Sub-picosecond measurements of primary electron transfer in *Rhodopseudomonas viridis* reaction centers using near-infrared excitation

Michael R. Wasielewski and David M. Tiede

Chemistry Division, Argonne National Laboratory, Argonne, IL 60439, USA

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Rate constants for the primary photoinduced electron transfer reactions within the reaction center protein of the photosynthetic bacterium *Rhodopseudomonas viridis* have been measured using transient optical absorption spectroscopy following excitation of the primary donor P960 directly with 950 nm laser flashes. The time resolution of the experiments was 0.45 ps. The reduction of BPheo *b* as indicated by absorption changes at 543 nm exhibits monophasic kinetics with a $1.7 \times 10^{11}\ s^{-1}$ rate constant following direct excitation of P960. Spectral changes at 805 and 835 nm due to perturbations of the intermediary BChl *b* occur synchronously with those at 543 nm. There is no evidence for formation of BChl b^- as a distinct chemical intermediate preceding the reduction of BPheo *b*.

Bacterial photosynthesis *Charge separation* *Ultrafast spectroscopy*

1. INTRODUCTION

The advent of an X-ray structure of reaction center protein crystals from the purple photosynthetic bacterium *Rhodopseudomonas viridis* has ended a long period of speculation as to the detailed placement of the chromophores within the protein (fig. 1) [1]. The chromophores are arranged within the protein along a C_2 symmetry element. Two of the BChl *b* molecules are closely associated spatially and electronically to form the primary donor P960. Another pair of BChl *b* molecules are placed nearly edge on relative to P960. These molecules in turn are adjacent to a pair of BPheo *b* molecules which in turn are next to the quinones.

Earlier spectroscopic studies have shown that excitation of P960 results in the formation of $P960^+$ and BPheo b^- in <8 ps followed by electron transfer from BPheo *b* to Q_A in about 230 ps [2]. The role of the intermediary BChl *b* molecule has remained unclear due to a lack of experimental data. One hypothesis for its role, which is suggested by the crystal structure, is that BChl *b* is reduced by P960 and acts as an intermediate electron carrier prior to the reduction of BPheo *b*. Recently, sub-picosecond spectroscopy has been applied to a similar problem in reaction centers from the related organism *Rps. sphaeroides* [3,4]. These measurements clearly illustrate that BChl a^- does not exist as a distinct chemical intermediate on a time scale of a few hundred femtoseconds prior to the formation of BPheo a^- in that organism. These results contrast with previous claims for the observation of BChl a^- using excitation pulses with 25–30 ps durations [5–9]. The use of such long pulses to determine events which occur on a substantially shorter time scale has been criticized [10]. In this paper we present transient absorption change data and the corresponding

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kinetics of electron transfer from P960 to BPheo *b* in *Rps. viridis* reaction centers for which the primary donor P960 has been directly excited with 950 nm, sub-picosecond pulses.

2. MATERIALS AND METHODS

Reaction centers from *Rps. viridis* were isolated with the detergent lauryl dimethylamine oxide (LDAO) as described [11,12]. The LDAO was replaced by Triton X-100 during chromatography on DEAE-Sephadex, and the reaction centers were eluted with 0.1% Triton, 250 mM NaCl, 10 mM Tris, pH 8. The reaction center concentration was adjusted to yield an absorbance of 0.8 mm^{-1} at 830 nm in 65% (v/v) glycerol. 1 mM sodium ascorbate was added to reduce the reaction center associated high-potential cytochromes *c*-558, which function as efficient electron donors to photo-oxidized P960 at room temperature [13,14]. The 10 Hz, 950 nm actinic laser pulses converted the reaction centers to the P960-Q_A^- redox state [15] and data collection started after a delay of at least 1.5 s.

