Primary Photochemistry in the Facultative Green Photosynthetic Bacterium Chloroflexus aurantiacus

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The mechanism of primary photochemistry has been investigated in purified cytoplasmic membranes and isolated reaction centers of Chloroflexus aurantiacus. Redox titrations on the cytoplasmic membranes indicate that the midpoint redox potential of P870, the primary electron donor bacteriochlorophyll, is $+362$ mV. An early electron acceptor, presumably menaquinone has E_m 8.1 $= -50$ mV, and a tightly bound photooxidizable cytochrome c554 has E_m 8.1 $= +245$ mV. The isolated reaction center has a bacteriochlorophyll to bacteriopheophytin ratio of 0.94:1. A two-quinone acceptor system is present, and is inhibited by o-phenanthroline. Picosecond transient absorption and kinetic measurements indicate the bacteriopheophytin and bacteriochlorophyll form an earlier electron acceptor complex.

Key words: Chloroflexus aurantiacus, primary photochemistry, reaction centers, bacterial reaction centers, bacteriochlorophyll, bacteriopheophytin, menaquinone, ubiquinone

Chloroflexus aurantiacus is a gliding thermophilic green photosynthetic bacterium, capable of living either aerobically or photosynthetically [1]. The 16S rRNA catalog of Chloroflexus indicates that it is only distantly related to either the purple or the anaerobic green photosynthetic bacteria [2]. Phylogenetically, Chloroflexus diverges very early from other photosynthetic procaryotes, and may be very important in tracing the evolution of the photosynthetic energy storage process.

The most prevalent pigment in Chloroflexus is bacteriochlorophyll (BChl) *c*, contained entirely in chlorosomes, oblong antenna bodies appressed to the inner side of the cytoplasmic membrane [3]. The green bacteria do not have the continuous cytoplasmic/intracytoplasmic membrane system commonly found in the purple pho-

tosynthetic bacteria [4]. Instead, all membrane-bound cellular functions are carried out by the cytoplasmic membrane. In spite of the close similarity of the pigment composition and membrane structure in the anaerobic and facultative green bacteria, the primary photochemical mechanism of the two groups appears to be quite distinct. The anaerobic green bacteria contain low-potential Fe-S proteins as early electron acceptors [5,6], which appear to be similar in many ways to the acceptors found in Photosystem I of oxygen-evolving photosynthetic organisms. The facultatively aerobic green bacteria have a cyclic photosystem more similar to that found in the purple bacteria [7]. Some aspects of the primary photochemistry in Chloroflexus are discussed in this report.

MATERIALS AND METHODS

Preparative Methods

Growth conditions were the same as those described by Sprague et al [8]. Isolation of whole membranes and purified cytoplasmic membranes was performed as described by Feick et al [9]. Reaction center isolation was performed using the procedure of Pierson and Thornber [10].

Instrumental Methods

Redox potentiometry was performed as described by Bruce et al [7]. Absorption spectra were obtained on a Cary 219 absorption spectrophotometer. Microsecond [7] and picosecond [11] laser-induced absorbance changes were performed as previously described.

Pigment Analysis

Pigment analysis and extinction coefficient determination were performed essentially as described by Straley et al [12]. Prior to extraction, the reaction center sample was incubated in the dark in the presence of 30 mM ascorbate to ensure that P870 was fully reduced. Small aliquots (50 μl) of a concentrated reaction center sample were extracted into 2.5 ml of acetone, centrifuged at 10,000g for 10 min and the absorption spectrum measured. In some cases, 25 μl of 10 M HCl was added to convert all the pigment to bacteriopheophytin (BPh). Extinction coefficients for extracted pigments and equations for calculating pigment ratios were taken from Straley et al [12]. Only the Qx absorption bands of BChl and BPh were used for the analysis, since apparent small shifts of the absorbance of the Qy band due to the small amount of water present in the acetone extracts gave inconsistent results in the red region.

