Transient Absorption Spectroscopy of Energy-Transfer and Trapping Processes in the Reaction Center Complex of *Chlorobium tepidum*

Hirozo Oh-oka,[†] Shoichiro Kamei,[†] Hiroshi Matsubara,[‡] Su Lin,[§] Paula I. van Noort,[§] and Robert E. Blankenship*[,§]

Department of Biology, Graduate School of Science, Osaka University, Osaka 560, Japan; Department of Biochemistry, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700, Japan; and Department of Chemistry and Biochemistry and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe, Arizona 85287-1604

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Reaction center complexes from the green sulfur bacterium *Chlorobium tepidum* were examined using picosecond absorption difference spectroscopy at room temperature. The complexes include the core proteins containing the primary electron donor P840 and associated core antenna pigments and also include some of the Fenna−Matthews−Olson (FMO) antenna protein. Upon excitation at 590 nm, long-lived absorption difference changes were observed that are interpreted to arise from a combination of the excited FMO protein and the P840-containing complex. Most of the FMO protein does not transfer energy to the core in this preparation. Excitation at 840 nm directly into the core complex gave an overall excited-state decay of 35 ps, forming the long-lived charge-separated state $P840^{+}A_0^{-}$ and no excited FMO complex. No antenna components were observed with absorption at wavelengths longer than the primary donor, in contrast to the situation in the related reaction centers from heliobacteria and photosystem I.

Introduction

Recent evidence strongly suggests that the reaction center of green sulfur bacteria is structurally and functionally related to photosystem 1 of cyanobacteria and higher plants (for reviews, see refs 1−3). On the acceptor side, they contain chlorophyll *a*-like pigments as primary acceptors and iron−sulfur centers as secondary and/or tertiary electron acceptors, respectively. The most prominent feature is that green sulfur bacteria appear to possess a homodimeric core complex, which forms a clear contrast to most other reaction centers with heterodimeric structure.[4] This is also the case for the heliobacteria.[5]

The initial energy-transfer processes and subsequent charge separation steps in photosynthetic systems have been studied extensively with ultrafast laser techniques. Although data concerning the energy-trapping processes have been accumulated in photosystems 1 and 2, purple bacteria and heliobacteria,[6] similar data in green sulfur bacteria are scarce. The major reason is the difficulty of preparing samples with stable charge separation activity as well as the presence of the large chlorosome antenna complex. The earlier measurement performed on the picosecond time scale (35 ps excitation flash) was that by Nuijs et al.,[7] who measured the reoxidation of the primary electron acceptor, now known to be a chlorophyll *a*-like pigment absorbing at 670 nm,[8] in membrane vesicles of *Prosthecochloris aestuarii*. Kramer et al.[9] have recently studied time-resolved difference absorption spectra using membranes of *P. aestuarii* with an improved time resolution of a few picoseconds and observed trapping of excitation energy by the reaction center core which exhibited a time constant of about 30 ps.

In this paper, we have studied picosecond transient absorption kinetics using the stable reaction center complex isolated from *Cb. tepidum*.[10] This preparation contains the reaction center protein, some of the FMO antenna protein, and a protein that contains iron sulfur centers and bound monoheme cytochromes. We describe here the initial excitation distribution within the core antenna system connected to the antenna BChl *a* of the FMO protein and the subsequent exciton trapping. We also interpret the kinetic components in the context of those obtained recently in photosystem I[11] and *Hc. mobilis*.[12]

Materials and Methods

Cb. tepidum cells were grown according to Wahlund et al.[13] in 1.2 L bottles for 2 days under tungsten lamps at 43 °C. The reaction center complex was isolated as reported recently by Oh-oka et al.[10] The samples were diluted with a buffer (50 mM Tris-HCl (pH 8.0), 2 mM DTT, 2 mM sucrose monolaurate, and the protease inhibitors (1 mM EDTA, 1 mM *p*-aminobenzamidine, 1 mM 6-amino-*n*-capronic acid, 0.2 mM PMSF, 2 μM E-64, 1 μM pepstatine)) supplemented with the glucose oxidase/catalase system (at a final concentration of 2 u/mL glucose oxidase, 20 u/mL catalase, 20 mM glucose) and 50 μM phenazine methosulfate (PMS). The samples were stable for up to 1 month at −80 °C. The absorption spectra of the samples were measured before and after the laser experiments and were unchanged.

