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EPR PROPERTIES OF THE REACTION CENTER OF *RHODOPSEUDOMAS GELATINOSA* IN SITU AND IN A DETERGENT-SOLUBILIZED FORM

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Summary

The photochemical reaction centers from a variety of purple photosynthetic bacteria are composed of a trimer of protein subunits. However, the recently isolated reaction center from *Rhodopseudomonas gelatinosa* appears to have only two subunits. In this paper we examine the EPR characteristics of the primary photochemical reactants in this species, and compare them with those of other species. Despite of the differences in protein composition, no dramatic differences in EPR properties are seen in vivo, although some interesting effects are seen upon solubilization of the reaction center, which may be related to the unusual lability of the isolated preparation. Perhaps the most noteworthy phenomenon seen in *Rps. gelatinosa* is the apparent ability of electrons on the reduced intermediary electron carrier to tunnel at low temperatures to the oxidized *c*-type cytochrome, which has not been seen in other species studied to date.

Introduction

The primary events of purple bacteria photosynthesis occur within the photochemical reaction center, where an actinic photon causes the oxidation of a bacteriochlorophyll dimer $(BChl)_2$ and the concomitant reduction of an intermediary carrier, I, which probably involves a bacteriopheophytin. The electron on I then proceeds to the classically defined "primary acceptor", a quinone · iron (QFe) complex, in a reaction which effectively renders the light reaction irreversible. A recent review on these early events, and the components involved, can be found in ref. 1.

Abbreviation: BChl, bacteriochlorophyll.

Reaction centers have been isolated from a variety of photosynthetic bacteria, including *Rhodopseudomonas sphaeroides*, *Rhodopseudomonas capsulata*, *Rhodospirillum rubrum* and *Chromatium vinosum* (see ref. 2 for a recent review), and indeed it has been the availability of these preparations which has allowed the elucidation of the primary events described above. The reaction centers from the various species share many common properties, and all those mentioned above possess three protein subunits when analyzed by polyacrylamide gel electrophoresis (see ref. 2). Recently Clayton and Clayton [3] have isolated the reaction center of *Rhodopseudomonas gelatinosa*. This reaction center is far less stable in isolated form than those of the other species, and apparently has only two protein subunits. Nevertheless the optical properties of the *Rps. gelatinosa* preparation are very similar to those of other species; in this paper we present the electron paramagnetic resonance (EPR) properties of the reaction center from *Rps. gelatinosa*, both in vivo in the chromatophore membrane, and in the isolated state. In vivo its properties are very similar to those of other purple bacteria, but the isolated reaction center displays some noteworthy differences.

Materials and Methods

Reaction centers (solubilized by 4% lauryl dimethylamine *N*-oxide) and chromatophores of the blue-green (carotenoidless) strain TG-9/EM1 of *Rps. gelatinosa* were prepared as previously described [3].

EPR spectra were measured with a Varian E-4 spectrometer, equipped with a flowing helium cryostat, as described elsewhere [4].

Results and Discussion

EPR properties of the reaction center in situ in the chromatophore membrane

The "primary donor". Fig. 1 shows the spectrum of the photooxidized primary donor; it is a gaussian signal centered close to g 2.0025 with a peak to peak linewidth of 9.8 G. This is very similar to the EPR spectra of the primary donors of other bacteriochlorophyll *a*-containing species [5], which are $\sqrt{2}$ narrower than that of the cation radical of monomeric bacteriochlorophyll *a*. It was this difference between the in vivo and in vitro EPR spectra which provided the first evidence that the primary donor was a dimer or special pair of bacteriochlorophylls [6].

Fig. 2 shows the EPR spectrum of the light-induced spin polarized triplet of the primary donor, seen when the prior reduction of the "primary acceptor" prevents normal photochemistry [1]. In chromatophores, the zero field splitting parameters are $D = 186 \cdot 10^{-4}$ cm^{-1} and $E = 28 \cdot 10^{-4}$ cm^{-1}, in good agreement with the values of other strains of this species [4,7].