Sub-picosecond time-resolved transient absorption measurements were obtained as follows: reaction centers were placed in 1 mm pathlength cells. The absorbances of the samples were 0.8 at 830 nm. A 2 mm diameter spot on the sample cell was illuminated with the pump and probe beams of the transient absorption apparatus. The 514 nm output of a mode-locked Ar^+ laser operating at an 82 MHz repetition rate was used to synchronously pump a rhodamine-6G dye laser. Addition of the saturable absorber dye DQOCI [16] to the rhodamine dye solution resulted in 611 nm, 0.4 ps, 0.5 nJ pulses from the dye laser. These pulses were amplified to 1.5 mJ using a 4-stage rhodamine-640 dye amplifier pumped by a frequency-doubled Nd-YAG laser operating at 10 Hz. The resulting 0.45 ps amplified laser pulse was split with a dichroic beam splitter. A 611 nm, 0.45 ps, 0.8 mJ pulse was focused into a 75 cm long high-pressure gas cell containing a 0.5 mm inner diameter capillary waveguide and 900 lb/$inch^2$ of CH_4 gas. A long-pass filter was used to isolate the resulting 90 μJ, 160 fs, 950 nm second Stokes Raman line which emerged from the gas cell. The remaining 611 nm, 0.45 ps, 0.7 mJ pulse was used to generate a 0.45 ps white light continuum probe pulse. Pulse lengths were determined by autocorrelation techniques. The total instrument response function was 0.45 ps. A 10 μJ, 950 nm pulse was used to excite the sample. Thus, the samples were excited with at most 1 photon per reaction center. Typically, 256 laser shots were averaged to obtain the data presented here. Absorbance measurements were made with a double-beam spectrometer which employed optical multichannel detection. Time delays between pump and probe pulses were accomplished with an optical delay line. Time constants for kinetic data were determined by iterative reconvolution using the Grinvald-Steinberg method [17].

3. RESULTS

Excitation of *Rps. viridis* reaction centers with 950 nm laser pulses at room temperature with Q_A reduced results in bleaching of the BChl *b* dimer band at 960 nm. The time response of this bleaching monitored at 950 nm is shown in fig.2. Since depletion of ground state P960 leads to

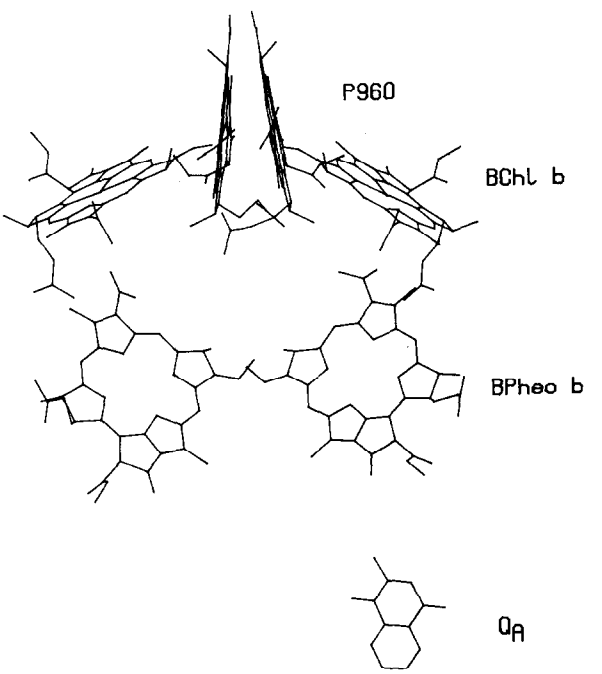


Fig.1. Chromophore placement within the reaction center protein from *Rps. viridis*. The long aliphatic groups of the chromophores have been removed for clarity.

