Time-Resolved Spectroscopy of Energy Transfer and Trapping upon Selective Excitation in Membranes of *Heliobacillus mobilis* at Low Temperature†

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Transient absorption difference spectra in the Q_y absorption band of bacteriochlorophyll (BChl) *g* and in the 670 nm absorption band of the primary acceptor A_0 in membranes of *Heliobacillus mobilis* (*Hc. mobilis*) were measured at 20 K upon selective excitation at 668, 793, 810, and 815 nm with a 5 nm spectral bandwidth. When excited at 793 nm, the spectral equilibration of excitations from shorter to longer wavelength-absorbing pigments occurred within 3 ps and mostly localized at the band centered around 808 nm. When excited at 668 nm, the excitation energy transfer from the 670 nm absorbing pigment to the Q_y band of BChl *g* took less than 0.5 ps, and the energy redistribution occurred and localized at 808 nm as in the case of the 793 nm excitation. All of the excitations were localized at the long wavelength pigment pool centered around 810 or 813 nm when excited at 810 or 815 nm. A slower energy transfer process with a time constant of 15 ps was also observed within the pool of long wavelength-absorbing pigments upon selective excitation at different wavelengths as has been observed by Lin et al. (*Biophys. J.* **1994**, *67*, 2479) when excited at 590 nm. Energy transfer from long wavelength antenna molecules to the primary electron donor P798 followed by the formation of P^+ took place with a time constant of 55−70 ps for all excitations. Direct excitation of the primary electron acceptor A_0, which absorbed at 670 nm, showed the same kinetic behavior as in the case when different forms of antenna pigments were excited in the Q_y region. This observation generally supports the trapping-limited case of energy transfer in which the excitations have high escape probability from the reaction center (RC) until the charge separation takes place. Possible mechanisms to account for the apparent "uphill" energy transfer from the long wavelength antenna pigments to P798 are discussed.

Introduction

Heliobacteria are a newly discovered group of non-oxygen evolving photosynthetic bacteria.[1−3] This family of organisms has a simple photosynthetic apparatus in which the antenna and the reaction center (RC) are organized as a single pigment−protein complex with approximately 35−40 BChl *g* molecules.[4,5] Recent studies indicate that the core protein complex in the RC of heliobacteria is a homodimer, in contrast to the heterodimeric core protein complexes found in most photosynthetic RCs.[6] The RC of the heliobacteria is broadly similar to Photosystem I (PS I) of the oxygen evolving organisms.[7] Therefore, heliobacteria may be considered as a bacterial model for PS I in the same way that purple bacteria are used as models for Photosystem II (PS II).[7−9] It is also the most primitive and organizationally the simplest photosynthetic organism yet examined.

There are three different forms of BChl *g* antenna pigments in heliobacteria which can be spectroscopically distinguished from each other at low temperature, that is, BChl *g* 778, BChl *g* 793, and BChl *g* 808.[10,11] The excitation energy transfer processes, within the core antenna system and from the antenna to the RC, have been studied by the absorption, fluorescence emission, and excitation spectra at both room temperature and low temperature. The results indicate that there is an efficient energy transfer pathway, that is, from the higher energy species BChl *g* 778 and BChl *g* 793 to the lower energy BChl *g* 808 and then to the primary electron donor P798 of the RC,[10−13] and all these processes take place on a picosecond time scale.[4,14,15]

The excitation energy transfer process in the membrane of *Hc. mobilis* has been studied using 590 nm excitation at both room temperature and low temperature.[16,17] By this excitation, all the different forms of antenna pigments are pumped to the excited states at the same time because it is near the 576 nm Q_x band of BChl *g*. The excitation redistribution of the antenna pigments can be observed over a broad wavelength region, but the energy transfer from the short wavelength absorbing pool to the long wavelength absorbing pool cannot be clearly distinguished. Recently, the excitation energy transfer steps of *Hc. mobilis* at room temperature have been reported using selective excitations at either 770 or 812 nm with a time resolution of 30 fs.[15] An uphill energy transfer with a 300 fs time constant and a downhill energy transfer with 100 and 500 fs components have been observed upon excitation at 812 nm (12 nm fwhm) and 770 nm (25 nm fwhm), respectively.

In order to further investigate the heterogeneity of antenna pigments, energy transfer among different forms of the antenna and trapping processes at 20 K and selective narrow-band (5 nm fwhm) excitations at 668, 793, 810, and 815 nm were carried out to directly excite the primary electron acceptor pigments, the BChl *g* 793 pool, the BChl *g* 808 pool, and the longest wavelength absorbing pigments.

