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A STUDY OF THE PRIMARY CHARGE SEPARATION IN GREEN BACTERIA BY MEANS OF FLASH SPECTROSCOPY

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A reaction-center pigment-protein complex of the green bacterium *Prosthecochloris aestuarii* was studied by means of nanosecond-flash spectroscopy. In this complex electron transfer between the primary and secondary acceptor is blocked. The spectra and kinetics of the absorption changes induced by a short flash indicated the formation of the radical pair P-840$^+$I$^-$, which decayed in 20–35 ns, mainly to the triplet state of the primary electron donor P-840. The absorption difference spectrum of the initial absorption change indicated that the primary acceptor I is either bacteriopheophytin *c* or another pigment with absorption maximum at 665 nm.

In previous publications from this laboratory we have reported on the properties of photochemically active pigment-protein complexes from the green photosynthetic bacterium *Prosthecochloris aestuarii*. By means of optical and ESR spectroscopy evidence was obtained that the light-induced electron transport may be written as:

$$\text{P-840} \xrightarrow{h\nu} \text{I} \rightarrow \text{X}_1 \rightarrow \text{X}_2 \rightarrow \text{X}_3$$

In this scheme X_2 and X_3 are iron-sulfur centers [1]; X_1 is a BChl *a* monomer with Q_y band at 814 nm [2]. When X_1 was accumulated in the reduced state, illumination still generated a spin-polarized triplet of P-840 [2]. This would imply the existence of a still earlier, 'intermediary' electron acceptor I. However, no information on the nature of the acceptor I was obtained.

In this paper we report the results of a nanosecond flash spectroscopic study on the so-called reaction-center pigment-protein complex. This complex contains about 35 BChl *a* molecules per reaction center [3]. Flash illumination of the reaction-center pigment-protein complex does not produce a stable charge separation in the μs time range, but results only in a high yield of triplet formation, indicating that secondary electron transport is severely impeded [4].

The reaction-center pigment-protein complex was prepared as described in Ref. 3. The sample was suspended in a medium containing 40% sucrose/10 mM ascorbate/10 mM phosphate (pH 7.4). It was contained in a 2 mm transparent plastic (perspex) cuvette and excited at 532 nm by a non-saturating 30 ps pulse of a frequency-doubled, mode-locked Nd-YAG laser. The laser was fired at the flat maximum of a 15 μs xenon flash. This flash was used as measuring light and detected through a monochromator by an avalanche photodiode, connected to a transient digitizer. The response time of the system was 2 ns. Two different methods were used to separate the signal of the nanosecond absorbance change from the microsecond measuring light pulse. In one set-up the photodiode was connected to a high-pass electrical

Abbreviation: BChl, bacteriochlorophyll.

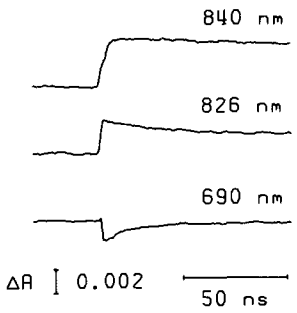

Fig. 1. Kinetics of absorbance changes at 275 K in the reaction-center pigment-protein complex (A_{814} = 0.45), excited by a 30 ps laser flash (λ = 532 nm). The traces were measured in different samples; each trace is the average of 10–20 measurements. Irregularities in the rising edge are caused by the response of the system.

Butterworth filter. This proved to be a good way for measuring the initial absorbance changes. Another method used an electrical delay line, which offered a slightly lower signal-to-noise ratio, but a much better kinetic representation of the absorbance change. A more complete description of the apparatus will be presented elsewhere. The spectrum of Fig. 3 was measured with a microsecond flash spectrometer [5].

Fig. 1 shows the kinetics of absorbance changes at a number of wavelengths induced by a laser flash. After an initial rapid change of absorbance, the rate of which was limited by the 2 ns response

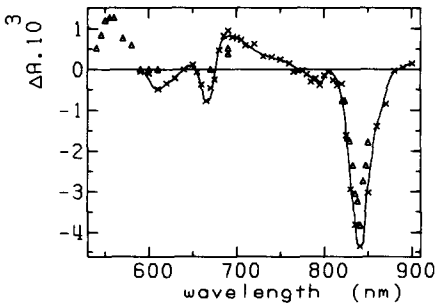

Fig. 2. Spectrum of absorbance changes in the reaction-center pigment-protein complex (A_{814} = 0.45). Each point is an average of 10–20 measurements. The spectrum of the initial absorbance change (crosses) was measured 7 ns after the laser flash. The triangles show the absorbance change measured 100 ns after the flash. They were obtained with a different sample, and are plotted relative to the amplitude of the initial absorbance change in the same preparation.