Gel Electrophoresis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was done according to Laemmli [13] with the following modifications. An 11.5–16.5% linear acrylamide gradient was stabilized by a 3–18% sucrose gradient. The separation gel buffer was adjusted to pH 8.7 and the electrode buffer contained 64.6 gr of glycine per liter. Prior to electrophoresis, samples were diluted 1:2(v/v) with "solubilization-buffer" containing 3.3% SDS, 0.125 M Tris, 6% Mercaptoethanol, and 20% glycerol at pH 6.8 and heated at 65°C for 30 min.
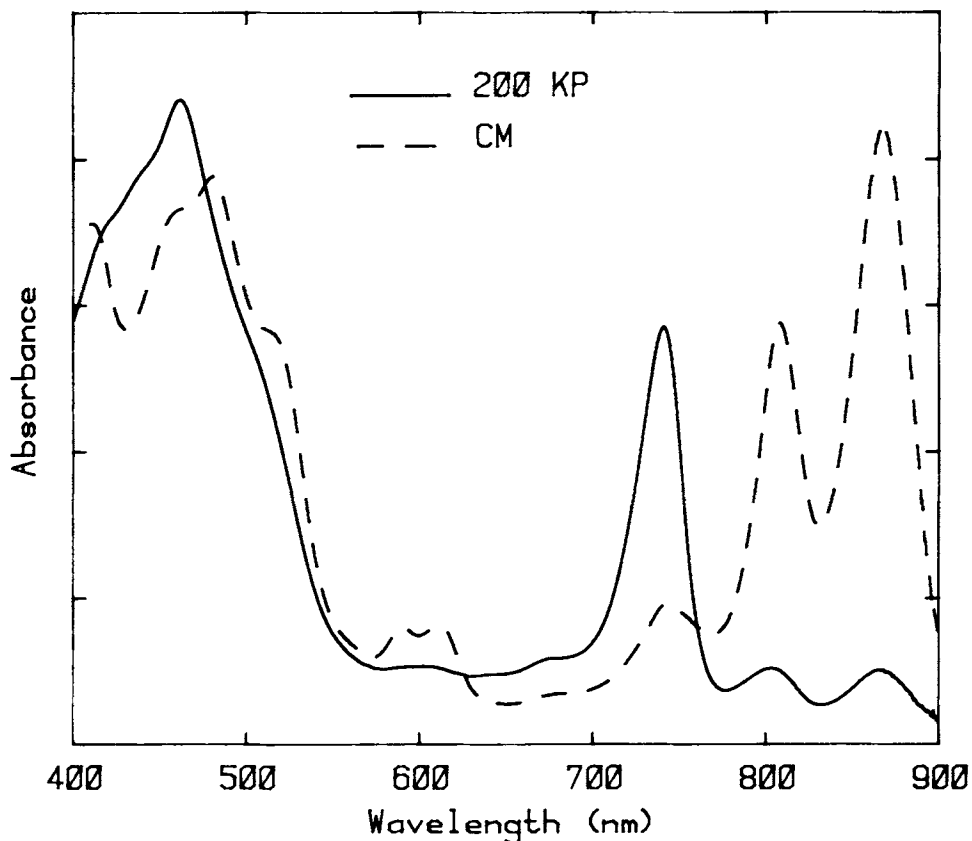
Fig. 1.   Absorption spectra of whole membranes, 200KP (——) and purified cytoplasmic membranes, CM (---) from Chloroflexus.

RESULTS

Absorption spectra of whole membranes (solid line) and purified cytoplasmic membranes (dashed line) are shown in Figure 1. The prominent absorbance at 740 nm in the whole membrane spectrum is due to the large quantity of BChl c contained in chlorosomes. The absorbances at 808 and 865 nm in both spectra are largely due to an antenna Bchl a pigment protein [14,15]. Chloroflexus apparently lacks the water-soluble Bchl a protein found in the anaerobic green bacteria [16,17]. The purified cytoplasmic membrane preparation is devoid of chlorosomes and BChl c yet still retains a tightly bound c-type cytochrome, full photochemical activity, and five respiratory enzyme activities [7,9].

Redox titrations at pH 8.1 of three components of the electron transport chain of Chloroflexus are shown in Figure 2. A titration of 865 nm photobleaching, induced by 1-μs, 583-nm laser flashes is shown in Figure 2A. The data fit a one-electron change with a midpoint redox potential of +362 mv. There is no apparent dependence

on mediator concentration. The apparent fall-off in photooxidation at potentials lower than 300 mv is due to reduction of cyt c554, which rapidly rereduces P870 on the ms time scale of these measurements. A titration of the flash-induced cytochrome photooxidation measured at the α band maximum of 554 nm is shown in Figure 2B. The cytochrome photooxidation at lower potentials is shown in Figure 2C. The data reveal that the cytochrome midpoint redox potential is +245 mv, and that the photooxidation disappears at low redox potentials, reflecting the reduction of an electron acceptor with a midpoint potential of -50 mV. This behavior is very similar to that observed in the quinone acceptor system of purple bacteria and quite distinct from the low potential acceptors found in the anaerobic green bacteria (Chlorobiaceae) [5,6,18].

