrum. The zero field triplet ESR spectrum resembles the spectra of purple bacteria [14, 15]. The triplet transition lines are rather narrow and only the $|D|$ and $|E|$ transitions

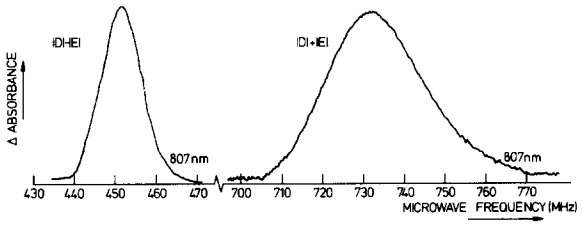

Fig.1. The zero field ESR ADMR spectrum at 1.2 K of the primary donor, detected at 807 nm, in isolated reaction centers of *Chloroflexus aurantiacus*, suspended in 10 mM Tris buffer (pH 8.0) containing 0.025% LDAO and diluted to 65% (v/v) ethylene glycol; 10 mM sodium dithionite (final concentration) was present. The spectrum is a single scan of 100 s taken with a response time of 0.3 s.

Table 1

Characteristics of the triplet state of the primary donor of several photosynthetic bacteria and monomeric BChl *a*

Species	$\|D\|$ ($\times 10^{-4}$ cm^{-1})	$\|E\|$ ($\times 10^{-4}$ cm^{-1})	k_x (s^{-1})	k_y (s^{-1})	k_z (s^{-1})	[Ref.]
Ch. aurantiacus	197.7 ± 0.7	47.3 ± 0.7	12660 ± 750	14290 ± 800	1690 ± 50	[here]
Rps. sphaeroides R-26 (whole cells)	187.2 ± 0.6	31.2 ± 0.6	9000 ± 1000	8000 ± 1000	1400 ± 200	[9]
BChl *a* in methyltetrahydrofuran	230 ± 2	58 ± 5	11950 ± 700	15900 ± 1300	1635 ± 50	[7]
Pr. aestuarii	207 ± 2	37 ± 1	3000 ± 400	3000 ± 400	1300 ± 150	[10]

are observed. Presumably, the 2 $|E|$ transition is weak because of the almost equal population of the two sublevels connected by the resonant microwave field at the corresponding frequency. The values of $|D|$ and $|E|$ and of the triplet sublevel decay rates are tabulated in table 1, together with those of some other bacteria and of BChl *a* in methyltetrahydrofuran.

The decay kinetics of the triplet sublevels of the primary donor were measured as a function of incident light intensity after a brief pulse of microwaves with frequency corresponding to the $|D| + |E|$ or the $|D| - |E|$ transition [7, 9, 11]. At low light intensity the kinetics were independent of the light flux. Hence, under those conditions the true triplet sublevel decay rates k_x, k_y and k_z are measured.

The value of $|D|$ for *C. aurantiacus* reaction centers falls in between that found for purple bacteria and that observed in *P. aestuarii*; it is about 15% lower than that of monomeric BChl *a*. The triplet sublevel decay rates correspond closely to those of BChl *a* in methyltetrahydrofuran, which are slightly faster than those of the purple bacterium *Rps sphaeroides* R-26, but they deviate considerably from those observed for *P. aestuarii*. These differences in $|D|$ and the decay rates may be due to small changes in the geometry of the primary donor, or to differences in ligands to the BChl pigments. It is known that changes in the ligands may change the triplet parameters of BChl *a* in vitro appreciably [7], so that at present, without information on the ligands in vivo, it is difficult to draw a conclusion.

In fig.2 the ADMR-monitored T–S spectrum is shown. The sign of the microwave transition at 887 nm corresponds to an increase in the ground state population under resonant conditions. The T–S spectrum shows bleachings at 887 nm, 606 nm and below 400 nm. Between 420 and 730 nm broad, rather unstructured bands appear, which we ascribe to triplet–triplet absorbance. In the 750–830 nm region a strong narrow band at 807 nm appears and a weak bleaching around 818 nm occurs, while at 770 nm an absorbance increase is observed.