Materials and Methods

The preparation of the *Hc. mobilis* membrane fragments and the setup for the transient absorption measurements are as described previously.[4,16] Samples were suspended in a buffer containing 20 mM Tris, pH 8.0, 10 mM sodium ascorbate, 100

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μM PMS, and 67% (v/v) glycerol in order to form clear glasses at low temperatures (13 and 20 K). The typical optical density of the sample was 1.2 at 788 nm at room temperature, in a sample cell with an optical path length of 1.2 mm used for low-temperature measurement. The cell was attached to the finger of an Air Products closed circulated helium refrigerator and cooled to the desired temperature.

Samples were excited at 793 and 810 nm where antenna BChl *g* 793 and BChl *g* 808 pigments absorb, respectively. Samples were also excited at 815 and 668 nm in which the longest absorbing antenna pigments and the primary electron acceptor A_0 absorb, respectively. The excitation pulses were generated as follows: a train of 590 nm, 200 fs, 100 μJ laser pulses at a repetition rate of 540 Hz was focused on a 0.5 in. thick rotating fused silica plate to generate a white light continuum. Because the sample cannot be flowed at low temperature, the fraction of closed RC can be estimated using the interval between excitation pulses in our laser system, 1.85 ms, and the observed recovery of the ground state at low temperature, 2.3 ms.[11,18] Using these values, at least 55% of the centers were open before the next flash, even if saturating pump flashes were used. The amount of open centers should be significantly higher than this, because the pump flashes were subsaturating, and not all excited reaction centers carry out stable charge separation at low temperatures. A series of narrow-band-pass interference filters with a fwhm of 5 nm (IF 680 tilted; IF 800, tilted; IF 810; and IF 820, tilted) in conjunction with the corresponding dyes which were pumped at 532 nm were used to produce the amplified excitation pulses at 668, 793, 810, and 815 nm, respectively. Transient absorbance changes were measured at the magic angle with the respect to the excitation polarization.

Data analysis techniques, including multiexponential kinetic analysis, global analysis, and generation of decay-associated spectra (DAS), were carried out as described earlier.[16] The DAS represents the amplitude spectrum of each decay component as function of wavelengths which provides information regarding energy transfer form one species to another. The negative amplitude shows the loss of population while the positive amplitude indicates gain of population.

Results

Excitation at 793 nm into the Q_y Band of BChl *g*. Transient absorption difference spectra of membranes of *Hc. mobilis* were measured in the 740−840 nm Q_y band region at 20 K upon excitation at 793 nm. This excitation is directly absorbed by the BChl *g* 793 antenna pigments, as well as the primary electron donor P798, which has maximum photobleaching at 794 nm at low temperature. Since the molecular ratio of P798 and antenna BChl *g* 793 pool is approximately 1:16,[10] the excitation is mostly absorbed by antenna pigments at this wavelength.

Figure 1A shows the time-resolved spectra (TRS) at early times (0−3 ps). The photobleaching band was developed initially around 798 nm in the first 0.9 ps with a small shoulder toward the longer wavelength-absorbing pigments (around 806 nm). Later, the 798 nm band decreased, and the photobleaching band at 806 nm grew in and shifted to 809 nm. This spectral evolution was completed within 3 ps and resulted in the major bleaching band centered around 809 nm with a minor shoulder at 798 nm. Therefore, the redistribution of the excitation energy from shorter to longer wavelength-absorbing pigments within the Q_y transition band is clearly demonstrated. Figure 1B shows the spectra taken at later times. The 809 nm bleaching band decreased and shifted to around 813 nm at 170 ps, whereas a bleaching band at around 790 nm remained.