The absorption spectra of purified reaction centers from Chloroflexus (solid line) and Rhodopseudomonas sphaeroides (dashed line) are shown in Figure 3A and

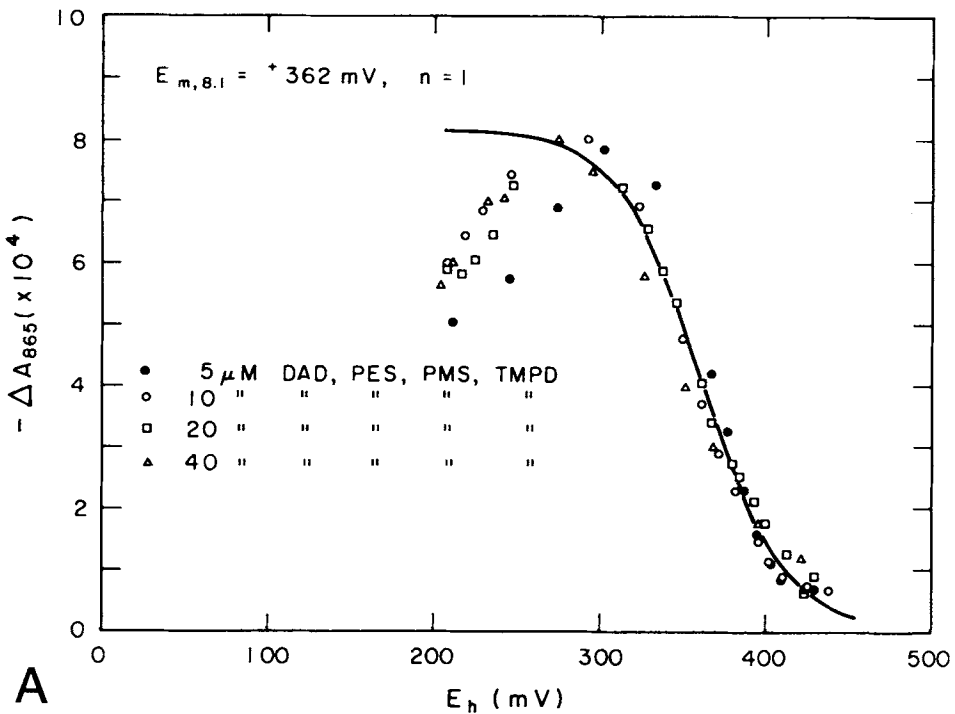Fig. 2. Redox titrations on cytoplasmic membranes from Chloroflexus. A) Titration of P870 photobleaching induced by 1-μs, 583-nm laser flashes. Redox mediators present were: DAD (2,3,5,6 tetramethyl-p-phenylenediamine), PES (phenazine ethosulfate), PMS (phenozine methosulfate), TMPD (N,N,N',N' tetramethyl phenylenediamine). Mediator concentrations were: ●, 5 μM; ○, 10 μM; □, 20 μM; △, 40 μM. The solid line is a theoretical n = 1 Nernst curve for $E_{m,8.1}$ = +362 mV. B) Titration of laser flash-induced cytochrome photoxidation, monitored at 554 nm. Mediators were: PES, PMS, DAD, and TMPD, each at 20 μM. ●, oxidative titration; ▲, reductive titration. The solid line is an n = 1 Nernst curve for $E_{m,8.1}$ = +245 mV. C) Redox titration of acceptor monitored by cytochrome oxidation at 554 nm. Mediators were: TMPD, PES, PMS, duraquinone, and Vitamin K_1, each at 20 μM. ■, oxidative titration; x, reductive titration. The solid line is an n = 1 Nernst curve for $E_{m,8.1}$ = -50 mV.