We ascribe the negative bands below 400 nm and at 606 and 887 nm to the disappearance of the singlet absorbance spectrum of P-865. The band at 770 nm in the T–S spectrum can be tentatively ascribed to triplet–triplet absorbance, but might also be due to a small increase of BPheo *a* absorbance in this region, while the small absorbance

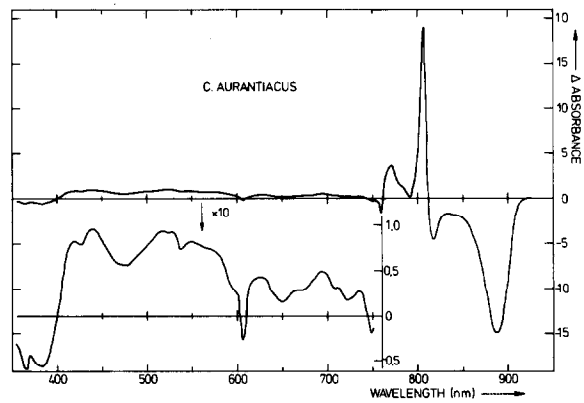

Fig.2. The triplet–singlet absorbance difference spectrum of isolated reaction centers of *C. aurantiacus* recorded by ADMR at 1.2 K. The frequency of the resonant microwaves was 735 MHz for the region 750–950 nm and 451 MHz for the region 350–750 nm. Spectral resolution is 3.2 nm. Further conditions as in fig.1.

changes observed around 755 nm might be due to band shifts of BPheo *a*.

Fig.3 shows on an expanded scale the triplet–singlet difference spectrum for 3 different microwave frequencies within the microwave transition line, in the 730–930 nm region. The position of the 887 nm band depends on the frequency of the monitoring microwaves, the maximum position ranging from 879 nm at 760 MHz to 895 nm at 711 MHz. A shoulder at the long-wavelength side as in reaction centers of *Rps. sphaeroides* R-26 and *Rps. viridis* [12, 13] is not observed. The other bands are much less sensitive to the microwave frequency.

For BChl *a* containing purple bacteria it is generally accepted on the basis of ENDOR data that the primary donor is a BChl *a* dimer [16, 17]. The T–S spectrum of the primary donor of *Rps. sphaeroides* R-26 is in agreement with this notion [6]. We have shown [6] that the T–S spectrum of the primary donor of *Rps. viridis* can also be interpreted in terms of a dimeric BChl *b* model [17].

The same applies to the T–S spectrum of *C. aurantiacus*. Because the integrated bleachings in the region 770–830 nm are much smaller than the integrated absorbance increases, they cannot be explained by a combination of spectral shifts. By analogy with our findings for *Rps. sphaeroides* and *Rps. viridis* [6] we ascribe the narrow band at 807 nm, which is correlated with the formation of PT-865, to the absorbance of a BChl *a* monomer of the P-865 dimer, in which the interaction in the singlet state has been broken by the formation of the triplet state.

It has been suggested that the 792 nm absorbance band of the isolated reaction center, which shifts to 787 nm at 4 K, might be the high-energy exciton transition of the primary donor [2]. However, comparison of the T–S spectrum at 1.2 K with the singlet–singlet absorbance spectrum obtained at 4 K [4] shows that this band is still present after triplet formation and thus cannot be due to the primary donor. The same conclusion was obtained from measurements of circular and linear