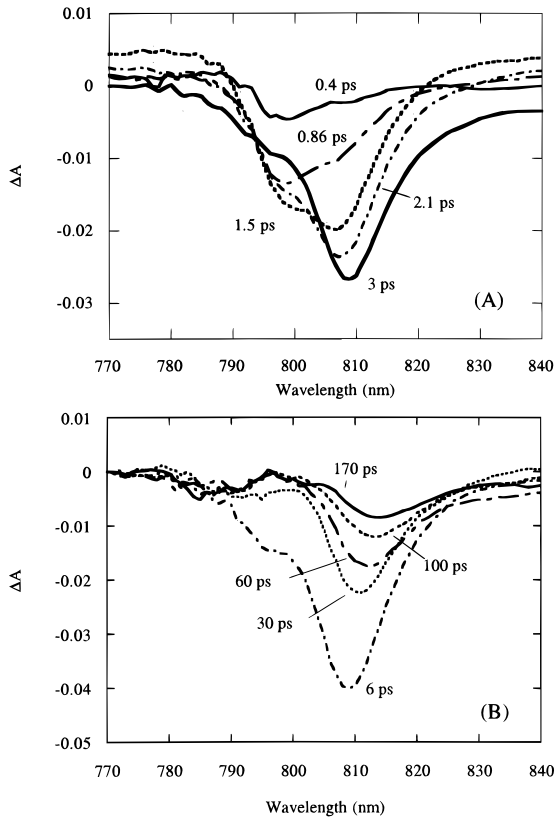

Figure 1. Time-resolved absorption difference spectra of membranes of *Hc. mobilis* at 20 K with 793 nm excitation: (A) at early times of 0.4, 0.86, 1.5, 2.1, and 3 ps; (B) at later times of 6, 30, 60, 100, and 170 ps.

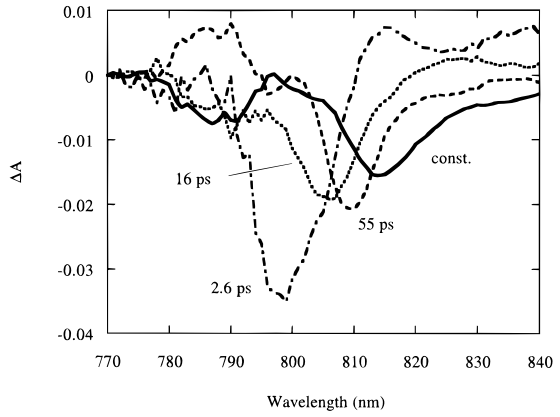

Figure 2. Decay-associated spectra of membranes of *Hc. mobilis* at 20 K in the BChl *g* Q_y region obtained from the fitting of the transient absorption difference spectra upon 793 nm excitation. Four decay components are resolved as indicated in the figure.

Transient absorption difference spectra taken on the 180 ps time scale were fit by global analysis with a sum of exponentials over the wavelength region of 770−840 nm. The fit revealed four components (2.6, 16, and 55 ps and a nondecaying component) as shown in the DAS in Figure 2. The 2.6 ps component has a large negative band centered at 798 nm and a small positive band around 813 nm. Part of this component is due to the energy transfer from shorter wavelength-absorbing pigments to longer wavelength-absorbing pigments. However, the positive and negative signals are not symmetric as the negative amplitude is much larger than the positive one. This suggests that some singlet−singlet annihilation occurs in this wavelength region. The 16 ps phase has a spectral shape reflecting the energy transfer from shorter wavelength pigments to longer wavelength pigments within the long wavelength BChl

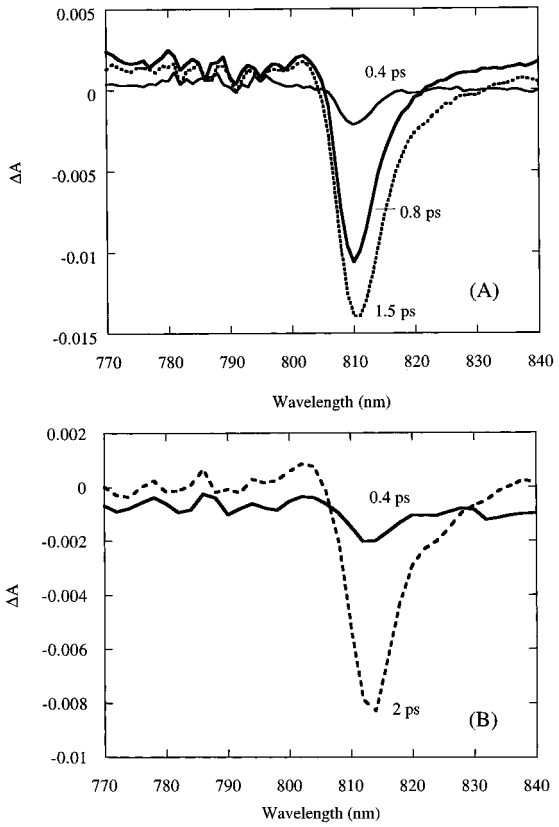

Figure 3. Time-resolved absorption difference spectra of membranes of *Hc. mobilis* at 20 K at early times: (A) 0.4, 0.8, and 1.5 ps upon 810 nm excitation; (B) 0.4 and 2 ps upon 815 nm excitation.