The "intermediary carrier". As we have discussed at length elsewhere [1] there is now ample evidence that an intermediary carrier, I, functions between what have been classically defined as the "primary donor" and the "primary acceptor". The I/I$^-$ couple apparently has a very negative midpoint potential, and I has not been chemically reduced in the dark in any bacteriochlorophyll *a*-containing species (cf. the bacteriochlorophyll *b* containing *Rhodopseu-*

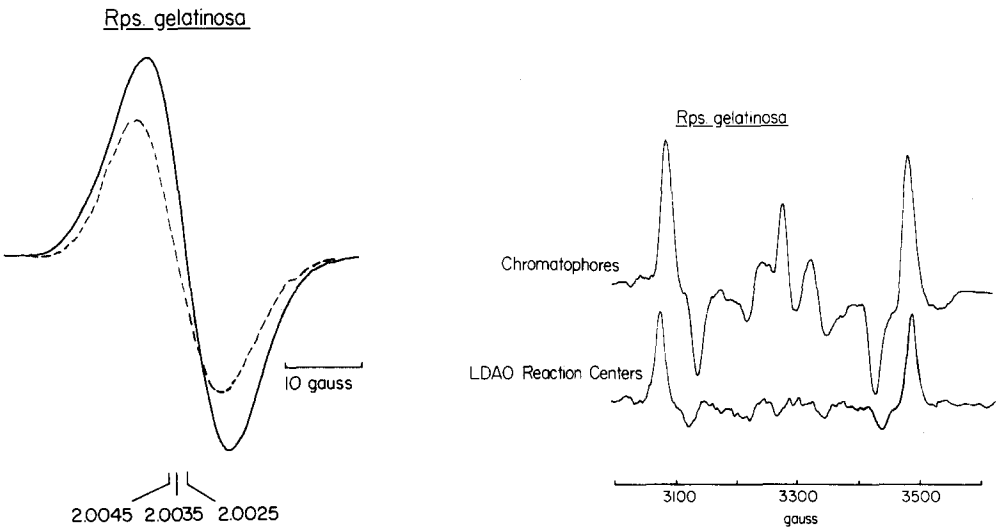

Fig. 1. Low temperature EPR spectra of the light-induced signals close to g 2 in the *Rps. gelatinosa* preparations when normal photochemistry can occur. The solid line represents the signal obtained with chromatophores. Same chromatophore sample as in Fig. 2. The "Primary acceptor" Q^-Fe spectrum was (40 μM). Both the spectra are light minus dark difference spectra, obtained at 11°K with a microwave power of 1 mW and a modulation amplitude of 5 G. The peak to peak line widths of the signals, discussed in the text, have been corrected for this rather large modulation amplitude.

Fig. 2. The light-induced triplet signals seen in the *Rps. gelatinosa* preparations. The samples used in Fig. 1 were thawed, dithionite was added, and the samples were refrozen. The signals represent light minus dark difference spectra, obtained at 6°K with a microwave power of 1 mW and a modulation amplitude of 5 G.

domonas viridis, see ref. 4). However, I can be readily trapped photochemically in its reduced form in those species which possess *c*-type cytochromes that are capable of donating electrons to the reaction center $(BChl)_2^+$ at cryogenic temperatures [8—10]. This photochemical trapping of I^- starts with the reaction centers in the state ferro cytochrome *c* $[(BChl)_2I]Q^-Fe$, which prolonged illumination converts to ferricytochrome *c* $[(BChl)_2I^-]Q^-Fe$. At low temperatures this latter state is quite stable, at least in *C. vinosum* [8] and *Rps. viridis* [10], where is has a half-life of tens of minutes at 200°K, and weeks at 77°K.

Rps. gelatinosa possesses two *c*-type cytochromes which are capable of donating electrons to $(BChl)_2^+$ at low temperatures [11], and this has allowed us to photochemically trap I in its reduced state (Fig. 3). However, even prolonged (10 min) illumination at 200°K resulted in only a small fraction (approx. 30%) of the total I becoming stably reduced, as judged by the diminution of the light-induced triplet signal after the low temperature illumination (cf. refs. 4, 8 and 10). Furthermore, even at 6°K some I^- could apparently be reversibly generated in the light, suggesting that electrons can tunnel from I^- back to the ferricytochrome. This has not been noted in *C. vinosum* [8], *Rps. viridis* [10], or *Thiocapsa pfennigii* [12].