Transient absorption difference spectra on the millisecond time scale were measured on a single-beam spectrophotometer as described in Kleinherenbrink et al.[14] Saturating excitation light at 532 nm with a pulse duration of 5 ns (fwhm) was provided by the frequency-doubled output of a Q-switched Nd:YAG laser (Surelite, Continuum). The measuring light was obtained from a 250 W tungsten halogen lamp and a mono-

* Corresponding author. Fax (602) 965-2747, e-mail Blankenship@asu.edu.
[†] Osaka University.
[‡] Okayama University of Science.
[§] Arizona State University.

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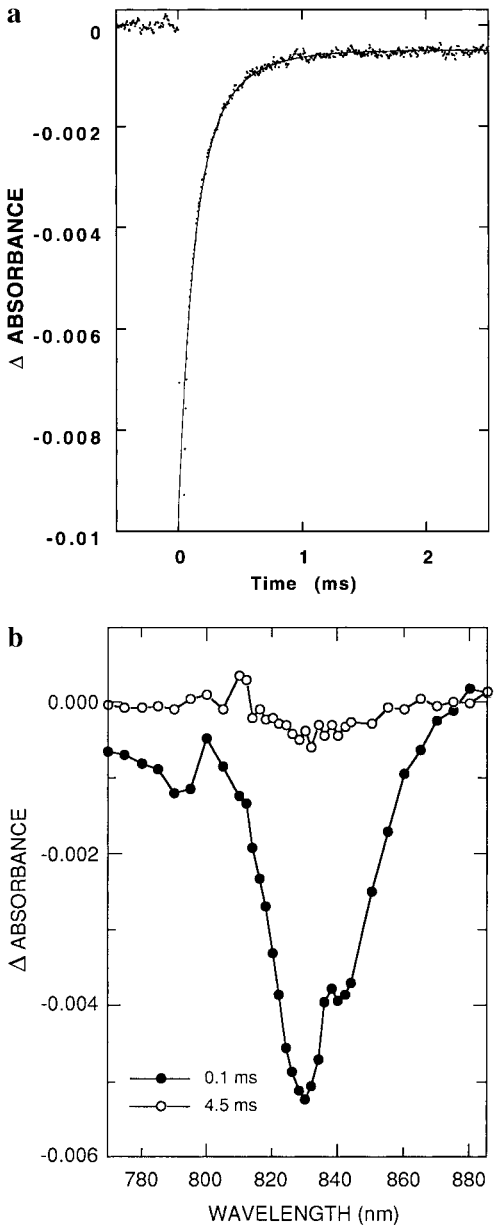

Figure 1. (a) Kinetic trace of the absorbance change at 830 nm from the reaction center complexes of *Cb. tepidum* at room temperature. The excitation was at 532 nm with pulse duration of 5 ns. The solid line is a theoretical fit with two exponentials plus a constant. The two exponentials have decay times of 116 μs (66%) and 331 μs (33%). (b) Absorbance difference spectra recorded at 0.1 and 4.5 ms.

chromator (SPEX 1681). The sample was contained in a 1 cm path length cuvette with a typical optical density of 1.5 at 810 nm.

The setup for transient absorption change measurements on the picosecond time scale was described in Lin et al.[12] and Hastings et al.[11,15] The excitation pulse was set at 590 or 840 nm with a pulse duration of 200 fs and a repetition rate of 540 Hz. The intensity of the excitation pulse was reduced using neutral density filters to avoid singlet−singlet excitation annihilation and had an energy of 1−3 μJ pulse^{-1}. The broadband probe and reference beams were sent through the excited and unexcited areas of the sample, respectively, and were set at the magic angle with respect to the polarization of the excitation pulse. Samples were loaded in a spinning cell with an optical path length of 2 mm. The cell was rotated at 2 Hz to ensure that a fresh sample area was excited by each excitation pulse. A typical optical density of the sample in the spinning cell was 1.5 at 810 nm.