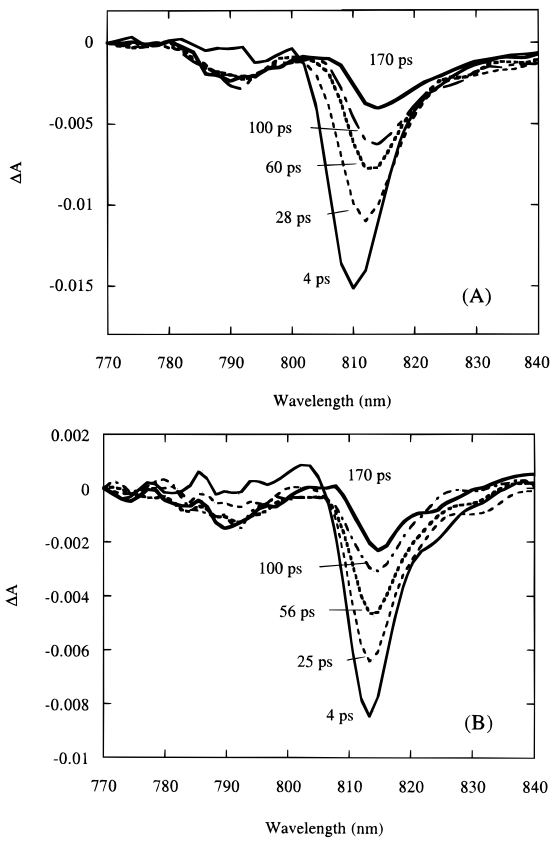

Figure 4. Time-resolved absorption difference spectra of membranes of *Hc. mobilis* at later times: (A) 4, 28, 60, 100, and 170 ps upon 810 nm excitation; (B) 4, 25, 56, 100, and 170 ps upon 815 nm excitation.

g 808 band. A similar component was observed in ref 17. The 55 ps component shows a negative amplitude around 810 nm and a positive band around 790 nm. Its profile reflects the energy transfer from long wavelength antenna BChl *g* to the primary electron donor P798 in the RC.[17] The nondecaying component represents the charge-separation state $P798^{+}A_0^{-}$ (centered around 792 nm) and some "stuck" low-energy excited states centered around 815 nm which do not decay on the picosecond time scale.

Excitations into the BChl *g* 808 Pool. Time-resolved spectra at early times (0−2 ps) in the Q_y region upon excitations at 810 and 815 nm are shown in Figure 3, A and B, respectively. When excited at 810 nm, a bleaching band centered at 810 nm grew in and was fully developed within 2 ps (Figure 3A). When the excitation was changed to 815 nm, the initial bleaching band appeared at 813 nm and reached a maximum at 2 ps (Figure 3B). The excitations were again localized on long wavelength pigments close to the wavelength of the excitation pulses. The uphill energy transfer equilibration from the BChl *g* 808 pool to the BChl *g* 793 pool was not observed at early times upon excitation at either 810 or 815 nm at 20 K. However, the measurements done recently by Liebl et al.[15] demonstrated the uphill transfer equilibration between the BChl *g* 808 pool and the BChl *g* 793 pool at room temperature upon excitation at 812 nm.

Time-resolved spectra taken at later times (up to 180 ps) upon excitation at 810 and 815 nm are shown in Figure 4, A and B, respectively. It shows clearly that the maximum bleaching (around 810 and 813 nm) at early time decreases and shifts to 815 nm at later times for both excitations. In addition, the bleaching around 790 nm builds up and reaches the maximum at 170 ps. This indicates that part of the excitation energy is trapped in the longest wavelength-absorbing pigments which decay very slowly, and part of the excitation energy still can be transferred to other pigments and captured by the photoactive pigments to give rise to charge separation. Figure 5A,B shows the DAS in the Q_y region upon 810 and 815 nm excitation. Three components are obtained for both excitations. The 15 ps phase has a spectral shape reflecting energy transfer from the short wavelength-absorbing pigments to long wavelength-absorbing pigments within the BChl *g* 808 pool. The 55−70 ps phase represents the trapping time for the energy transfer from long wavelength-absorbing pigments to the P798 in RC, and the nondecaying phase reflects the charge-separation state and long-lived excited state of the antenna at 815 nm. The 1−2 ps fast component, due to the energy redistribution from short BChl *g* pool to long BChl *g* pool,[17] is not observed at 20 K when the excitations are directly into the long wavelength BChl *g* pool. The energy redistribution probably occurred only within the BChl *g* 808 pool upon excitations at 810 and 815 nm. No evidence was observed, suggesting that energy transfer to the shorter wavelength forms of the antenna BChl *g* pool at 20 K took place, apparently because of the uphill transfer becomes more difficult at low temperatures. This redistribution is a subpicosecond process as shown by Liebl et al.[15] when excited at 812 nm for the same species at room temperature.