$E_{m, 8.1} = {}^+245\ mV, \quad n = 1$

B

$E_h\ (mV)$

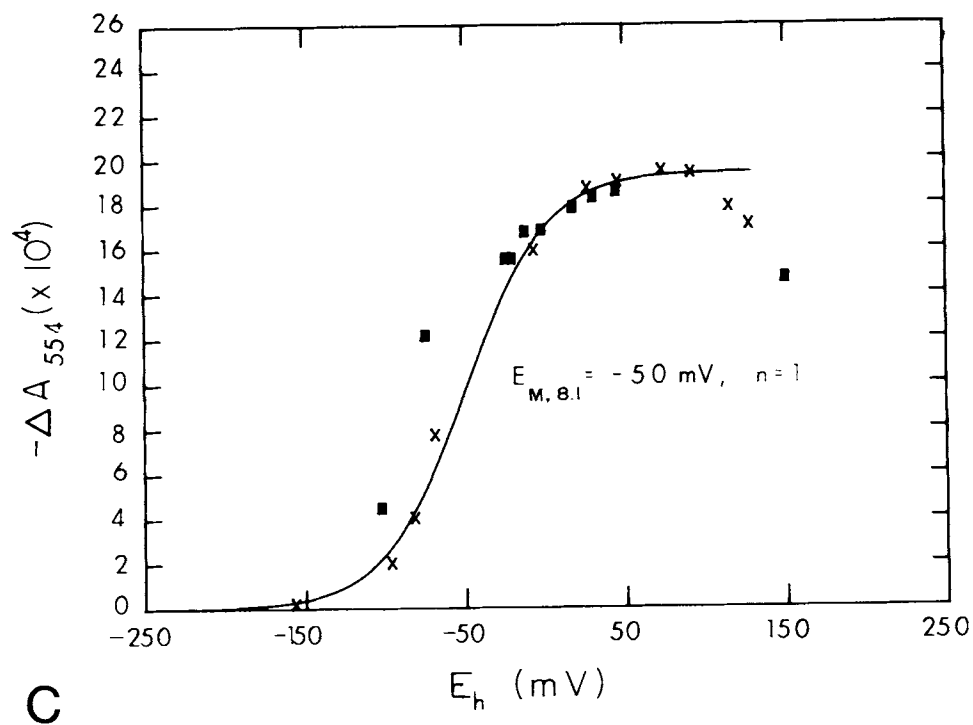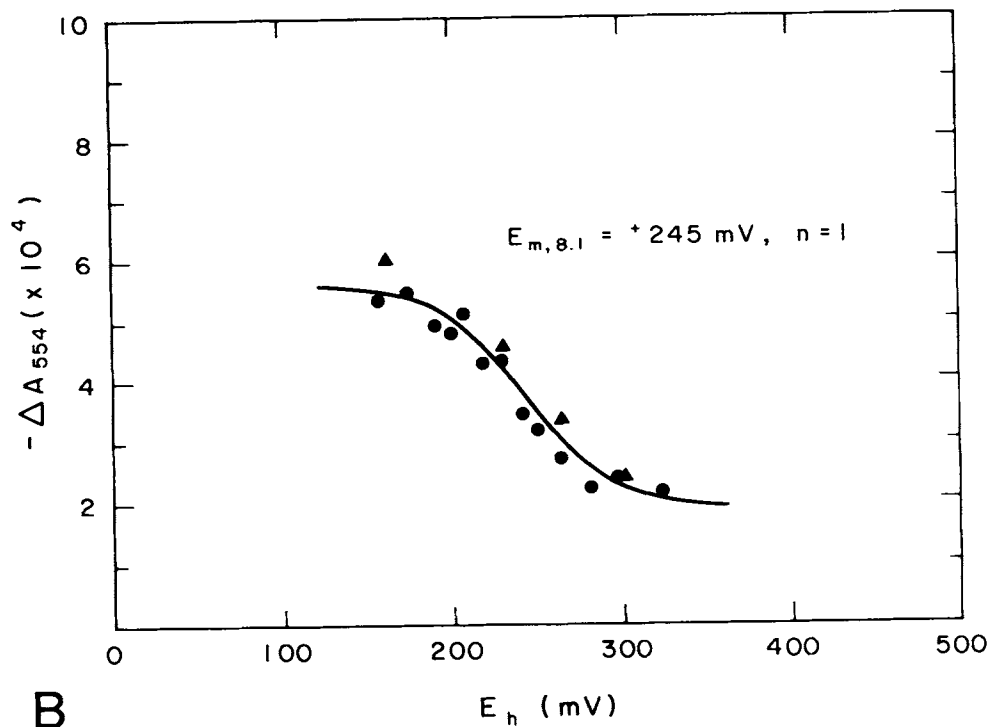$E_{M, 8.1} = -50\ mV, \quad n = 1$

C

$E_h\ (mV)$

Fig. 2. (Continued)

their respective polypeptide profiles after SDS polyacrylamide gel electrophoresis are shown in Figure 3B.

Isolated reaction centers from Chloroflexus heated at 65°C in the solubilization buffer for 30 min exhibited a single major band with an apparent molecular weight of M_r 26,000, similar in its mobility to the M subunit of Rps sphaeroides reaction centers. Upon lowering the temperature to 30–40°C, the minor band seen at the 50,000 molecular weight position increased while the 26,000 band decreased. Under these mild conditions, a pigmented band at M_r 50,000 was observed, which had an absorption spectrum similar to the native complex. It thus seems likely that the smallest functional reaction center of Chloroflexus consists of a complex of two ~26,000 polypeptides and associated pigments.