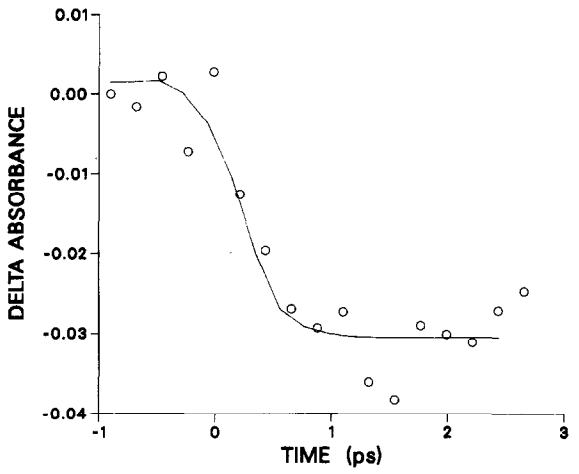
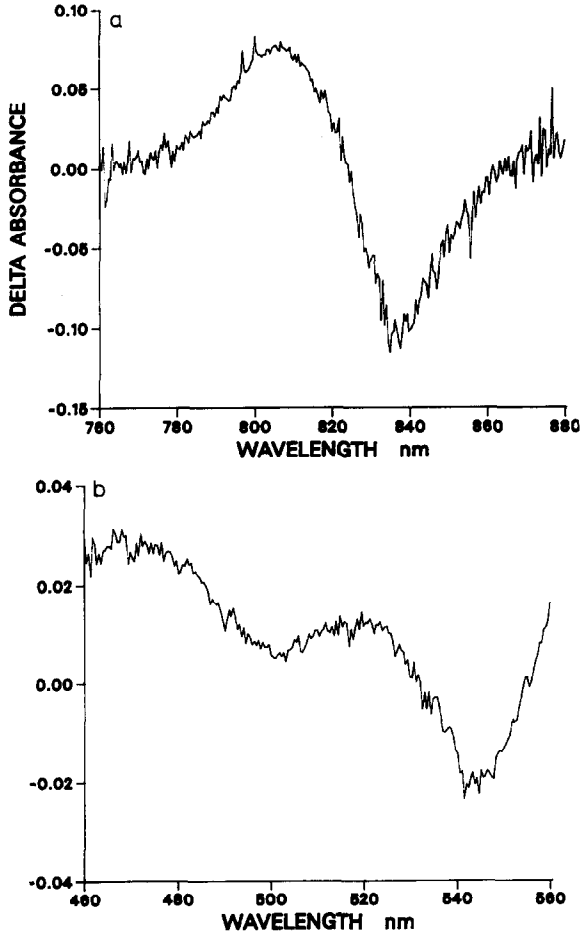


Fig.2. Transient absorption change at 950 nm for *Rps. viridis* reaction centers excited with 950 nm, 160 fs laser pulses.



bleaching of this band, the formation time of the bleach is indicative of the 0.45 ps instrument response time.

Fig.3a,b shows the transient absorption changes that occur in the near infrared and blue-green region of the spectrum, respectively, 25 ps after excitation. The infrared absorbance changes are dominated by a strong positive absorbance change at 805 nm and a strong bleach at 835 nm. These changes involve both the intermediary BChl *b* and the BPheo *b*. On the other hand, the bleach at 543 nm in fig.3b is assigned solely to reduction of the BPheo *b* by electron transfer from P960 [2].

Fig.4a presents the kinetics for the formation of the bleach at 543 nm following 950 nm excitation. The data are fitted smoothly with a single exponential function with $\tau = 6.0 \pm 0.9$ ps. There is no indication of additional kinetic processes occurring within the 0.45 ps time resolution of the measurement. Figs 4b,c shows respectively the positive absorbance change at 805 nm and the appearance of the bleach at 835 nm following the 950 nm laser flash. These data can also be fitted quite well with single exponential functions that yield $\tau = 6.1 \pm 0.9$ ps at 805 nm and 5.8 ± 0.9 ps at 835 nm. Thus, the observed absorption changes at 543, 805 and 835 nm exhibit the same kinetics within experimental error.

4. DISCUSSION

The infrared absorption changes observed with 950 nm excitation are all very similar to those observed for *Rps. viridis* reaction centers previously [18]. The absorption change at 543 nm due to reduction of BPheo *b* has never been reported on this time scale. However, the position of the bleach agrees well with that reported on a nanosecond time scale [2].

The synchronous appearance of the bleach at 543 nm and the spectral changes at 805 nm and at 835 nm suggest that the near-infrared spectral changes at 835 nm are primarily due to perturbation of the electronic structure of BChl *b* by the formation of $P960^+$ BPheo b^-. The perturbation

Fig.3. Transient absorption spectrum of *Rps. viridis* reaction centers obtained 25 ps after 950 nm, 160 fs laser pulse excitation: (a) near-infrared spectral region, and (b) blue-green spectral region.