Excitation at 668 nm into the Q_y Band of the Primary Electron Acceptor A_0. In order to obtain direct information regarding the formation of the primary charge-separated state, excitation was done into the absorption band of the primary electron acceptor, A_0. Kinetics were measured in the region of 640−840 nm, where all different forms of antenna and photoactive pigments absorb. Figure 6A shows the TRS at early times (0−3 ps) when excited at 668 nm at 20 K. As shown in the 0.3 ps spectrum, there was a bleaching band at 670 nm, but there was no instantaneous bleaching signal in the Q_y band region of BChl *g*. The 670 nm bleaching band reached a

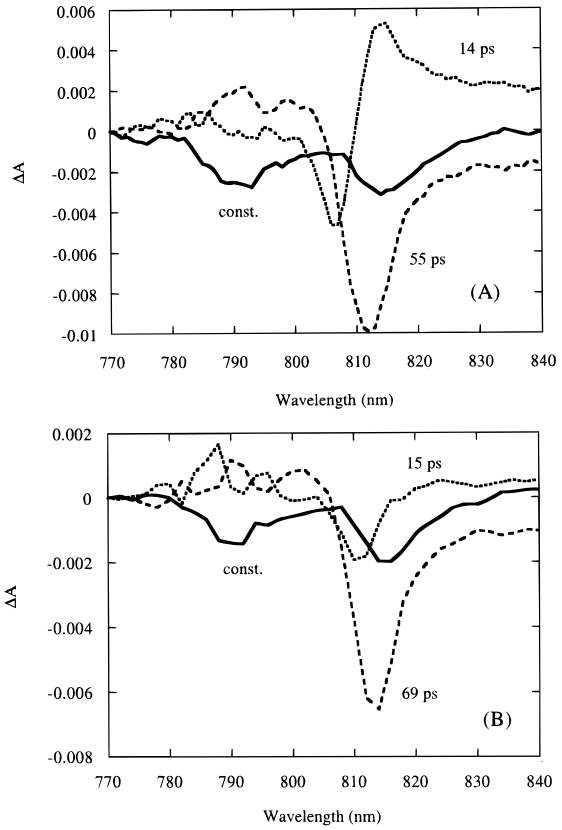

Figure 5. Decay-associated spectra of membranes of *Hc. mobilis* at 20 K in the BChl *g* Q_y region obtained from the fitting of transient absorption difference spectra (A) upon 810 nm excitation and (B) upon 815 nm excitation.

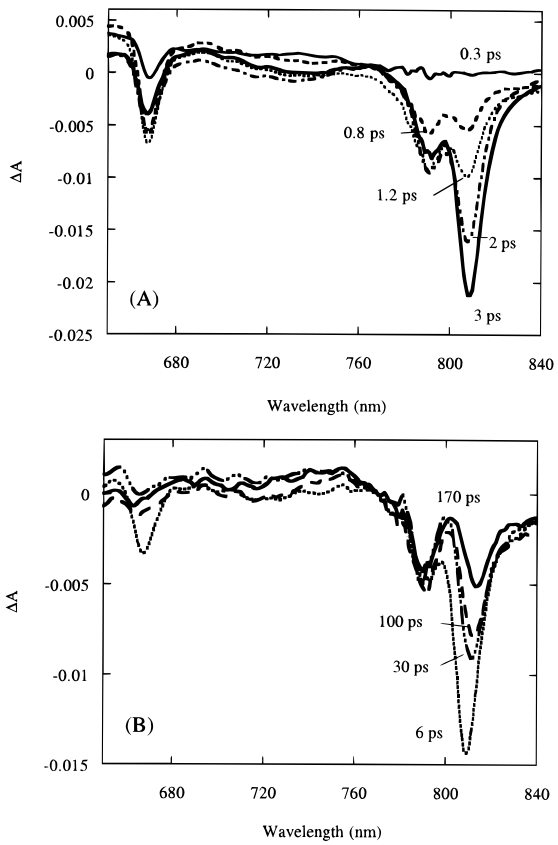

Figure 6. Time-resolved absorption difference spectra of membranes of *Hc. mobilis* at 20 K with 668 nm excitation: (A) at early times of 0.3, 0.8, 1.2, 2, and 3 ps; (B) at later times of 6, 30, 60, 100, and 170 ps.

maximum at around 1 ps with two new bleaching bands in the Q_y region of BChl *g* centered around 790 and 808 nm growing in. The 808 nm bleaching band reached a maximum at 3 ps as the 670 nm bleaching band partly decreased. This clearly shows the excitation energy transfer is from A_0 to the neighboring antenna pigments, probably via P798, and is finally mostly located on the long wavelength BChl *g* pool after the first few picoseconds. Figure 6B shows the TRS taken at later times. The bleaching band centered around 808 nm at 6 ps decreased and further shifted to 815 nm at 170 ps while the bleaching band around 790 nm remained relatively unchanged. The 670 nm bleaching band also decreased at later times and shifted to 666 nm, reflecting the decay of the excitation energy and the formation of the primary charge-separated state, $P798^+A_0^-$.