The principal difference between the two absorption spectra in Figure 3A is the smaller absorbance of the Chloroflexus reaction center in the 800-nm region (813 nm in Chloroflexus) and increased absorbance at 760 nm. These peaks have been assigned to BChl and BPh in the purple bacteria [19]. Pierson and Thornber [10] proposed that the increased BPh absorption and decreased BChl absorption reflected a difference in pigment composition in the reaction centers. Figure 4 shows the results of acetone extraction experiments designed to determine the pigment ratio. Eight determinations (four in neutral solution and four in which the amount of BPh was measured before and after HCl addition) gave a BChl:BPh ratio of 0.94:1 (± 0.1).

The results of an experiment designed to determine if the Chloroflexus reaction center has a two-quinone acceptor system similar to that found in purple bacteria are shown in Figure 5. The kinetics of charge recombination of P870$^+$ and the reduced acceptor were monitored at 865 nm after a 1-μs laser flash. Trace A shows the behavior with no additions; the τ of the recombination is 60 ms. Trace B shows the kinetics after addition of vitamin K_1 (2-methyl-3-phytyl-1, 4-naphthoquinone), where the decay time is lengthened to 550 ms. Addition of o-phenanthroline, a specific inhibitor of the electron transfer between the two quinone acceptors in purple bacteria [20], restores fast electron recombination from the first acceptor to P870. This experiment is strong evidence in favor of a two quinone acceptor system similar to that found in purple bacteria. This confirms the similar conclusion reached by Bruce et al [7] using the cytoplasmic membrane preparation. A naphthoquinone was used in the experiment of Figure 5 since Chloroflexus has been shown to contain menaquinone but no ubiquinone [21].

The possible existence of earlier electron acceptors was investigated using picosecond spectroscopy. Figure 6 shows difference absorption spectra obtained 53 ps and 2 ns after a half saturating 35-ps, 600-nm laser flash. The 2-ns spectrum is similar to that obtained under continuous illumination and reflects primarily the