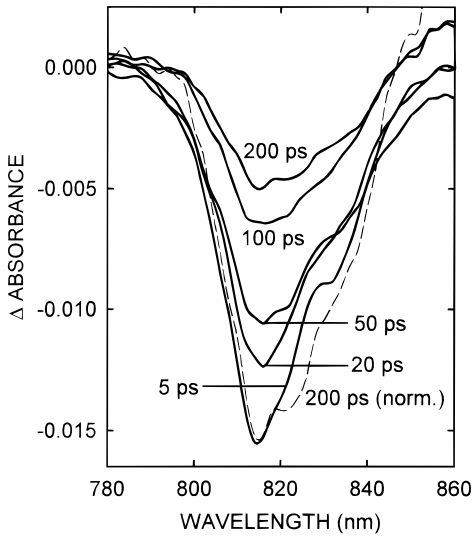

Figure 2. Time-resolved absorbance difference spectra of *Cb. tepidum* at room temperature using 590 nm excitation with 200 fs pulse duration. Spectra were recorded at 5, 20, 50, 100, and 200 ps after the laser excitation, as indicated. Also shown is the 200 ps difference spectrum after normalization to the maximum of the 5 ps difference spectrum (dashed line). The spectral resolution was 2 nm.

Results

Absorbance Changes on a Millisecond Time Scale. Millisecond pump−probe experiments were performed on the reaction center complex of *Cb. tepidum* at room temperature using saturating 532 nm excitation pulses with pulse duration of 5 ns. A kinetic trace at 830 nm recorded on a millisecond time scale is shown in Figure 1a. A fast decay with lifetime of about 0.1 ms and a slow decay of 30 ms were obtained from a two-exponential fitting.[16] Figure 1b shows spectra recorded at 0.1 and 4.5 ms constructed from the kinetic traces taken at various wavelengths. The spectrum at 0.1 ms has a bleaching maximum at 830 nm and a shoulder at about 840 nm. A small bleaching around 790 nm is also seen. The spectrum at 4.5 ms shows a decrease of the overall amplitude around the 830 nm bleaching band. According to previous work,[10] the fast phase is due to cytochrome donation and the slower phase is due to recombination.

Absorbance Changes with 590 nm Excitation. Transient absorbance changes were measured with picosecond time resolution using 200 fs excitation pulses at 590 nm. Time-resolved spectra at different time delays are plotted in Figure 2. The initial bleaching is centered at 815 nm with a shoulder at 835 nm. The spectrum at 5 ps presents mostly the excited state of the antenna molecules. The spectrum taken at 200 ps shows more than 60% recovery of the bleaching band. However, the spectral profile is still very similar to that taken at 5 ps as shown in Figure 2 by comparison of the spectra at 5 and 200 ps after normalization at the maximum. There is a very slow further recovery (probably on the nanosecond time scale) of the absorbance change, and the spectral profile remains basically the same on the time scale of hundreds of picoseconds. This spectrum shows significantly different features from the one obtained on the millisecond time scale (Figure 1b). The result indicates that 590 nm excitation excites some molecules absorbing at wavelength shorter than 830 nm which do not decay on the nanosecond time scale.

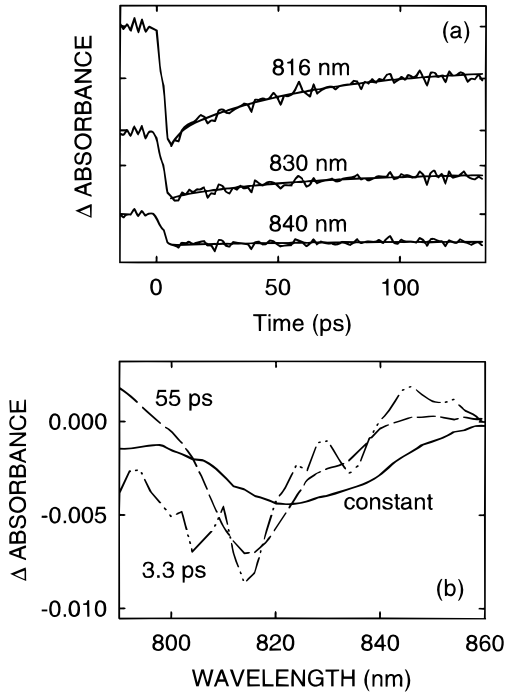

Figure 3. (a) Kinetics at 816 nm (upper), 830 nm (middle), and 840 nm (lower) with 590 nm excitation. The smooth curves are the theoretical fittings using three kinetic components. The time resolution is 1.5 ps. (b) Decay associated spectra obtained from global analysis.

