Fast Energy Transfer and Exciton Dynamics in Chlorosomes of the Green Sulfur Bacterium *Chlorobium tepidum*

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The excited-state structure and energy-transfer dynamics, including their dependence on temperature and redox conditions, were studied in chlorosomes of the green sulfur bacterium *Chlorobium tepidum* at low temperatures by two independent methods: spectral hole burning in absorption and fluorescence spectra and isotropic one-color pump−probe spectroscopy with ∼100 fs resolution. Hole-burning experiments show that the lowest excited state (LES) of BChl *c* aggregates is distributed within approximately 760−800 nm, while higher excitonic states of BChl *c* (with absorption maximum at 750 nm) possess the main oscillator strength. The excited-state lifetime determined from hole-burning experiments at anaerobic conditions was 5.75 ps and most likely reflects energy transfer between BChl *c* clusters. Isotropic one-color absorption difference signals were measured from 720 to 790 nm at temperatures ranging from 5 to 65 K, revealing BChl *c* photobleaching and stimulated emission kinetics with four major components, with lifetimes of 200−300 fs, 1.7−1.8 ps, 5.4−5.9 ps, and 30−40 ps at anaerobic conditions. The lifetimes are attributed to different relaxation processes of BChl *c*, taking into account their different spectral distributions as well as limitations arising from results of hole burning. Evidence for at least two spectral forms of BChl *c* in chlorosome is reported. There is a striking similarity between the spectrum and kinetics of the 5.4−5.9 ps component with those of the LES determined from hole burning. A pronounced change of isotropic decays was observed at around 50 K. The temperature dependence of the isotropic decays is correlated with temperature-dependent changes of BChl *c* fluorescence emission. Further, the temperature decrease leads to an increase in the relative amplitude of the 200−300 fs component. At aerobic conditions, both hole burning and pump−probe spectroscopy show that the lifetime of the LES shortens to ∼2.6 ps, as a result of excitation quenching by a mechanism presumably protecting the cells against superoxide-induced damage. This mechanism operates on at least two levels, the second one being characterized by a 14−16 ps lifetime.

Introduction

Chlorosomes are the main light-harvesting systems of green bacteria. The term green bacteria involves two otherwise not closely related bacterial families, *Chlorobiaceae* and *Chloroflexaceae*, possessing similar chlorosomes.[1] Strictly anaerobic green sulfur bacterium *Chlorobium (Chl.) tepidum* is a member of the first group and contains bacteriochlorophyll (BChl) *c* as a major antenna pigment. Chlorosomes differ from other photosynthetic antennae due to their unusually large size and internal structure. They form oblong bodies 100−150 nm long and 30−40 nm in diameter.[1] The envelope of the chlorosome presumably consists of a lipid monolayer containing BChl *a* and some associated protein. The whole chlorosome contains up to 10 000 BChl *c*, *d*, or *e* and about 100−200 BChl *a* molecules.[2] Electron microscope studies revealed that inside the chlorosomes there are rod elements (∼10 nm diameter) which fill the entire length of the chlorosome.[3] Evidence based on the results of various methods suggests that BChl *c*, *d*, or *e* is organized in these rods in highly ordered aggregated states where the geometrical arrangement and photophysical properties are due to pigment−pigment rather than pigment−protein interactions.[4] Chlorosomes lacking any significant amount of a protein but nevertheless retaining the spectroscopic properties of intact chlorosomes have been prepared.[5] This is in contrast with other photosynthetic antennae in which pigment−protein interactions dominate in maintaining the structure. The proper structure of aggregates is still a matter of debate.[4,6] There is now a general agreement that aggregation both in the chlorosomes and in large in vitro aggregates involves the interaction of the 3^1-OH oxygen of one BChl with the Mg of a second BChl and that the OH hydrogen should be hydrogen-bonded to the 13^1 keto group of a third BChl.[4,6] As the length of hydrogen bond is ∼4 Å, a close interaction between BChl's forming the aggregate results in strong excitonic coupling. The whole chlorosome is, in the case of *Chlorobiaceae*, attached to the inner side of the cytoplasmic membrane via a crystalline Fenna−Matthews−Olson (FMO) protein complex.[1] The cytoplasmic membrane contains the core complex which includes the core antenna and the reaction center (RC).[2]

