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Picosecond measurements of the primary photochemical events in reaction centers isolated from the facultative green photosynthetic bacterium *Chloroflexus aurantiacus*

Comparison with the purple bacterium *Rhodopseudomonas sphaeroides*

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The primary charge separation process in reaction centers isolated from the green photosynthetic bacterium *Chloroflexus aurantiacus* has been investigated with picosecond transient absorption techniques. Transient spectra and kinetics are compared with those obtained under identical conditions on reaction centers from the purple bacterium *Rhodopseudomonas sphaeroides*. An early electron acceptor complex in both organisms appears to involve interacting bacteriopheophytin-*a* and bacteriochlorophyll-*a* molecules. However, there are significant spectral and kinetic differences in the two reaction center preparations.

Photosynthesis *Picosecond spectroscopy* *Reaction center* *Green bacteria*

1. INTRODUCTION

The primary electron transfer reactions in photosynthetic organisms occur in pigment–protein complexes called reaction centers. The initial charge separation process has been studied most extensively in reaction centers isolated from the purple non-sulfur bacteria, especially *Rhodopseudomonas sphaeroides* [1]. Reaction centers from this species contain 4 bacteriochlorophyll (BChl), 2 bacteriopheophytin (BPh), and 1 or 2 quinones (Q) [2–4].

Picosecond studies on reaction centers from several species of purple bacteria have shown that the photochemical oxidation of the primary electron donor (P) results in the reduction of an intermediary electron carrier (I), followed by rapid (200 ps) transfer of an electron to Q [1,5–8]. P is thought to be a complex involving two BChl molecules [1]; I appears to be composed of a BPh strongly interacting with another of the BChl molecules (B) [9–12]. The reduction of B may precede the reduction of BPh [7].

Here, we report on picosecond transient absorption studies of reaction centers isolated from the facultative green photosynthetic bacterium *Chloroflexus aurantiacus*. Previous millisecond optical and kinetic measurements and redox titrations [13] have indicated that the electron acceptor system in *Chloroflexus* may be similar to that in the purple bacteria and in photosystem II of plants, where pheophytin and quinones are early acceptors [1]. This is in contrast to the anaerobic green bacteria, the Chlorobiaceae [14,15], which appear to have iron–sulfur centers as early electron acceptors, making them similar to photosystem I of plants. It has been suggested on the basis of the optical absorption spectrum that

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reaction centers from *Chloroflexus* contain 3 BChl-*a* and 3 BPh-*a* [16]. Menaquinone is the only quinone detectable in *Chloroflexus* [17].

To compare our results on *Chloroflexus* with previous studies on reaction centers from purple bacteria, we have carried out measurements on *Rps. sphaeroides* under identical conditions. Some features of the transient spectra in *Chloroflexus* are similar to those observed in *Rps. sphaeroides*. However, some marked differences in the spectral and kinetic behavior have been found as well. These may reflect different interactions between the pigments in *Chloroflexus* from those that occur in purple bacteria, and/or a different pigment content. Such comparisons may furnish additional information on the nature of the primary reactions in the various bacterial species.

2. MATERIALS AND METHODS

Reaction centers from *Chloroflexus* [16] and *Rps. sphaeroides* (strain R-26) [18] were prepared essentially as described. The concentration of *Chloroflexus* (20 mM Tris (pH 8), 0.05% LDAO, 5 mM ascorbate) was adjusted for an A_{815} of 0.8 (4 mm path cell). Samples of *Rps. sphaeroides* (10 mm Tris (pH 8), 0.05% Triton X-100, 10 μM EDTA) were adjusted for the same absorbance at 800 nm.

The dual-beam picosecond transient absorption spectrometer has been described in [19]. The 600 nm excitation flashes were generated from the 532 nm second harmonic of a modelocked Nd:YAG laser via stimulated Raman scattering in C_6D_{12}. These flashes were ~35 ps duration and were reduced to 150 μJ of energy. Under these excitation conditions, bleaching in the 870 nm band was about half the ground state absorption (half-saturating). The laser system was operated at a 10 Hz repetition rate. Samples were flowed through a 4 mm pathlength cell and maintained at ~20°C. Millisecond time-scale measurements and absorption spectra were acquired as in [13].