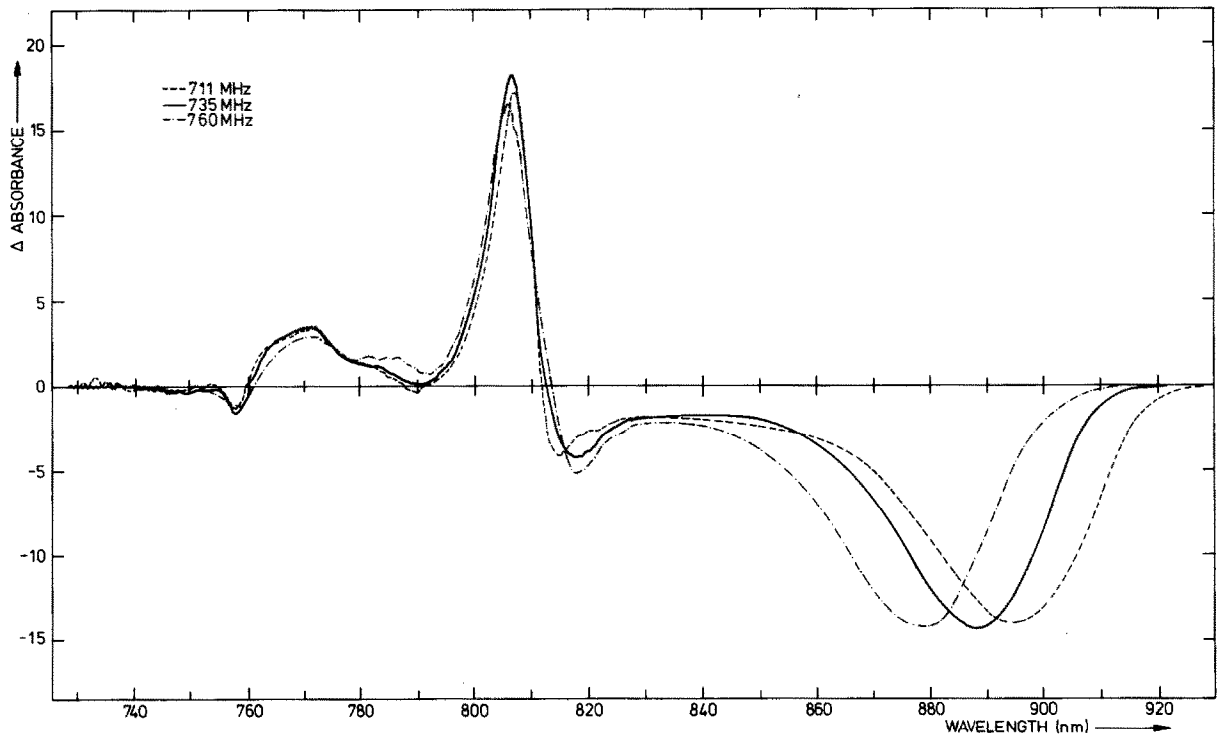

Fig.3. The triplet–singlet absorbance difference spectrum in the region 730–930 nm of isolated reaction centers of *C. aurantiacus* recorded by ADMR at 1.2 K with resonant microwaves at 711 (– – –), 735 (——) and 760 MHz (·····). Further conditions as in fig.2.

dichroism [4]. Most likely the band around 815 nm in the T–S spectrum is caused by a shift of the absorbance band of an additional BChl *a* pigment, absorbing at 813 nm.

The interpretation of the 807 nm absorbance band as the appearance of monomeric BChl *a* absorbance is consistent with the small amplitude of the bleachings in the Soret region and in the Q_x region (at 606 nm), as compared to the oxidized–reduced absorbance difference spectrum of P-865 [1]. If we assume that 2 pigments absorb at 887 nm and 4 pigments near 600 nm, and that the 887 nm band bleaches completely upon triplet formation, then we obtain bleaching near 600 nm corresponding to 2.0 ± 0.3 pigment molecules upon oxidation and 0.5 ± 0.3 pigments upon triplet formation of P-865. Thus, triplet formation produces significantly less bleaching at 600 nm than oxidation of the primary donor.

Similar estimates have been obtained for isolated reaction centers of *Rps. sphaeroides* R-26 and *Rps. viridis* [6]. The fact that both for *C. aurantiacus* and for *Rps. sphaeroides* the number obtained is less than 1.0 might be caused by an intensity redistribution between the Q_x and Q_y transitions of BChl *a* [Scherz, A., private communication]. Nevertheless, the low number obtained supports the notion that the triplet state of P-865 is localized on one of the constituent pigments of the dimer on the time scale of optical measurements.

ACKNOWLEDGEMENTS

The authors would like to thank Dr K. Schmidt for a gift of cultures of *C. aurantiacus*, Mrs L.M. Blom for her help in preparing the reaction centers, A.H.M. de Wit for culturing the bacteria and C.J.M. Bauer for his help in preparing the figures. The 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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