Figure 3a shows kinetic traces at 816, 830, and 840 nm after the 590 nm excitation with theoretical fitting curves at the corresponding wavelengths. Global analysis of the absorbance change surface on the 150 ps time scale gives three kinetic components: 3.3 ps, 55 ps, and a nondecaying component. The decay associated spectra are plotted in Figure 3b. The 3.3 ps component shows negative amplitude at wavelengths shorter than 840 nm and positive amplitude longer than 840 nm which represents excitation energy transfer and the buildup of the stimulated emission within the antenna system shortly after the laser excitation. Similar processes were also observed in heliobacteria[12] and in photosystem I.[11] The 55 ps component shows a negative band at 815 and 835 nm which is probably due to the decay of the excitation energy within the antenna system. The nondecaying component has a broad band centered around 820−830 nm. Its profile is similar to that of the time-resolved spectra measured at 100 ps and later but is different from the spectrum taken on the millisecond time scale.

Absorbance Changes with 840 nm Excitation. To observe energy transfer from the core antenna to the reaction center, samples were excited at 840 nm, which is directly into the core antenna of BChl *a* and the reaction center. The process of excitation redistribution among different absorption bands was observed from the spectral evolution during the first few picoseconds shown in Figure 4a. The spectra taken at early times (0−0.6 ps) show a major bleaching band centered at 835 nm and a broader band at 820 nm. The amplitude of the 820 nm band increased more than that of the 835 nm band at later times (1.2−3.2 ps), indicating that more excitations are distributed in this band. The spectral profile at 3.2 ps with 840 nm excitation is significantly different from that with 590 nm excitation (compare with the 5 ps spectrum shown in Figure 2); the latter shows a major bleaching at 815 nm and a shoulder at 835 nm.

Time-resolved spectra taken at later times (on 200 ps time scale) with the 840 nm excitation are shown in Figure 4b. There

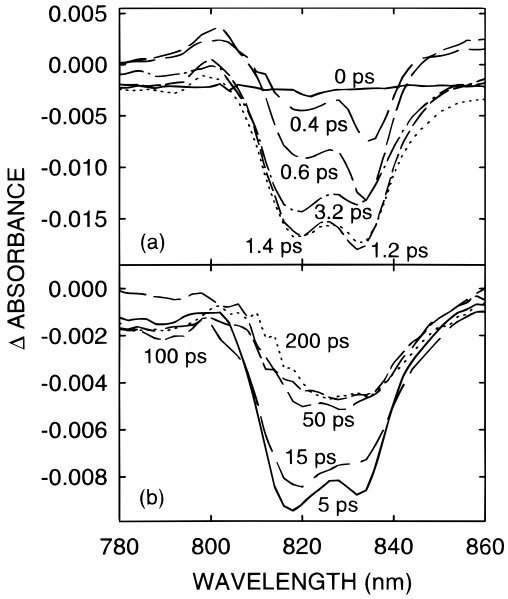

Figure 4. Time-resolved absorbance difference spectra of *Cb. tepidum* at room temperature using 840 nm excitation (a) at early times and (b) at later times.

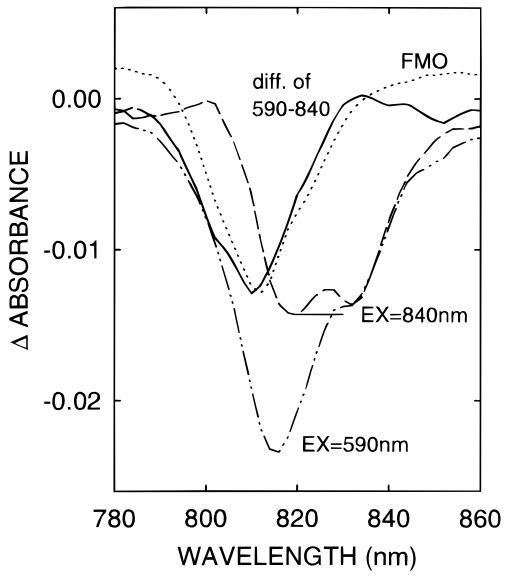

Figure 5. Comparison of the time-resolved spectra recorded at 5 ps after the laser excitation with 590 nm excitation (dot-dot−dashed line) and 840 nm excitation (dashed line). These two spectra were normalized at 835 nm. The solid line is the difference spectrum obtained by subtracting the spectrum with the 840 nm excitation from the one with the 590 nm excitation. The dotted line is the time-resolved spectrum of isolated FMO protein with 590 nm excitation. These two spectra were normalized at 810 nm.

is a fast absorbance recovery up to 50% of the initial bleaching within the first 100 ps, and the spectrum remains essentially unchanged after that time. The spectrum at 200 ps has a little broader features than that measured at 0.1 ms, with major bleaching bands at 825 and 835 nm and a weak band around 790 nm.