The efficiency and energy-transfer (ET) rates in chlorosomes of green sulfur bacteria are strongly dependent on redox conditions, probably in order to protect RC's against damage

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by singlet oxygen at aerobic conditions.[7,8] At low potential there is evidence for energy transfer from chlorosomal BChl to BChl *a* within 30−40 ps for BChl *c* containing bacteria. Longer lifetimes were observed for bacteria containing BChl *d* and *e* due to a smaller spectral overlap with BChl *a*.[9] Under oxidizing conditions these lifetimes are shortened to 10−15 ps, and no characteristic rise is observed in BChl *a*.[8,10] According to current knowledge, quinones are involved in redox-regulated quenching.[11] The internal energy transfer within chlorosomes of green sulfur bacteria is far from being well understood. A component of ∼11 ps lifetime (15 ps at 77 K) observed in chlorosomes of *Chl. vibrioforme* (containing BChl *d*) was attributed to energy transfer from short- to long-wavelength absorbing oligomers.[12] At least two different spectral forms with different lifetimes of the LES (2.1−5.3 ps, depending on redox conditions and spectral form) were observed for *Chl. limicola* (BChl *c*).[13] For isolated chlorosomes of *Chl. tepidum* (BChl *c*) major PB/SE isotropic decay components were found to be typically 1−2, 5−10, and 35−50 ps, the last one due to BChl *c*-to-BChl *a* energy transfer.[14] The 1−2 ps component was interpreted as perhaps due to a single energy-transfer step. Spectral inhomogeneity of antenna was suggested to play a role.[14] The kinetics in the FMO protein complex consisting of trimers of seven strongly coupled BChl *a* is extensively studied,[15,16] and interstate relaxation between particular exciton levels is assumed to occur with 30 fs−2.5 ps kinetics.[16]

We have used two complementary methods to investigate excited-state structure and energy-transfer dynamics in chlorosomes of BChl *c* containing *Chl. tepidum*. Particular attention was dedicated to the dependence on temperature and redox conditions. A combination of direct femtosecond pump−probe and indirect spectral hole-burning techniques appears to be a powerful tool for study and interpretation of relaxation processes. As hole burning is a low-temperature method, time-resolved experiments were performed between 5 and 65 K to enable direct comparison of both methods. Remarkable differences compared to room-temperature behavior[14] were observed and are discussed. Several relaxation processes are reported and interpreted in the frame of excitonic relaxation and energy transfer within the chlorosome.

Experimental Section

Whole cells of *Chl. tepidum* stored under strictly anaerobic conditions were harvested by centrifugation and then dissolved in a 40:60 mixture of the growth medium and glycerol forming a transparent low-temperature glass. When experiments were performed at anaerobic conditions, sodium dithionite was added up to a total concentration of 20 mM. To obtain aerobic conditions, samples were bubbled with air for ∼10 min. Whole cells were studied rather than isolated chlorosomes to prevent lifetime artifacts.[10]

Low-temperature absorption spectra were measured by passing the light of a wolfram lamp light through a double-grating monochromator (J. Yvon HRD 1, spectral resolution ≈ 0.5 cm^{-1}). After transmission through the sample placed in a bath cryostat, the signal was detected with a cooled photomultiplier RCA C34031 and fed into a lock-in amplifier. Fluorescence was obtained by exciting the sample with the 488 nm line of a CW argon ion laser. The main absorbing pigment at this wavelength is carotenoid chlorobactene. The hole-burning spectra were measured in both absorption and fluorescence. (The method is described in detail elsewhere.[17]) The dye laser used for burning (Spectra Physics 375B with Pyridine 1) was equipped with a three-plate birefrigent filter and a thin Etalon