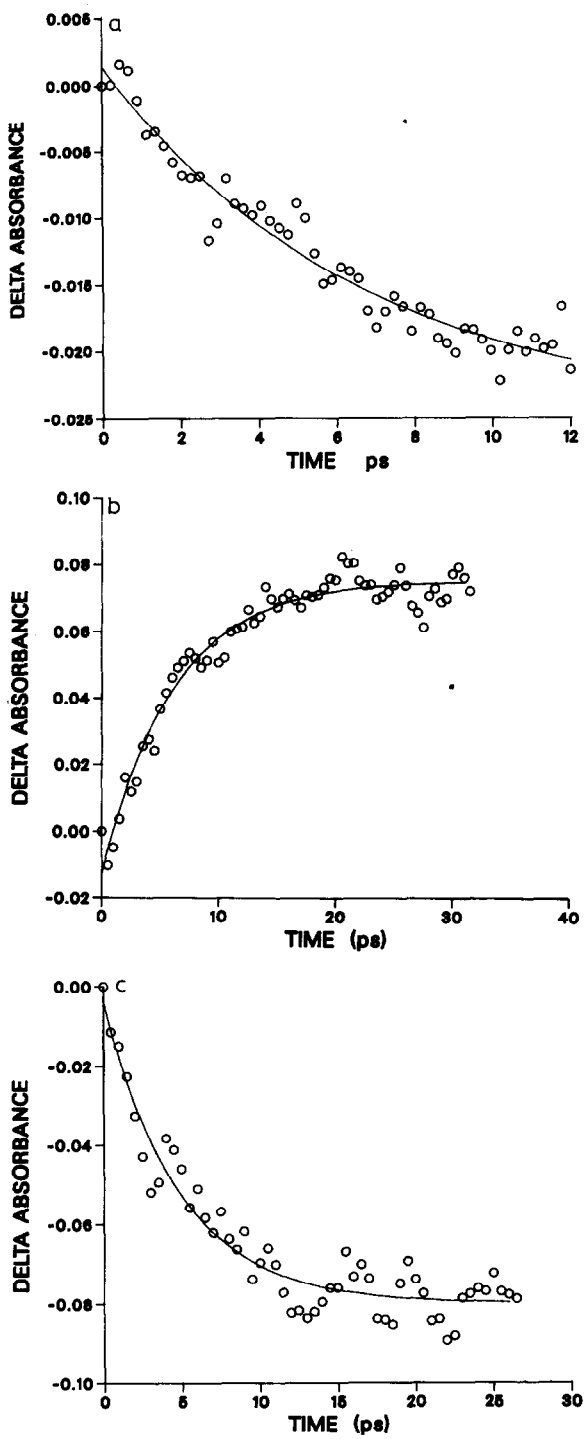


Fig.4. Transient absorption changes of *Rps. viridis* reaction centers following 950 nm, 160 fs laser pulse excitation monitored at (a) 543 nm, (b) 805 nm, and (c) 835 nm.

of the optical absorption spectrum of the BChl *b* molecule by the formation of BPhe b^- has also been seen in spectra obtained for the reduced acceptor I^- obtained previously [19]. In these spectra a large 835 nm bleach or bandshift is observed adjacent to a much smaller bleach at 800 nm due to formation of BPheo b^-.

The role of the intermediary BChl *b* molecule in *Rps. viridis* reaction centers may be to lower the overall energetic requirements for electron transfer by strong mixing of its π-π^* states with those of P960 or by the formation of charge transfer states involving P960. Similar interactions may occur between the BChl *b* and BPheo *b* molecules. The exact nature of these interactions remains to be determined. Nevertheless, while our data suggest that the BChl *b* and BPheo *b* molecules are strongly coupled electronically, direct excitation of P960 results in reduction of BPheo *b* without the intermediate formation of BChl b^- as a distinct chemical intermediate with a lifetime longer than 0.45 ps.

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