3. RESULTS AND DISCUSSION

Ground state absorption spectra normalized at 865 nm for *Rps. sphaeroides* and *Chloroflexus* are shown in fig.1. The most striking features are the reduced absorption attributable to BChl in the 800 nm region and the increased absorption due to BPh near 750 nm for *Chloroflexus* compared to *Rps. sphaeroides*. On the basis of these differences and those in the Q_x bands of BPh near 535 nm and BChl near 600 nm, Pierson and Thornber [16] have suggested that *Chloroflexus* reaction centers contain three molecules each of BChl and BPh, compared to 4 BChl and 2 BPh for *Rps. sphaeroides*. The peaks due to BChl in *Chloroflexus* (605 and 815 nm) are red shifted from those in *Rps. sphaeroides* (595 and 800 nm), while the bands due to BPh are slightly blue shifted in *Chloroflexus* (534 *vs* 536, 756 *vs* 760) ([16] and fig.1).

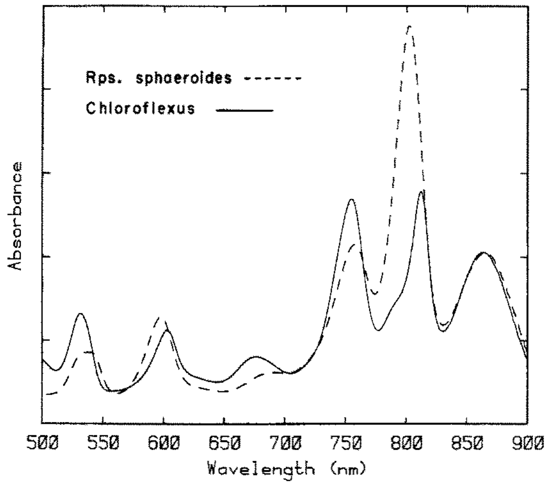

Fig.1. Ground state absorption spectra of *Chloroflexus* (——) and *Rps. sphaeroides R-26* (– – –), normalized at 865 nm.

Fig.2A–C show transient absorption spectra for *Chloroflexus* at time delays before (C), during (A), and after (B) the 600 nm excitation flash. For comparison, transient spectra acquired under the same conditions with *Rps. sphaeroides* reaction centers are shown in fig.3A–C. The spectra for both *Rps. sphaeroides* and *Chloroflexus* exhibit bleaching in the long-wavelength band of P centered near 870 nm and complex absorption changes at shorter wavelengths.

The 1 ns difference spectrum obtained for *Chloroflexus* (dashed curve in fig.2B) is unchanged at longer delays, and is essentially the same as that observed under continuous illumination or after 1-μs 583-nm flashes (fig.4). On the basis of such comparisons and other near-UV spectral data (un-

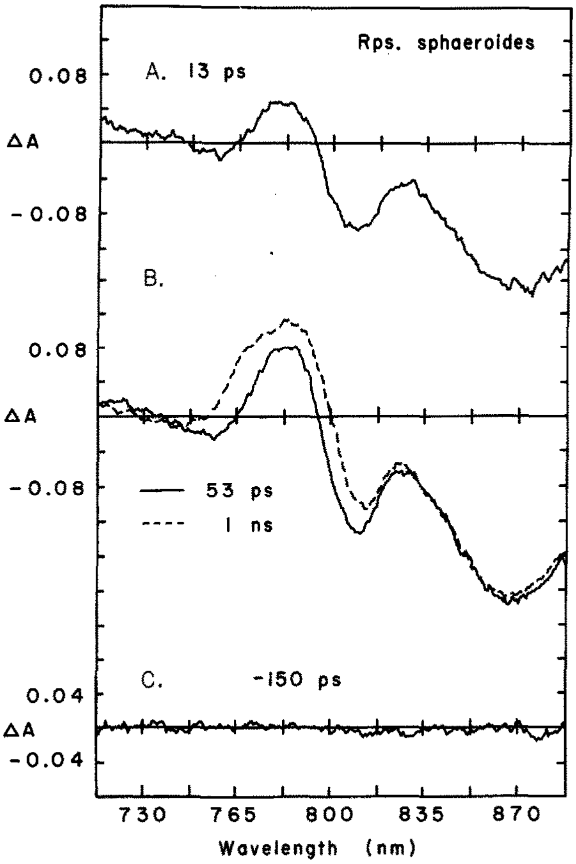

Fig.2. Near-infrared transient difference spectra for *Chloroflexus* reaction centers at the specified time delay between the subsaturating 600 nm excitation flash and the weaker broad-band monitoring flash. The 13 ps spectrum in (A) shows the absorption changes measured before the 35 ps excitation flash has reached its peak amplitude. Each transient spectrum, acquired over a 170 nm wavelength interval with the two-dimensional detection system, represents the average of ~600 spectra. Standard deviations in ΔA are <0.01, as indicated in (C), in which the monitoring flash has arrived at the sample before the excitation flash.