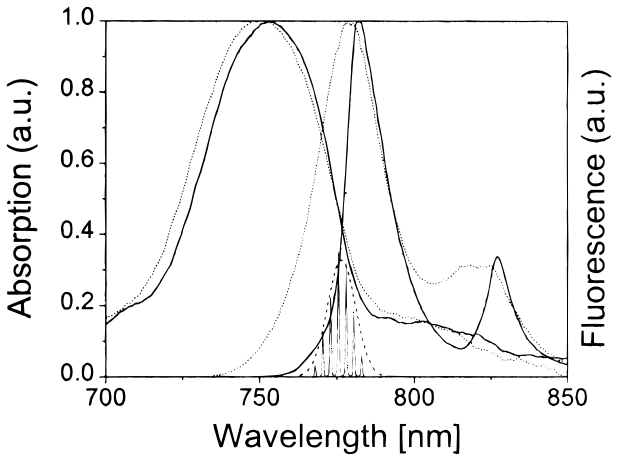

Figure 1. Near-IR steady-state absorption and fluorescence spectra of *Chl. tepidum* at low temperature (dotted line, 65 K; solid line, 5 K) together with the ZPH action spectrum obtained in absorption. Its envelope is fitted by a Gaussian (- - -). The action spectrum is not to scale: the relative depth of the most intense hole at 775.5 nm is 4%.

(spectral width ∼ 0.3 cm^{-1}). Spectral data were processed and stored in a PC. The obtained hole profiles were deconvoluted from the monochromator slit function and from the burning laser profile by the Simplex optimization method.

One-color isotropic absorption difference signals were measured using a Ti:sapphire laser (Tsunami 3960-L3S) providing ∼100 fs pulses with a repetition rate of 82 MHz. Pulses with ∼10 nm spectral bandwidth were tuned in the range 720−790 nm. Further, the pulses were split into pump and probe pulses with intensity ratio ∼10:1 and further attenuated to excitation intensities $<1 \times 10^{14}$ photons $pulse^{-1}$ cm^{-2}. Varying the pump pulse intensity by more than 1 order of magnitude did not lead to annihilation effects. Both pump and probe beams were focused onto the sample, which had a 1 mm path length in the gas flow cryostat. The autocorrelation function of the pump and probe pulses was recorded by replacing the sample by a KDP crystal. The intensity of the probe beam was detected by a photodiode connected to a lock-in amplifier and accumulated in a PC. The temperature dependence of isotropic kinetics was studied at seven different temperatures between 5 and 65 K at wavelengths above 735 nm. The obtained profiles were fitted by a Simplex optimization method.

Results and Discussion

A. Steady-State Absorption and Fluorescence Spectra. Figure 1 shows absorption and fluorescence spectra of whole cells of *Chl. tepidum* at 5 and 65 K. The main absorption band in the near-infrared is the Q_y band of BChl *c* at ∼750 nm. The absorption at $\lambda > 790$ nm is mainly due to BChl *a* in various pigment−protein complexes. The fluorescence spectrum at 5 K shows an intense BChl *c* band centered at ∼780 nm and a less intense band at ∼830 nm due to BChl *a* emission. At 65 K both bands are remarkably broader and blue-shifted compared to the spectrum at 5 K.

B. Hole-Burning Experiments. In hole-burning experiments in absorption we succeeded to observe zero phonon holes (ZPH) only within a narrow spectral range around 775 nm, where BChl *c* absorption is overlapped with its fluorescence, similarly as was observed for other green bacteria.[18,19] The so-called hole-burning action spectrum in Figure 1 represents the depths of the holes burned at different wavelengths at constant burning power and time at 4.2 K. However, in fluorescence, which is a zero-background method, the resonant ZPH's could be