The Spectral Difference between 590 and 840 nm Excitations. Spectra taken at 5 ps with 590 and 840 nm excitations are compared in Figure 5. The two spectra were normalized at 835 nm. A very good correspondence can be seen at 835 nm and longer wavelengths. The difference spectrum of 590−840 nm excitation shows a bleaching band centered at 812 nm. This difference spectrum is very similar to the absorbance change

spectrum measured from purified FMO protein at the same time delay. The excited-state kinetics of the FMO protein has been determined to be multiexponential, with one lifetime of 60 ps and a second one on the order of 1−2 nanoseconds.[17] Global analysis has shown that the ratio of the amplitudes of the two kinetic components at 810 nm is about 4 to 6. Similar kinetic decay components (80 and 1400 ps) have recently been obtained upon flash excitation of the FMO protein isolated from *P. aestuarii*.[9] When the sample was excited at 590 nm, part of the excitation was into the FMO protein, about 60% of which does not decay on the time scale measured. Therefore, even at 200 ps after the laser excitation, there are still some excitations in the antenna system that are not transferred to the reaction center to form charge-separated states. This explanation agrees with the measurement shown in Figure 2 in which the time-resolved spectrum at 200 ps still has a major bleaching band at 815 nm. It is suggested that, with the 840 nm excitation, the excitation is most directly into the core antenna and the reaction center, with no excitation into the FMO protein, which has negligible absorption at 840 nm. The absorbance changes taken at early times represent the excited antenna molecules, and the later one is mostly due to the formation of the charge-separated state $P840^+$.

The Kinetics of the Formation of Charge-Separated State. Figure 6a shows kinetic traces in the Q_Y transition band region with 840 nm excitation. At 816 nm, a bleaching is observed within the time resolution of 2.5 ps per point. The recovery of this bleaching is biphasic. The bleaching at 840 nm is essentially constant. Global analysis in the wavelength region from 800 to 860 nm returns at least two kinetic components: a fast component of 35 ps and a nondecaying phase. The fast component with 3 ps lifetime as in the 590 nm excitation measurement was not observed. It is possible that the energy redistribution is faster when excited at 840 nm or that the faster phase was not observed because the time resolution was 2.5 ps instead of the 1.5 ps in the 590 nm excitation case. The decay associated spectrum (shown in Figure 6b) with the 35 ps lifetime has negative bands centered at 815 and 830 nm and positive amplitudes at wavelengths shorter than 805 nm and longer than 840 nm. This component most likely represents the decay of the excitation within the core antenna system. The decay-associated spectrum of the long-lived component shows a broad band peaking between 825 and 835 nm, which is compared in Figure 6c with the $P840^+$/P840 difference spectrum (see Figure 1b) by normalizing at 830 nm. There is probably little contribution of A_0^- in this spectral region, because it absorbs maximally at 670 nm.[8]