Kinetics of absorbance changes at 668, 790, 810, and 816 nm upon 668 nm excitation at 20 K are plotted in Figure 7. The smooth curves are the fits at the corresponding wavelengths obtained from global analysis. The DAS in the Q_y region of BChl *g* upon 668 nm excitations at 20 K are shown in Figure 8A and consist of four kinetic components with 1.6, 8.6, and 71.5 ps and a constant. The 1.6 ps phase has a positive amplitude over a broad wavelength region and is assigned to the excitation redistribution among different forms of antenna pigments. The 8.6 ps component shows the spectral profile reflecting the energy transfer from the short wavelength-absorbing pigments to the long wavelength-absorbing pigments within the BChl *g* 808 pool. The 71.5 ps phase is ascribed to the trapping time of the energy from the long wavelength pool around 810 nm to the primary electron donor P798 in the RC. The long-lived component represents the charge-separation state. The DAS derived from measurements at 13 K upon 668 nm excitation also shows similar components, except that the component for the energy transfer from short wavelength-

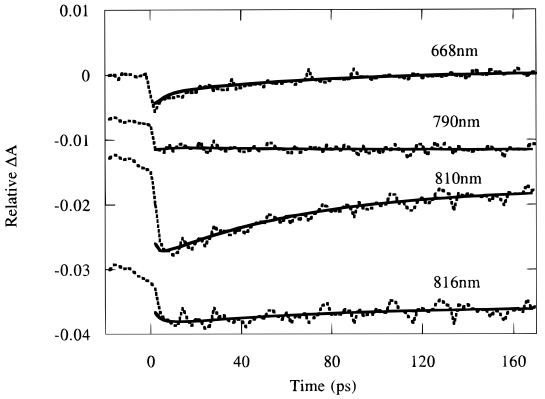

Figure 7. Decay profiles at 668, 790, 810, and 816 nm of membranes of *Hc. mobilis* at 20 K upon 668 nm excitation. Solid lines were fits obtained from four components global analysis.

absorbing pigments to long wavelength-absorbing pigments within the BChl *g* 808 pool is 15 ps instead of 8.6 ps as shown in Figure 8B. The 15 ps phase is probably more accurate to describe this energy transfer process based on the fact that the 20 K measurement upon 668 nm excitation has considerable noise before the background correction at early times. The previous measurements done at 20 K upon 590 nm excitation also reveal a 15 ps phase for this energy transfer process.[17]

Discussion

Energy Transfer within the Q_y Band. The rate of energy transfer from the BChl *g* 793 pool to the BChl *g* 808 pool upon 793 nm excitation is observed to be less than 2.6 ps at 20 K. This energy transfer rate cannot be determined accurately on

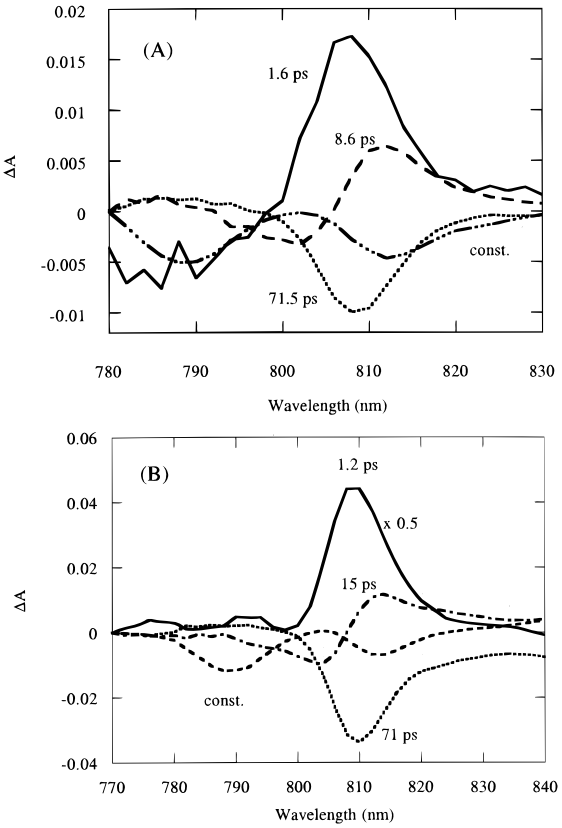

Figure 8. Decay-associated spectra of membranes of *Hc. mobilis* in the BChl *g* Q_y region obtained from the fitting of transient absorption difference spectra upon 668 nm excitation (A) at 20 K and (B) at 13 K.