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Fig. 3.   Absorption spectra and gel electrophoresis of purified reaction centers from Chloroflexus and Rps sphaeroides. A) Absorption spectra, normalized at 865 nm, of Chloroflexus (——) and Rps sphaeroides R-26 (- - -). B) Gel electrophoresis. Lanes 1–4, 11.5–16.5% gradient gel. Lanes 5 and 6, 10% gel. Lane 1, standard proteins; lane 2, Chloroflexus whole membranes; lane 3, Chloroflexus reaction centers; lane 4, Rps sphaeroides reaction centers; lane 5, Chloroflexus reaction centers; lane 6, Rps sphaeroides reaction centers. The molecular weight scale for lanes 1–4 is different than lanes 5 and 6. The L,M, and H subunits of Rps sphaeroides reaction centers are indicated in lane 6.
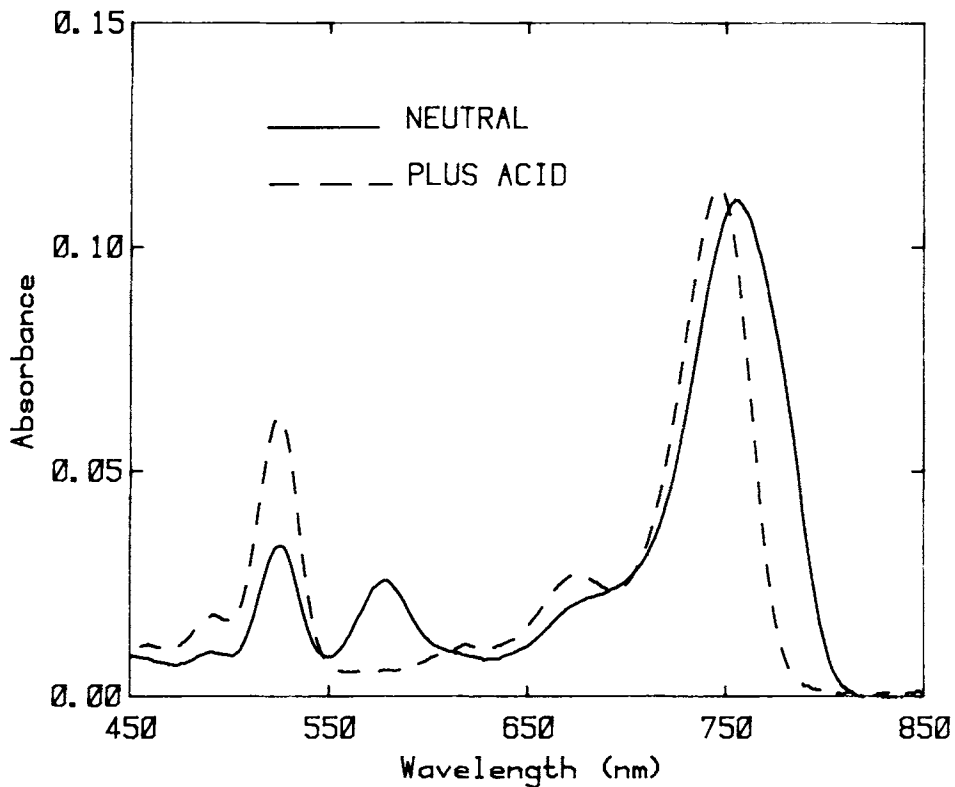
A
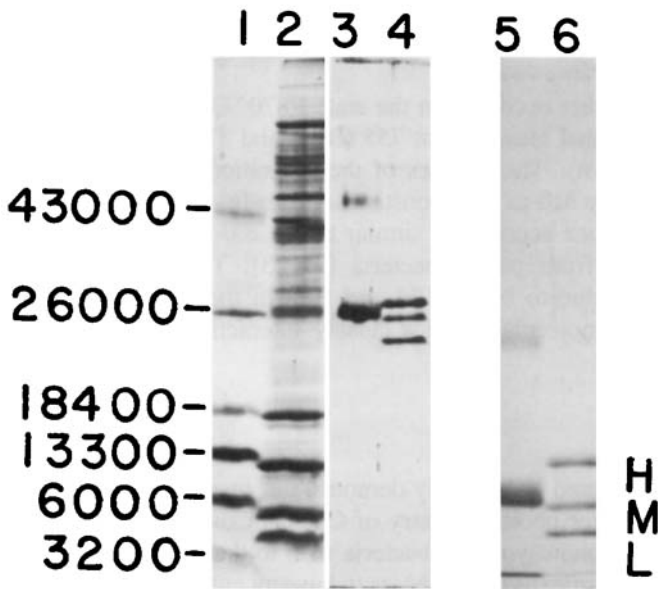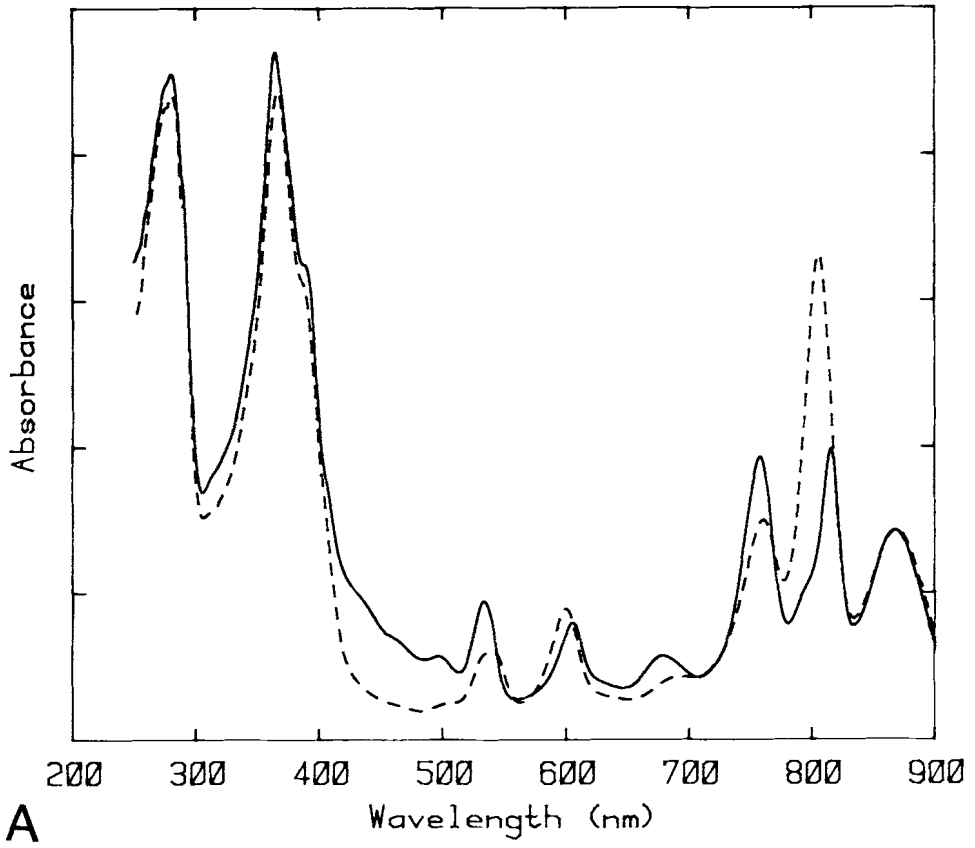
B