The EPR spectrum of I^- in chromatophores of *Rps. gelatinosa* is very similar to that seen in *C. vinosum* [8] and *T. pfennigii* [12], with the majority of the electron spins being present in a broad signal split by 83 G about g 2.0025. Just as in the other species [8,10,12], this signal is difficult to saturate at low tem-

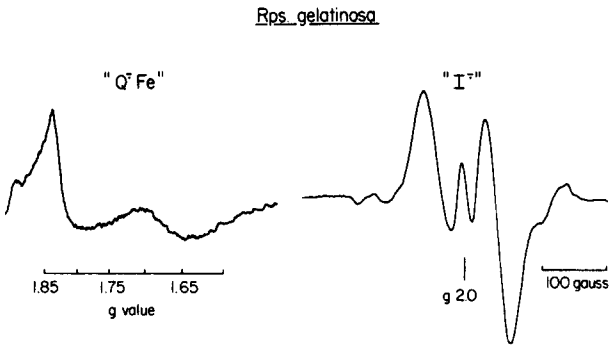

Fig. 3. The EPR spectra of the "primary acceptor" and intermediary electron carrier in *Rps. gelatinosa* chromatophores. Some chromatophore sample as in Fig. 2. The "Primary acceptor" Q^-Fe spectrum was obtained at 6.5°K at a microwave power of 5 mW and a modulation amplitude of 25 G. The I^- spectrum was obtained under similar conditions, and represents the changes induced by a 5 min illumination of the chromatophore sample at 200°K.

peratures, but is undetectable above 20°K. As we have discussed elsewhere, these relaxation properties are similar to those of the "primary acceptor" Q^-Fe, and there is a considerable body of evidence to suggest that the broad I^- signal is the result of I^- interacting, both by exchange and dipolar coupling, with Q^-Fe [8,10,12].

The "primary acceptor". Fig. 3 also shows the EPR spectrum of the reduced primary acceptor. In fact Fig. 3 was obtained after chemical reduction by dithionite, but a similar signal is seen in the light if normal photochemistry can occur. The signal is very similar to that seen in other species (see refs. 1 and 2), characterized by a prominent signal at g 1.82 and g 1.64. It is generally accepted that this signal reflects a semiquinone in close association with a ferrous iron.

EPR properties of the reaction center in a detergent-solubilized form

The EPR properties of the detergent-solubilized reaction centers show some striking differences from those measured in the chromatophore. Fig. 1 shows the light-induced signal near g 2. Unlike the symmetrical signal centered at g 2.0025 seen in chromatophores, which was assigned to $(BChl)_2^+$, the signal seen in the isolated reaction centers is an asymmetric signal centered close to g 2.0035. Perhaps the best explanation of this is that the quinone of the "primary acceptor" QFe has become uncoupled from the iron, so that the photo-produced semiquinone appears at g 2.0045 [13,14,9]. The light-induced signal would then be the sum of the $(BChl)_2^+$ signal at g 2.0025 and a Q^- signal centered at g 2.0045. In support of such an idea, we have been unable to detect a significant level of the g 1.82 Q^-Fe signal even in dithionite-reduced reaction centers of *Rps. gelatinosa*, although this center was readily detected in companion experiments with reaction centers from *Rps. sphaeroides* R-26 and with chromatophores of *Rps. gelatinosa.*

A further difference between the reaction center in situ and in the isolated state is seen in the light-induced spin polarized triplet, shown in Fig. 2. The signal in the isolated reaction centers is somewhat broader with zero field splitting parameters of $D = 194 \cdot 10^{-4}$ cm^{-1} and $E = 34 \cdot 10^{-4}$ cm^{-1}. Although isolated

reaction center preparations often have slightly larger D and E values than in vivo (e.g. see ref. 10), these values measured in *Rps. gelatinosa* are by far the most dramatic demonstration of this phenomenon. Since the magnitude of D is a qualitative measure of the average distance between the unpaired electrons of the light-induced triplet state [7], this change in D upon detergent solubilization may be related to the unusual lability of reaction centers from *Rps. gelatinosa* [3].

The detergent-isolated reaction centers lack *c*-type cytochromes [3] so we have been unable to trap I^- at low temperatures.

In conclusion it seems that despite of the unusual protein subunit composition of the reaction center of *Rps. gelatinosa*, the EPR properties of the photochemical reactants are remarkebly similar to those of the most studied organisms, *Rps. sphaeroides* and *C. vinosum*. Some differences are apparent in the isolated form; these may be related to the relative instability of isolated reaction centers from *Rps. gelatinosa* and/or the high levels of detergent needed to solubilize the chromatophore membrane. Perhaps the most noteworthy property of the reaction center of *Rps. gelatinosa* is the apparent reversibility of light-induced electron flow from the low potential cytochrome *c*, via $(BChl)_2$, to I, because this may imply that direct electron tunneling from I^- to the ferricytochrome can occur. In this respect it is curious that *Rps. gelatinosa* is the only organism so far noted to have reversible oxidation of the high potential cytochrome *c* at low temperature [11].

Acknowledgments

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