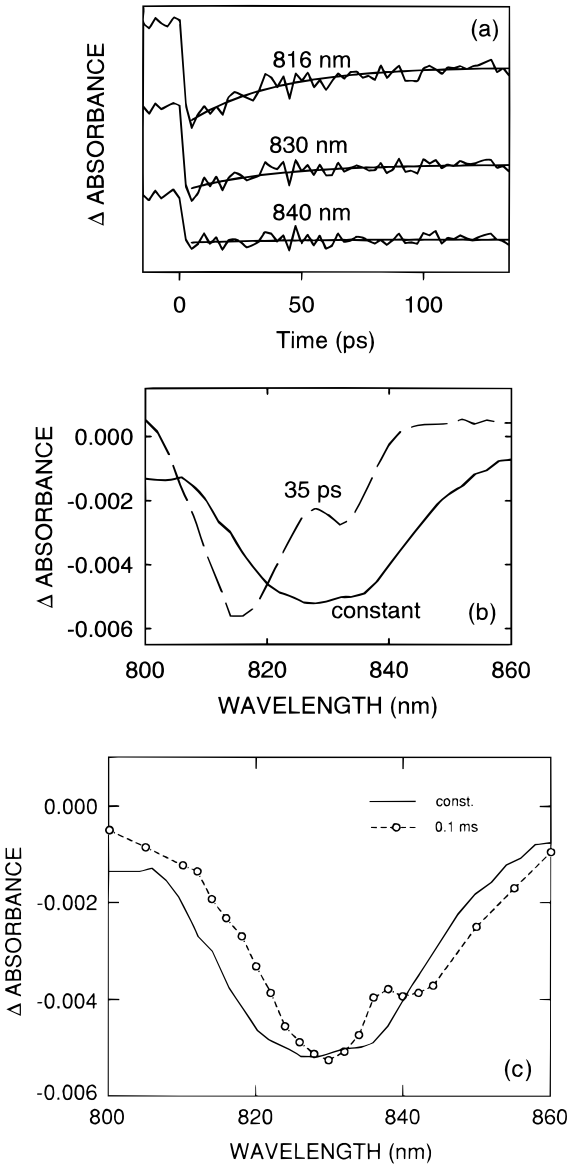

Figure 6. (a) Kinetic traces of the absorbance change in the Q_Y transition region with the theoretical fittings at corresponding wavelengths (see text for details): upper panel, kinetics at 816 nm; middle panel, kinetics at 830 nm; lower panel, kinetics at 840 nm. The excitation wavelength was at 840 nm. The time resolution of the kinetic curve is 2.5 ps per point. (b) Decay associated spectra of *Cb. tepidum* obtained from global fitting of the absorbance change surface. The wavelength region was from 800 to 860 nm with a spectral resolution of 2 nm. The time scale was 250 ps with 2.5 ps resolution. (c) Comparison of the nondecaying spectrum shown in (b) (solid line) and the $P840^+$/P840 difference spectrum shown in Figure 1b (dashed line). Both spectra were normalized at 830 nm.

Discussion

Energy Transfer from FMO to Reaction Center. Since the *Chlorobium* reaction center preparation used here contains the FMO protein, an efficient energy transfer had been anticipated to occur from the FMO antenna BChl *a* to the core antenna. However, when the sample was excited at 590 nm, the excited spectrum at 5 ps, which shows a major 815 nm bleaching band, decayed to a constant level on the hundreds of picoseconds time scale with no marked change of the spectral shape (Figure 2). The bleaching at 815 nm can be ascribed in large part to a transient absorption spectrum of the excited FMO protein, as revealed in the spectral analysis shown in Figure 5. This indicates clearly that a part of the excited energy within the FMO protein was not transferred to the core antenna or reaction center and decayed by itself with two kinetic components (60 ps and 1−2 ns) as in the isolated FMO protein.[17] As for the sample used here, the FMO protein is copurified with the fraction rich in the photoactive reaction center P840. However, the interaction between the FMO and core proteins might be disturbed during purification or by the harsh treatment such as solubilization with the detergents, resulting in the breaking of the energy link between them. The efficiency of energy transfer from the FMO protein to the core has recently been shown to be 35% in a detergent-solubilized FMO−core complex from *Cb. tepidum* and also 10−20% even in the isolated membranes from *P. aestuarii* at low temperature.[18] Zhou et al.[17] have also reported the redox effects on the laser-flash absorption kinetics of the FMO protein, although the function of the so-called redox active groups within its protein, whose X-ray structure has recently been resolved at a 2.2 Å resolu-

tion,[19] remains to be determined. There might be, therefore, another possibility that the redox regulation system exists to adjust energy-transfer efficiency from the FMO antenna to the core antenna pigments. However, reducing conditions seemed to have no effect to recover the high energy-transfer efficiency between them.[18] It will therefore be necessary to study how the energetic coupling between the FMO protein and the core is controlled in more detail, especially with respect to distance and/or orientation among the antenna pigments to each other.