this time scale due to the influence of stimulated emission which also occurred on this time scale as well as the time resolution of the data (2 ps per point). The same energy transfer process in the membrane of heliobacteria has recently been reported to be around 100−500 fs at room temperature upon 770 nm excitation with 25 nm fwhm pulses.[15] When excited at 810 or 815 nm, excitation equilibration within the BChl *g* 808 pool still occurred since the energy transfer from short wavelength-absorbing pigments to long wavelength-absorbing pigments within the BChl *g* 808 pool can be resolved with a rate constant of 15 ps as shown in the DAS of Figure 5. No uphill energy transfer from the BChl *g* 808 pool to the BChl *g* 793 pool was observed at 20 K. This is because that the smaller thermal energy, k_BT (≈14 cm^{-1}), could not overcome the energy barrier from E808 to E793 (≈234 cm^{-1}) at 20 K. However, the uphill energy transfer processes do occur from BChl *g* 808 to BChl *g* 793 with a rate constant of 300 fs at room temperature ($k_BT \approx$ 210 cm^{-1}) when the membrane samples were excited at 812 nm with 12 nm of fwhm.[15] The uphill energy transfer rate of 0.3 ps in heliobacteria at room temperature is approximately an order of magnitude faster than that of PS I in which the uphill energy transfer from the red- to blue-absorbing pigments in the core antenna occurred with a rate of 3−4 ps upon 712 nm excitation.[19]

Energy Transfer from RC to Antenna Pigments. When the primary electron acceptor A_0 was directly excited at 670 nm, energy was transferred rapidly from the core of the RC to the antenna pigments, and the excitation energy redistribution was mainly observed in the BChl *g* 793 pool and the BChl *g* 808 pool. The excitations were mostly localized into the BChl *g* 808 pool within 3 ps as shown in Figure 6. There are two possible pathways for the excitation energy to localize in the BChl *g* 808 pool as supported by the geometry of electron transfer chain near A_0 from the crystal structure of PS I at 4 Å resolution.[20] One possibility is that the excitation energy escapes from A_0 to the neighboring antenna pigments through the accessory pigments closely coupled to A_0. Another possibility is that the excitation energy is first transferred from A_0 back to P798 and then escapes immediately to the antenna pigments without being trapped by P798. In both cases, it indicates that the excitation energy has a very high escape probability from the RC, because the overall trapping process still takes place with a time constant of 55−70 ps at 20 K. From the TRS of Figure 6A at early times, it is clear that the 793 nm bleaching grows in first and the 808 nm bleaching develops later. This implies that the energy transfer sequence is most likely from A_0 to P798 and then to the long wavelength antenna pigments. The observation of a high escape probability from the core of the RC to the antenna suggests that the kinetics of excitation transfer are "trap-limited" rather than "special trap-limited" or "diffusion-limited".[21−25]

Equilibration in the BChl *g* 808 Antenna Pigments. The excitation energy is mostly localized in the BChl *g* 808 pool within a few picoseconds upon various wavelength excitations. After that, the observed absorption changes no longer depend on the excitation wavelength as shown in the TRS at later times in Figures 1B, 4, and 6B. However, the absorption changes over the first few picoseconds depend strongly on the excitation wavelength as indicated in the TRS at early times in Figures 1A, 3, and 6A. Once the excitation energy is localized in the BChl *g* 808 pool, a slower energy transfer process from short wavelength-absorbing pigments to long wavelength-absorbing pigments within the BChl *g* 808 pigments is clearly observed with a transfer time of 9−15 ps for various wavelength excitations at 20 and 13 K. This energy transfer process is not dependent on the wavelength of excitation, which indicates that some of the far red pigments within the BChl *g* 808 pool are apparently surrounded by pigments absorbing at shorter wavelengths in the antenna. Such an arrangement of low-energy pigments would result in the observed slow transfer process and serves as an intermediate excitation trap. The equilibration process before trapping was also observed in the PS I of cyanobacteria and the membrane of heliobacteria at room temperature.[15,19] The fact that we observe the equilibration process in the BChl *g* 808 band independent of the excitation wavelength at 20 K suggests that the long wavelength pigments are functionally coupled at room temperature, and the different spectral forms of BChl *g* are spatially distributed within the antenna and do not form an excitation funnel toward the RC. This conclusion rests on the assumption that the structure of the complex has not changed significantly upon cooling.