published), we assign the absorption changes shown 1 ns after excitation in fig.2B to the formation of state P^+Q^-, which in *Chloroflexus* has a lifetime of 62 ms at room temperature (fig.4, inset). The absorption changes 1 ns after excitation for *Rps. sphaeroides* (fig.3B) have been assigned previously to the formation of P^+Q^- [5,6]. State P^+Q^- in *Rps. sphaeroides* has a lifetime of 110 ms at room temperature (not shown).

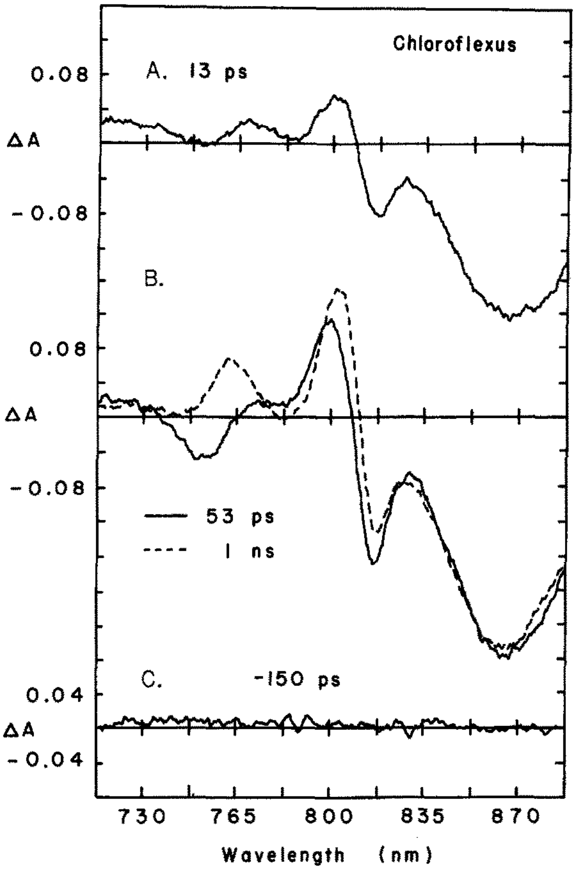

Fig.3. Near-infrared transient difference spectra for *Rps. sphaeroides* reaction centers. Other conditions as in fig.2.

The 53 ps difference spectrum for *Chloroflexus* also shows bleaching at 870 nm due to the oxidation of P to P^+, but there are marked differences from the 1 ns spectrum in the 730–830 nm region (fig.2B) and near 535 nm (fig.5A). The 53 ps spectrum exhibits absorption decreases in the BPh bands at 535 and 750 nm, and a weak positive absorption change from 765–785 nm. The apparent blue shift in the 800 nm region is at shorter wavelengths in the 53 ps spectrum than in that at 1 ns (fig.2B). The differences are seen more clearly in fig.6 (dashed spectrum), where the absorption changes observed at 53 ps minus those seen at 1 ns are plotted. The corresponding spectrum calculated from the absorption changes of fig.3B for *Rps. sphaeroides* is also shown in fig.6 (solid spectrum). If we assume that the 53 ps spectrum is due to P^+I^- minus PI, and that the 1 ns spectrum