Fig. 4.   Absorption spectra of neutral (——) and acidified (---) acetone extracts of Chloroflexus reaction centers. The 522 nm absorbance in both spectra is due to bacteriopheophytin a, while the 576 nm peak in the neutral solution is due to bacteriochlorophyll a. The long wavelength peaks are due to BPh only (acid solution) and BPh plus BChl (neutral solution).

absorption changes that occur when the state $P870^+Q^-$ is formed. The 53-ps spectrum exhibits additional bleaching at 755 (BPh) and 815 (BChl), and also at 535 nm (BPh, data not shown). The kinetics of the relaxation at 763 nm are shown in the inset of Figure 6. The 310-ps time constant for transfer of the electron from the earlier acceptor to the quinone acceptor is similar to the 200-ps time for the analogous step in reaction centers from purple bacteria [22,23]. The observation of absorption decreases in bands due to both BChl and BPh in the 53 ps spectrum of Figure 6 suggests that these molecules form a closely interacting electron acceptor complex [11,24,25].

DISCUSSION

The data presented here clearly demonstrate, in agreement with previous work, that the reaction center photochemistry of Chloroflexus is much more similar to that found in the purple photosynthetic bacteria than to that found in the anaerobic green bacteria [5-7]. Chloroflexus also appears to have an antenna protein whose absorption
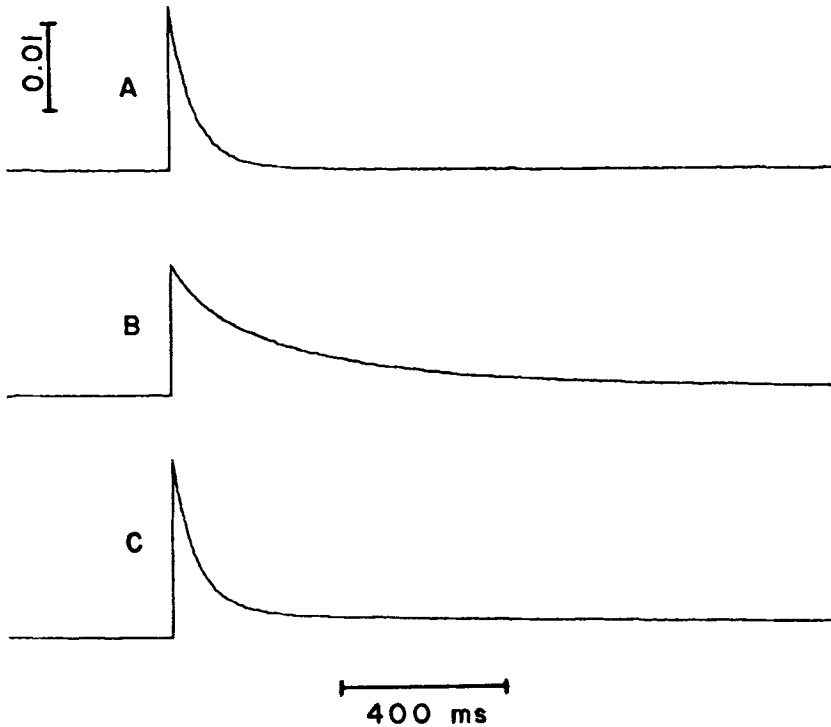
Fig. 5. Kinetics of recombination in Chloroflexus reaction centers. The traces show the absorbance changes at 865 nm induced by saturating 1-μs, 583-nm laser flashes. A) No additions; B) 170 μM vitamin K_1 added; C) 170 μM vitamin K_1 and 1.3 mM o-phenanthroline added. An upward deflection is an absorbance decrease.

spectrum is like the B800-850 complex found in purple bacteria, while the gross membrane structure and composition and the membrane-associated chlorosomes are characteristic of the anaerobic green bacteria.

While the reaction center appears similar to those found in purple bacteria in the overall mechanism of its photochemistry, there are some important differences. Chief among these differences are the peptide and pigment composition of the reaction center. Chloroflexus contains equimolar ratios of BChl and BPh, while in all purple bacteria analyzed the BChl:BPh ratio is 2:1 [12,19]. The peptide composition of Chloroflexus reaction centers is unusual, in that they contain at most two peptides, while purple bacteria usually contain three.

Pierson et al [26] have recently reported a similar polypeptide composition for the Chloroflexus reaction center. While our results are generally in agreement with theirs, there are some slight differences. Pierson et al [26] observed two distinct bands at M_r 26,000 and 30,000, while our gel shows only a single band at M_r 26,000. The difference is not simply one of resolution, since the Rps sphaeroides reaction center polypeptides are resolved equally well in the two gel systems.