With the excitation at 840 nm, which is only into the reaction center core, the excitation redistribution proceeded within a few picoseconds, definitely exhibiting energy transfer from the 835 nm absorption band to the 820 nm one (Figure 4b). These two bands may correspond to the 837 and 818 nm ones, respectively, in the calculated low-temperature absorption spectrum of the core complex.[18] The excited state decayed subsequently with the time constant of 35 ps concomitant with the formation of the primary charge-separated state (see below). The excited energy never appears to be transferred back into the FMO antenna system. This is likely due to the fact that most of the FMO protein seems to be kinetically uncoupled from the core, as observed in Figures 2−5. This step is also energetically unfavorable.

An interesting difference between the green bacterial reaction centers examined in this paper and those of both photosystem I and heliobacteria is that the latter two systems each contain long wavelength absorbing pigments as minor components in the antenna system, while our data show little evidence for their existence in *Cb. tepidum*. These pigments have maximum absorption at wavelengths longer than the photoactive chlorophyll. The origin and possible function of these long-wavelength pigments have been the subject of considerable discussion.[6]

Charge Separation Process. The global analysis of spectral changes and kinetics after the excitation at 840 nm revealed two components: a fast decay component of 35 ps and a nondecaying one, respectively (Figure 6b). The spectrum of the 35 ps component which displays the 815 nm bleaching band with a rather prominent shoulder at 830 nm results from the overall decay of excitation in the core antenna due to trapping in the reaction center. This time constant ($\tau_{ex} = 35$ ps) is comparable to those previously reported for the reaction center of *Hc. mobilis*[12,20] and photosystem I.[11,21] This time represents a combination of energy and electron transfer. If the number and spectral distribution of the antenna pigments and spectral properties of the trap are known, the intrinsic time for electron transfer can be estimated.[22−24] The presence of the apparently kinetically uncoupled FMO protein in the purified green bacterial reaction center complex makes such a determination uncertain so we have not made such a quantitative estimate with the current set of data. However, the similarity of the excited-state decay time and the fact that there are a similar number of core antenna pigments suggest that the intrinsic electron-transfer time constant will be of the same order of magnitude in the green sulfur bacteria as in heliobacteria and photosystem I, where times of 1−2 ps have been estimated.[11,12,21]

The shapes and kinetics of the transient spectra after laser excitation at 590 nm somewhat resemble those reported in membrane vesicles ("complex I") of *P. aestuarii* [7] which were measured with 35 ps excitation flash at 532 nm. However, the peaks (and shoulders) of the latter spectra shifted by 10−15 nm to the red. The same authors reported that the decay kinetics of excitation state of BChl *a* in the core complex had the time constant of 70 ± 10 ps, which corresponds reasonably well with the 55 ps time reported in Figure 3. On the other hand, the excitation at 670 nm, whose band is derived from the chlorophyll *a*-like pigments contained in the core, has recently revealed a relatively different spectrum, as a 30 ps component, ascribable to trapping of the excitation energy by the reaction center in membranes of *P. aestuarii*.[9]

Radical Pair Formation. The nondecaying spectrum shown in Figure 6b represents a state that does not decay on the time scale considered here. This spectrum exhibits a broad-band bleaching around 825−835 nm, which is similar in shape to the difference spectrum of $P840^+/P840$[25,26] or that shown in Figure 1b. However, some other components bleaching around 820−840 nm seem to be present in addition to the $P840^+$ formation on this time scale (see Figure 6c). In fact, Vasmel et al.[27] demonstrated the triplet-minus-singlet spectrum of a pigment other than P840 in their Reaction Center Pigment Protein (RCPP) complex with the absorbance-detected magnetic resonance (ADMR) technique. It is therefore necessary to measure absorption difference spectra in the 800 and 860 nm regions on a nanosecond time scale in order to trace the decay profile of this long-lived component.

The present data demonstrate the primary charge separation process followed by the formation of $P840^+$ state which lasts until 0.1 ms, implying that the acceptor side of the present reaction center preparation used here is relatively intact. Further detailed analysis using this preparation will clarify the electron-transfer process from A_0 to the subsequent acceptors.

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References and Notes

(1) Feiler, U.; Hauska, G. In *Anoxygenic Photosynthetic Bacteria;* Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995; pp 665−685.

(2) Sakurai, H.; Kusomoto, N.; Inoue, K. *Photochem. Photobiol.* **1996,** *64*, 5.

(3) Olson, J. M. *Photochem. Photobiol.* **1998,** *67*, 61.