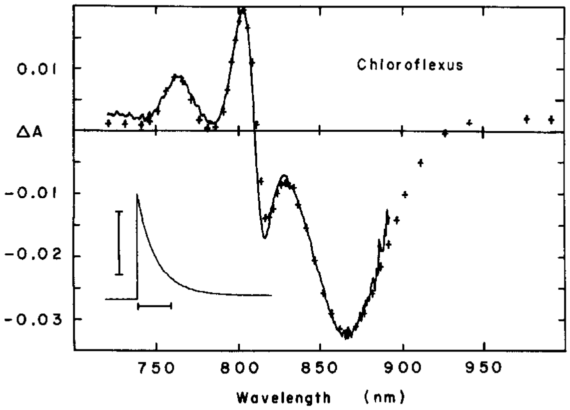

Fig.4. Near-infrared light minus dark difference spectra for *Chloroflexus*. The solid line is a continuous light-induced spectrum obtained by recording absorption spectra in the dark and under subsaturating blue illumination, followed by computer subtraction. (+) Absorption changes induced by saturating 583 nm laser flashes. The photobleaching at 865 nm was 0.0236 in the continuous light spectrum and 0.0325 in the flash-induced spectrum. The inset shows the kinetics of the flash induced transmittance change at 865 nm. The vertical bar represents 0.02 absorbance change and the horizontal bar represents 100 ms along the time axis. The first order time constant deduced from such measurements is 62 ms.

is due to P^+Q^- minus PQ, then the difference–difference spectra shown in fig.6 reflect mainly the absorption changes accompanying the reduction of the intermediary electron carrier I. The subtraction procedure assumes that the reduction of Q does not contribute significantly to the near-infrared absorption changes. Similar spectra calculated from the picosecond data for *Rps. sphaeroides* [8] have correlated well with the spectra obtained under conditions where I^- can be trapped (continuous illumination at low potential in the presence of cytochrome) [9–11].

The calculated spectrum for I^- minus I for *Chloroflexus* (fig.6) is in general similar to the corresponding spectrum for *Rps. sphaeroides*, exhibiting absorption decreases both in the regions of absorption due to BPh (760 nm) and B (805 nm). This similarity indicates that the intermediary electron acceptor I in *Chloroflexus* is a complex involving interacting BPh and B, as suggested for reaction centers from purple bacteria [9–12]. The results of fig.6 lend credence to the view that the 53 ps spectrum of fig.2B for *Chloroflexus* is due to P^+I^-. The major difference between the transient spectra for the formation of I^- in fig.6 is that for *Chloroflexus* the wavelength separation of the negative features due to BPh and B is about 15 nm greater than in the case of *Rps. sphaeroides*, and the region between is positive.

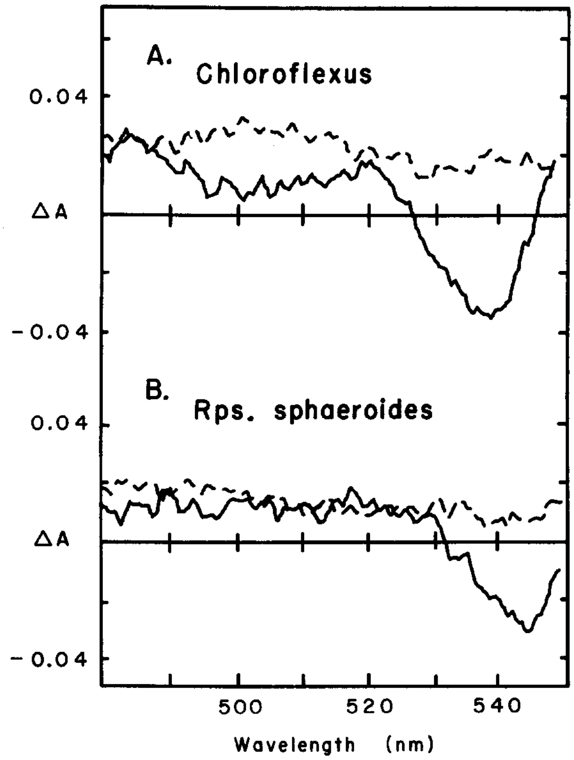

Fig.5. Transient difference spectra at 53 ps delay (——) and 1 ns delay (– – –) in the region of the BPh ground state Q_X band for *Chloroflexus* (A) and *Rps. sphaeroides* (B). The features near 500 nm at 53 ps in (A) may reflect an electrochromic shift on some carotenoid present [21] in the *Chloroflexus* reaction center.