The P870 midpoint redox potential is somewhat less positive than the value of $\sim$ +450 mv usually found in the purple bacteria [18]. The photooxidizable cyto-
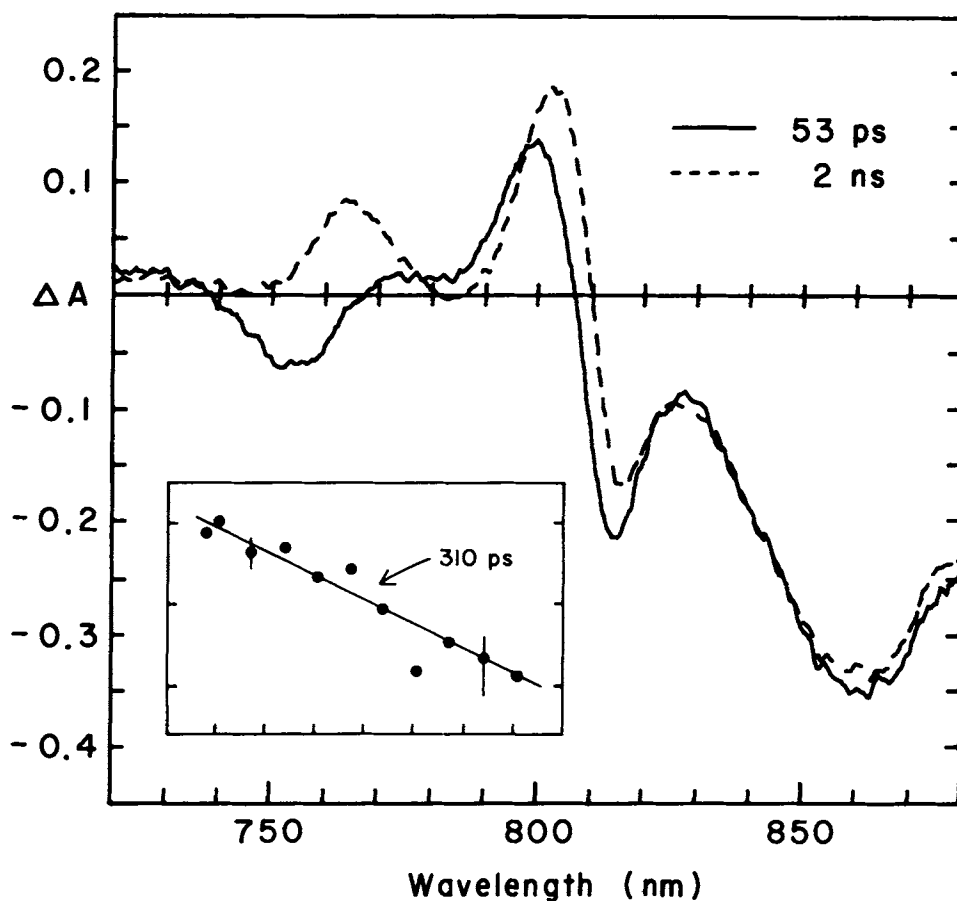
Fig. 6. Absorption difference spectra in Chloroflexus reaction centers induced by half saturating 35-ps, 600-nm laser flashes. Spectra are shown at 53 ps (———) and 2 ns (- - -) after the flash. The inset shows the kinetic behavior at 763 nm; each division along the time axis is 50 ps, and each division along the y axis is 0.5 units of $\ln|\Delta A-\Delta A_\infty|$.

chrome c midpoint potential is also somewhat less positive than the +300–350 mV values found for most c-type cytochromes in photosynthetic bacteria [27]. The cytochrome is unusual in that it is membrane-bound but does not remain bound to the reaction center during reaction center purification as do the tightly bound cytochromes of Chromatium vinosum and Rps viridis [28]. No information is available as to whether cytochrome c-554 has multiple hemes; if multiple hemes are present, the flash-induced redox titrations may be slightly distorted from steady-state titrations [29]. The molecular weight of purified c-554 from Chloroflexus is ~44,000 (Phan Huynh and R. Blankenship, unpublished results).

The picosecond absorption measurements (Fig. 6 and [11]) indicate that BPh and BChl are involved in an early electron acceptor complex. There is some indication at short times (< 10 ps) after the flash that there may be some differences in the

earliest states following photon absorption between Chloroflexus and Rps sphaeroides [11], but further studies are needed to explore this possibility.

ACKNOWLEDGMENTS

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