Energy Trapping Process. A small portion (≈15%) of the excitations in the BChl *g* 808 pigments is trapped by some of the far red pigments, while the majority of excitations from the BChl *g* 808 pigments gives rise to radical-pair formation at 20 K. This trapping process is again independent of the excitation wavelengths. The mechanism of this apparent uphill transfer from BChl *g* 808 to P798 is not clear, because the energy gap between the absorption band of P798 (maximum bleaching at 793 nm at 20 K) and the long wavelength-absorbing pigments at around 810 nm is approximately 250 cm^{-1}, corresponding to approximately 18 k_BT at 20 K while the bandwidth of excitation pulses at fwhm ranged from 75 to 112 cm^{-1} for the different excitation wavelengths used in this study. A similar situation of uphill energy transfer from long wavelength antenna to primary electron donor is also found in PS I and the RC of some purple bacteria.[26] In order to have an efficient uphill energy transfer from long wavelength-absorbing pigments to

the primary electron donor at 20 K, the spatial position of this long wavelength-absorbing pigment in the BChl *g* 808 band must be close to P798, and the overlap factor between P798 and the long wavelength-absorbing pigments must be sufficiently large. The overlap factor cannot be measured accurately because the relevant fluorescence and absorption spectra cannot be obtained independently of the other pigments. However, a conservative estimate is that it is at least a factor of 10−20 smaller than at room temperature. This is compared with the slowing by only about a factor of 2 of the trapping time (from ∼30 ps at room temperature to ∼60 ps at low temperature). One possible pathway is that the long wavelength-absorbing pigments transfers its excitation energy directly to form the $P798^+A_0^-$ radical pair, which has lower energy than the long wavelength-absorbing pigments. This would constitute a new type of tunneling process in which the initial state is an electronic excited state and the final state is a charge-separated state. A somewhat similar proposal was made by Smit et al.[11] on the basis of slower time scale measurements. This is also similar to an intervalence charge transfer process.

One of the major differences between the results obtained by this study and results by a previous study with 590 nm excitation is the apparent yield of $P798^+$. Comparing the amplitude of $P798^+$ bleaching at 792 nm at 170 ps with the bleaching at 808 nm at 3 ps, a ratio of 1:1 is obtained with 590 nm excitation, while the ratio is only 1:5 in this study. We consider it unlikely that the extra energy in the 590 nm photons could raise the local temperature of the sample, therefore providing a higher possibility for energy transfer from 808 nm-absorbing pigments to the 792 nm absorption of P798. Previous experiments[17] show a rather weak temperature dependence of the formation of $P798^+A_0^-$. Also, no evidence of this sort of local heating effect has been observed in other photosynthetic reaction centers at low temperatures. One possible explanation for the lower yield of charge-separated states in these experiments is that, despite the estimates of the fraction of open reaction centers given in the Materials and Methods, a larger fraction of the centers were oxidized prior to the flash. Oxidized reaction centers quench the antenna excited states with a similar efficiency as open ones but do not lead to charge separation. The results reported here were reproducible, with several different samples from different batches of cells all showing similar behavior.

The hypothesis that the $P798^+A_0^-$ is formed directly from B808* at low temperature can possibly be rationalized by assuming that one of the B808 molecules is located between P798 and A_0, mediating the formation of the final charge-separated state $P798^+A_0^-$. A proposed reaction scheme is shown as follows:

$$P798B808^*A_0 \rightarrow P798B808^+A_0^- \rightarrow P798^+B808A_0^-$$

It is indicated in the scheme that the primary charge-separated state $P798^+A_0^-$ is formed by a two-step electron transfer sequence involving transient oxidation of the B808 pigment. The excited antenna pigment B808* drives an electron to A_0 and the P798 then donates an electron to B808. It is quite possible that the reaction is downhill energetically and therefore can occur at low temperatures. This type of reaction was observed in several synthesized chemical compounds in a goal to mimic the function of photosynthetic reaction centers, e.g., in a C−P−Q triad.[27] A different kinetic rate for the formation of A_0^- was observed at room temperature when the amplitude of the 670 nm bleaching band was independently measured.[15] This rate is faster (10 ps) than that of the 30 ps overall decay of the antenna, which could be due to the difference between the rate of the two steps. The recent structural data of the PS I reaction center have indicated the existence of an accessory chlorophyll *a* molecule between the primary donor P700 and the primary acceptor A_0.[20] However, this possible mechanism is very speculative, and additional experimental and theoretical studies are required to explore it in more detail.

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