PHOTOOXIDATION AND TRIPLET FORMATION OF THE PRIMARY ELECTRON DONOR OF THE GREEN PHOTOSYNTHETIC BACTERIUM *PROSTHECOCHLORIS AESTUARII*, OBSERVED WITH ESR SPECTROSCOPY

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1. Introduction

Relatively little is known about the photochemical reaction in green photosynthetic bacteria. The high antenna bacteriochlorophyll to reaction center ratio of 1000–2000 BChl *c* + *a* per reaction center [1,2] and the difficulty of obtaining purified reaction center preparations has impeded the study of the electron transport by means of optical and ESR spectroscopy. In [3], we reported the isolation of photochemically active preparations of *Prosthecochloris aestuarii*. Starting from the membrane vesicle preparation complex I [2,4,5], the photosystem pigment (PP) complex and the reaction center pigment–protein (RCPP) complex were isolated, with antenna BChl *a* to reaction center ratios of ~75 and 35, respectively [3]. At room temperature both complexes show photooxidation of P-840, the primary electron donor, and of 1 cytochrome *c*-553/reaction center [3]. Upon flash excitation the formation of the triplet state of P-840 was observed with optical difference spectroscopy in both complexes [6]. At <120 K the amount of P-840 triplet formed in a saturating flash was 0.4–0.5 in the PP complex and close to 1/reaction center in the RCPP complex.

Here, we report on the kinetics of photooxidized P-840 and its triplet state monitored at 5 K with ESR and optical spectroscopy. Depending on the redox potential, the photooxidation of P-840 is reversible in the PP complex, yielding a dimer type BChl *a* free radical ESR signal at $g = 2.0025$. The triplet of P-840 is found to be spin polarized; the zero-field splitting parameters and the decay times of the different triplet levels are calculated. An average decay time of 280 μs was found. This, and the value of the zero field parameters suggest that the BChl *a* dimer in green bacteria is structurally different from that in purple bacteria.

2. Materials and methods

Prosthecochloris aestuarii strain 2 K, was grown anaerobically in a mixed culture known as '*Chloropseudomonas ethylica*' [7] as in [8]. The membrane preparation complex I and the pigment–protein complexes PP and RCPP complex were prepared as in [3]. The light-induced absorbance changes were measured at 5 K with the apparatus described in [9], equipped with a xenon flash-tube (duration at half-maximum intensity, 13 μs).

ESR experiments were performed with a Varian E-9 spectrometer, having an instrumental response time of 20 μs, as in [10]. Continuous illumination with white light provided by a 1000 W projection lamp, filtered by 5 cm water and a Balzers Calflex C filter or xenon flashes (duration at half-maximum intensity, 5 μs) were used as actinic illumination. The samples were contained in quartz tubes of 3 mm internal diameter. Before freezing to 5 K, the samples were frozen to 80 K in a nitrogen gas-flow cryostat. All samples contained glycerol (50%, v/v) to prevent crystallization on cooling.

3. Results and discussion

Fig.1 shows the ESR spectrum of the light-induced changes in the $g = 2$ region, obtained by illumination at 5 K of the PP complex prepared from *P. aestuarii*. The spectrum shows a free radical signal, symmetrically