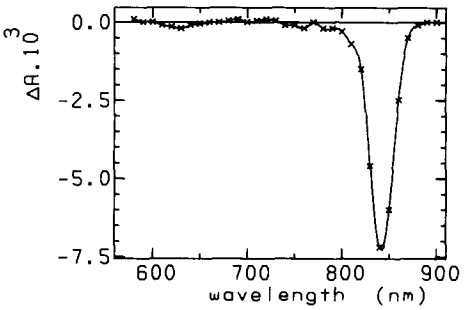

Fig. 3. Spectrum of the 80 μs decay component of the absorbance change at 300 K induced by a 532 nm laser flash (A_{814} = 0.50).

time of the detector, a single exponential decay was observed to a level that remained constant on the measured time scale (100 ns). As will be discussed below, we interpret the absorbance changes as resulting from the formation of the primary radical pair P-840$^+$I$^-$ which is converted to a secondary state (probably the reaction center triplet P-840^T) with a lifetime much longer than 100 ns. The decay time of the radical pair, determined from the average of several experiments as shown in Fig. 1, was 20–35 ns, depending on the sample.

The difference spectrum of the initial absorbance change at 275 K, measured with the high-pass electrical filter, is shown by the crosses in Fig. 2. The bleaching at 840 nm and the minimum at 600 nm are clearly due to bleaching of BChl *a* with Q_y band at 840 nm. The absence of a band or shoulder at 814 nm indicates that the reduction of X_1 [2] does not occur. This is in agreement with the notion that secondary electron transport is inhibited in the reaction-center particle [4]. The relatively short lifetime of about 25 ns indicates that the initial absorbance change is due to the generation of the primary radical pair P-840$^+$I$^-$, rather than to formation of the reaction-center triplet, which decays with a half-time of 90 μs at room temperature [4].

The triangles in Fig. 2 show the amplitude of the absorbance changes measured 100 ns after the flash by use of the electrical delay line. These absorbance changes showed a bleaching of a somewhat narrower band near 840 nm. The amplitude of this band amounted to about 60–90% of that of the initial absorbance change, depending on the

sample. In the region 600–820 nm, the absorbance changes which were still present after 100 ns appeared to be quite small, and no bleaching at 665 nm was observed. The bleaching at 840 nm showed a decay time of approx. 80 μs; the absorption difference spectrum of this component after a saturating flash is shown in Fig. 3. This spectrum, like the one measured at 100 ns, showed a negative band at 840 nm and only small absorbance changes below 800 nm. We conclude that both the shape of the difference spectrum and the lifetime of the absorbance change indicate the formation of the triplet of P-840, which is produced with high yield from the radical pair $P\text{-}840^+I^-$. The higher temperature at which the spectrum of Fig. 3 was recorded may explain the absence of the shoulder at 825 nm that was observed in the triplet difference spectra at 80 K [4]. The increase in absorbance around 560 nm in the 100 ns spectrum might be due to a carotenoid triplet. This triplet may be formed by a mechanism similar to that observed in some purple bacteria when the carotenoid is directly excited by the laser flash [6,7]. Its lifetime appeared to be less than 3 μs; the band was absent in difference spectra measured a few microseconds after the flash (not shown).

The relatively high amplitude of the bleaching at 840 nm after 100 ns as compared to that of the initial absorbance change indicates that the initial absorbance change at 840 nm is solely due to the formation of $P\text{-}840^+$, and that above 800 nm there is no significant contribution from the reduction of I in the spectrum of the initial absorbance change. Thus, the only features in the absorption difference spectrum, at least in the region above 580 nm, that can be attributed to reduction of the primary acceptor are the negative band at 665 nm and possibly the broad increase in absorbance at 680–760 nm. This suggest that the primary acceptor is bacteriopheophytin *c*, or possibly an unidentified pigment that was reported to have an absorption band at 665 nm (Ref. 8 and T. Braumann and H. Vasmel, unpublished results). Both pigments are known to occur in the reaction-center complex and in the photosynthetic membrane of *P. aestuarii*. If one assumes that the bleaching at 665 nm is partly offset by a positive absorbance change (possibly caused by the anion formed) at this wavelength, the extent of the negative band is not inconsistent with the reduction of one molecule of bacteriopheophytin *c* per P-840, since in organic solution the Q_y band of bacteriopheophytin *c* is 2–3-times weaker than that of BChl *a* [9]. The difference spectrum for bacteriopheophytin *c* reduction in vitro shows a bleaching at 665–670 nm, and a broad positive absorbance change at longer wavelength [11].

Finally, it should be noted that the initial difference spectrum in the region 760–830 nm is much simpler than that of $P\text{-}840^+$ as measured at longer time-scales [3]. The negative band at 830 nm is lacking, and the bands near 795 and 800 nm are considerably reduced. These bands are probably due to band shifts of neighboring BChl *a* molecules caused by the electrical field which is formed when an electron is transferred from P-840 to a secondary acceptor [10]. The absence or reduced amplitude of these bandshifts in the spectrum of the initial absorbance changes (Fig. 2) indicates that these pigments are subjected to a smaller field when the charge separation is confined to the primary radical pair. This effect is probably due to the shorter distance between the positive and negative charges of the dipoles formed.

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