The time constant for conversion of P^+I^- to P^+Q^- is longer in *Chloroflexus* than in *Rps. sphaeroides*. The time constant (1/e) for this step measured at 763 nm in *Chloroflexus* is 310 ± 30 ps (● in fig.7), while measurements at 766 nm in *Rps. sphaeroides* give 200 ± 25 ps (▲ in fig.7). The latter value is in agreement with the results of previous room temperature measurements on *Rps.*

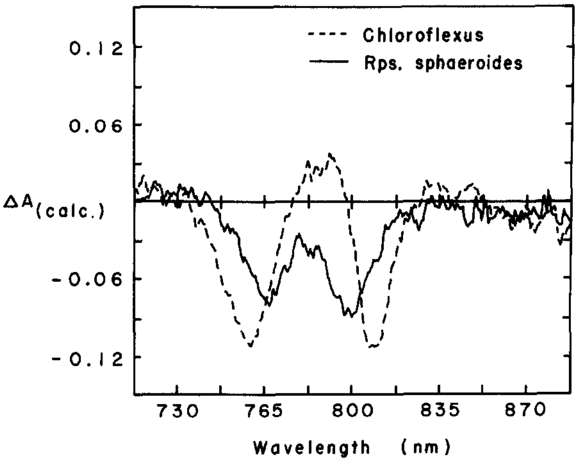

Fig.6. Difference spectra obtained by subtracting the 1 ns transient difference spectrum from that at a 53 ps delay for *Chloroflexus* (– – –) and *Rps. sphaeroides* (——). The data used to calculate these spectra are from fig.2B and 3B, respectively.

sphaeroides and other strains of purple bacteria [1,5–8]. The longer time constant in *Chloroflexus* could reflect either a greater distance between the photoactive BPh and Q than in *Rps. sphaeroides*, or a difference in the energetics for this step in the two types of reaction centers.

There is evidence from the transient spectra during the excitation flash (fig.2A,3A), that the initial time constant for the reduction of I (most probably the reduction of BPh) is slower in *Chloroflexus* than in *Rps. sphaeroides*. The absorption changes at a 13 ps delay for *Rps. sphaeroides* (fig.3A) all differ from those at 53 ps (fig.3B) by a multiplicative factor (because of the ∼35 ps duration of the excitation flash). This observation is consistent with previous picosecond studies placing a time constant of < 10 ps on the reduction of BPh [1,5–8]. However, except for the smaller amplitude, the 13 ps spectrum for *Chloroflexus* (fig.2A) is more similar to the 1 ns spectrum in fig.2B than to the 53 ps spectrum. This is particularly noticeable in the region of BPh ground state absorption near 760 nm. Because we do not know the detailed spectral differences for P* and P^+, at present we cannot tell whether this observation reflects a slower rate of electron transfer from P* to BPh in *Chloroflexus* or the involvement of an intermediary step. It has been suggested that B is an electron carrier prior to BPh in *Rds. rubrum* reaction centers [7].

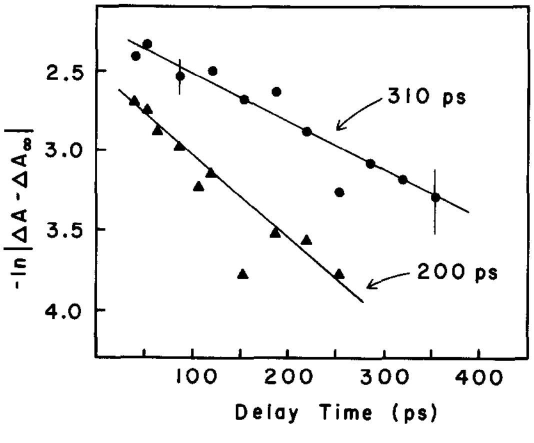

Fig.7. Kinetics for the conversion of state P^+I^- to P^+Q^- in *Chloroflexus* (●) and *Rps. sphaeroides* (▲) reaction centers. The time constants measured at 763 and 766 nm are 310 ± 30 ps (●) and 200 ± 25 ps (▲), respectively.

The apparent blue shift in the 800 nm region is not as great for *Chloroflexus* (fig.2B) as for *Rps. sphaeroides* (fig.3B) and the absorption increase is much narrower. Possibly the fourth BChl in *Rps. sphaeroides* contributes substantially to these absorption changes. This BChl might be analogous to the third BPh in the same or similar environment in *Chloroflexus*. A hypsochromic shift on this BPh due to the oxidation of P (and/or reduction of Q) then might appear in *Chloroflexus* as the substantial increase in absorption at 763 nm and trough at 783 nm (1 ns spectrum in fig.2B). The blue shift near 800 nm in *Chloroflexus*, due to only one BChl, would be less than in *Rps. sphaeroides*, where it could be due to two.

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