(4) Büttner, M.; Xie, D. L.; Nelson, H.; Pinther, W.; Hauska, G.; Nelson, N. *Proc. Natl. Acad. Sci. U.S.A.* **1992,** *89*, 8135.

(5) Liebl, U.; Mockensturm-Wilson, M.; Trost, J. T.; Brune, D. C.; Blankenship, R. E.; Vermaas, W. *Proc. Natl. Acad. Sci. U.S.A.* **1993,** *90*, 7124.

(6) Van Grondelle, R.; Dekker: J. P.; Gillbro, T.; Sundstrom, V. *Biochim. Biophys. Acta* **1994,** *1187*, 1.

(7) Nuijs, A. M.; Vasmel, H.; Joppe, H. L. P.; Duysens, L. N. M.; Amesz, J. *Biochim. Biophys. Acta* **1985,** *807*, 24.

(8) Van de Meent, E. J.; Kobayashi, M.; Erkelens, C.; Van Veelen, P. A.; Otte, S. C. M.; Inoue, K.; Watanabe, T.; Amesz, J. *Biochim. Biophys. Acta* **1992,** *1102*, 371.

(9) Kramer, H.; Aartsma, T. J.; Amesz, J. *Photochem. Photobiol.* **1996,** *64*, 26.

(10) Oh-oka, H.; Kamei, S.; Matsubara, H.; Iwaki, M.; Itoh, S. *FEBS Lett.* **1995,** *365*, 30.

(11) Hastings, G.; Kleinherenbrink, F. A. M.; Lin, S.; Blankenship, R. E. *Biochemistry* **1994,** *33*, 3185.

(12) Lin, S.; Chiou, H. C.; Kleinherenbrink, F. A. M.; Blankenship, R. E. *Biophys. J.* **1994,** *66*, 437.

(13) Wahlund, T. M.; Woese, C. R.; Castenholz, R. W.; Madigan, M. T. *Arch. Microbiol.* **1991,** *156*, 81.

(14) Kleinherenbrink, F. A. M.; Chiou, H. C.; LoBrutto, R.; Blankenship, R. E. *Photosynth. Res.* **1994,** *41*, 115.

(15) Hastings, G.; Kleinherenbrink, F. A. M.;. Lin, S.; McHugh, T. J.; Blankenship, R. E. *Biochemistry* **1994,** *33*, 3193.

(16) Oh-oka, H.; Kakutani, S.; Kamei, S.; Matsubara, H.; Iwaki, M.; Itoh, S. *Biochemistry* **1995,** *34*, 13091.

(17) Zhou, W.; LoBrutto, R.; Lin, S.; Blankenship, R. E. *Photosynth. Res.* **1994,** *41*, 89.

(18) Francke, C.; Otte, S. C. M.; Miller, M.; Amesz, J.; Olson, J. M. *Photosynth. Res.* **1996,** *50*, 71.

(19) Li, Y.-F.; Zhou, W.; Blankenship, R. E.; Allen, J. P. *J. Mol. Biol.* **1997,** *271*, 456.

(20) Van Noort, P. I.; Gormin, D. A.; Aartsma, T. J.; Amesz, J. *Biochim. Biophys. Acta* **1992,** *1140*, 15.

(21) Holzwarth, A. R.; Schatz, G.; Brock, H.; Bittersmann, E. *Biophys. J.* **1993,** *64*, 1813.

(22) Pearlstein, R. M. In Sybesma, C., Ed. *Advances in Photosynthesis Research*; Martinus Nijhoff/Dr. W. Junk Publishers: The Hague, 1984; Vol. I, pp 13−20.

(23) Renger, G. In Barber, J., Ed. *The Photosystems: Structure, Function and Molecular Biology;* Elsevier: Amsterdam, 1992; pp 45−99.

(24) Trissl, H. W. *Photosynth. Res.* **1993,** *35*, 247.

(25) Swarthoff, T.; Amesz, J. *Biochim. Biophys. Acta* **1979,** *548*, 427.

(26) Franken, E. M.; Amesz, J. *Biochim. Biophys. Acta* **1997,** *1319*, 214−222.

(27) Vasmel, H.; Den Blanken, H. J.; Ton Dijkman, J.; Hoff, A. J.; Amesz, J. *Biochim. Biophys. Acta* **1984,** *767*, 200.