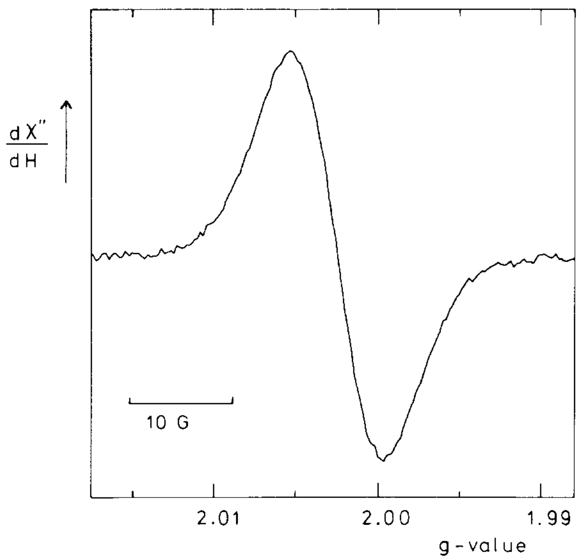

Fig.1. Light minus dark ESR difference spectrum obtained upon illumination in the $g = 2$ region at 5 K of the PP complex (A_{810} = 2.1 mm^{-1}) at a redox potential of +70 mV: modulation amplitude 5 G; microwave power 0.2 mW; average of 4 scans.

centered at $g = 2.0025 \pm 0.0002$ with a peak-to-peak linewidth $\Delta H_{pp} = 9 \pm 0.2$ G that is due to photooxidized P-840, the primary electron donor. The g-value is indicative of a BChl radical, the linewidth indicates that P-840 is a *dimer* of BChl *a*, similar to the primary electron donor in purple bacteria. This is in agreement with measurements of P-840 photooxidation in complex I [11], a membrane vesicle preparation from *Chlorobium limicola f. thiosulfatophilum*. About 20% of P-840 appeared to be irreversibly oxidized at 5 K in the PP complex frozen at a redox potential of +70 mV. If the suspension contained in addition 100 μM PMS and 10 mM dithionite at pH 7.7, which poised the sample at –420 mV, the same light-induced ESR spectrum was obtained. In this case, however, P-840 oxidation was completely reversible. This indicates that at –420 mV forward electron transfer is blocked because of reduction of a secondary electron acceptor. This acceptor may be responsible for the complete irreversibility of P-840 oxidation in 'chromatophores' prepared from *C. limicola f. thiosulfatophilum* [12,13]. In [13] a partial reversal of P-840 oxidation in complex I of *C. limicola* was observed; however, lowering the redox potential to –590 mV did not eliminate the irreversible signal [13]. The decay of the P-840$^+$ signal at 5 K after a short flash had a halftime of 13 ms, probably due to the back reaction between P-840$^+$ and a reduced acceptor, as observed at 77 K [6]. Flash-induced absorbance changes at 5 K, measured at 606 nm yielded the same halftime.

As was reported [6], only in ~50% of the reaction centers in the PP complex a stable charge separation is produced at <120 K. In the other half, electron transport at the acceptor side appears to be inhibited, resulting in the formation of the BChl triplet of P-840, which decayed with a halftime of 165 μs at 120 K [6]. The ESR spectrum of the BChl *a* triplet, together with the P-840$^+$ signal around $g = 2$, obtained upon illumination of the PP complex at 5 K is shown in fig.2. The AEEAAE polarization pattern, where A and E denote signals in absorption and emission, respectively, shows that the triplet is spin polarized by S–T_0 mixing, which is thought to be brought about by the radical pair mechanism [14,15]. Similar polarization patterns of the reaction center BChl *a* triplet were observed [16, 17] in cells and preparations of purple bacteria. The zero-field splitting parameters calculated from the triplet spectrum, $|D| = (207 \pm 2) \cdot 10^{-4}$ cm^{-1} and $|E| = (37 \pm 1) \cdot 10^{-4}$ cm^{-1} are, however, ~15% larger than those found for the reaction center BChl *a* triplet in a number of purple bacteria [18].

The decay of the triplet signal at the canonical x, y, z directions of P-840 was measured at 5 K at a microwave power of 2 μW. We assume that the number of transitions between T_0 and $T_{\pm}$, induced by this low level of microwave power is negligible during the

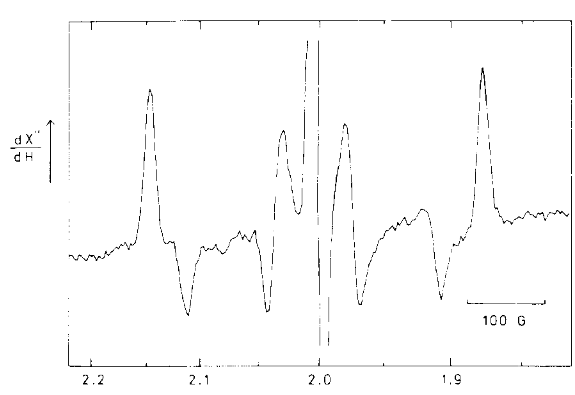

Fig.2. Light-induced ESR difference spectrum at 5 K obtained upon illumination of the PP complex (A_{810} = 2.1 mm^{-1}) at a redox potential of +70 mV: modulation amplitude 20 G; microwave power 10 mW; average of 4 scans.

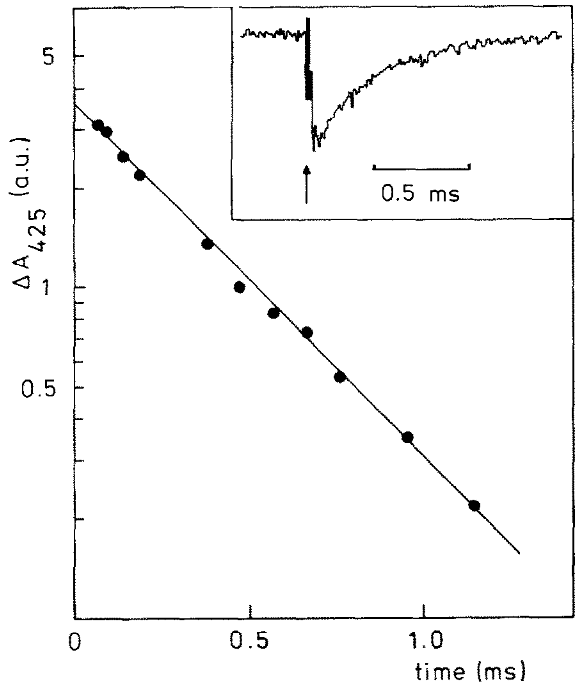

Fig.3. Absorbance change at 425 nm in the RCPP complex upon excitation at 5 K with infrared flashes provided by a combination of Schott RG 715 and RG 780 filters. The trace (inset) is the average of 128 flashes separated by 15 s. The semi-logarithmic analysis of the decay kinetics yields a half-time of 280 μs.

lifetime of the triplet state. Therefore, the measured decay times of the different triplet levels represent the actual molecular decay rate constants k_x, k_y and k_z [19]. Rate constants of $1300 \pm 150\ s^{-1}$ for the z-component and $3000 \pm 400\ s^{-1}$ for the x- and y-component were measured under these conditions. These values for k_x and k_y differ considerably from those measured in purple bacteria [19–21].

Fig.3 shows the kinetics of the absorbance change at 425 nm, induced by a short flash in the RCPP complex at 5 K, which is ascribed to the triplet of P-840 [6]. Semilogarithmic analysis yielded a monophasic decay within the limits of error, with a halftime of ~280 μs, which is in good agreement with the average halftime (290 ± 30 μs) measured with ESR. In contrast to the ESR technique, where one can magneto-select the x, y and z directions, optical spectroscopy does not discriminate between the 3 triplet levels and the decay is a super position of the 3 decay channels. As the rates differ only by a factor of 2, a quasi-exponential decay with average halftime is observed. This situation contrasts with the optically measured low temperature decay of the triplet state of purple bacteria, where a biphasic decay is observed [22], due to the much larger spread in the rate constants.

The above results suggest that the relative orientation of the 2 BChl *a* molecules in P-840 in green bacteria differs from that in the primary electron donor of BChl *a* containing purple bacteria, resulting in a structurally different special pair [23]. This is also indicated by the difference in position of the maximal bleaching of the Q_y-bands (830–840 nm in green bacteria [2,5,6] and 870–890 nm in purple bacteria).

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