observed within the entire BChl *c* band (760−795 nm, likewise for *Chl. limicola*[13]), even at wavelengths where the holes cannot be detected in absorption spectra due to a minor contribution of BChl *c* absorption to the total signal. Since in both absorption and fluorescence spectra the ZPH's can be observed resonantly at the burning frequency, these holes arise from elimination of transitions between ground and the lowest excited state (LES), and the hole-burning action spectrum thus represents a site distribution function of this transition. Due to higher sensitivity of detection in fluorescence, the hole-burning action spectrum obtained in emission includes that obtained in absorption and covers the whole spectral region of BChl *c* fluorescence. This proves that the lowest excited states (LES) of BChl *c* are distributed within spectral range from approximately 760−800 nm, with a maximum at ∼780 nm. As the maximum of absorption is around 750 nm, the direct consequence is that the absorption into higher excitonic states of BChl *c* aggregates possess the main oscillator strength. Such interpretation explains an unusually large spectral shift between the maxima of absorption and fluorescence of BChl *c* (>500 cm^{-1}). This is also an important criterion for selection of an appropriate model of the BChl *c* aggregates, which excludes for example pure J-aggregates.[20] Weak absorption of BChl *c* at around 780 nm, where the LES has maximum of its distribution, suggests that this transition is only weakly allowed. An example of the structure satisfying this condition is a circular organization of the excitonicaly coupled BChl's in which the symmetry makes the LES optically forbidden. For B850 antenna of purple bacteria, which possess such a conformation, the narrow ZPH's can be burnt also only in the red part of the absorption band,[21] and similarly as in our interpretation, the holes are assigned to the LES. The LES contributes to the total absorption by intensity of ∼5% because the B850 dipoles are not perfectly in plane. Both for B850[22] and chlorosomal[18,19] antennae it was shown that the main part of absorption exhibits significant homogeneous broadening due to exciton level structure and ultrafast interexciton level relaxation processes. However, these similarities do not necessarily mean that the rods within the chlorosome contain BChl c aggregates of the same structure as that of the B850 ring, where proteins play the significant role in maintaining the structure.

For direct comparison with time-resolved absorption difference measurements, we studied the ZPH's in absorption. The hole widths extrapolated to zero burning time and burning power (Figure 2) yielded values $\delta_{HB} = 1.85$ cm^{-1} at anaerobic and $\delta_{HB} = 4.0$ cm^{-1} at aerobic conditions and were independent of wavelength and of temperature up to 25 K. The independence of temperature indicates a negligible contribution of strongly temperature-dependent pure dephasing time,[23] and the hole widths thus reflect the lifetime of the LES. At anaerobic conditions, which are natural for these bacteria, this lifetime is equal to, or at least not shorter than, 5.75 ps. The lifetime of the LES shortens to 2.65 ps at aerobic conditions due to the redox-dependent quenching of excitation.

C. Isotropic Decays. Figure 3 summarizes one-color isotropic difference signals measured at anaerobic conditions at 65 K. The maximal absolute values of absorption difference signals ($\Delta A(t)$) were typically ∼1% of total absorbance at a particular wavelength. No hole burning was observed under conditions of pump−probe experiments. Similarly as observed for isolated chlorosomes of *Chl. tepidum* at room temperature,[14] the zero-crossing point between regions where $\Delta A(t)$ is dominated by excited-state absorption (ESA) and photobleaching/stimulated emission (PB/SE) occurs near 735 nm. However,

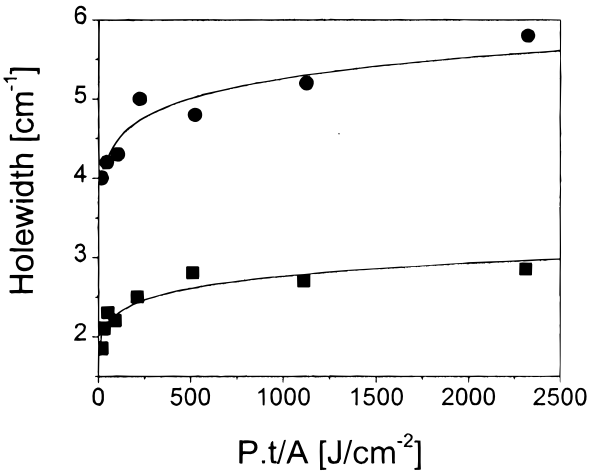

Figure 2. Hole widths measured in absorption of *Chl. tepidum* as a function of the burning fluence density $\delta_{HB} = \delta_{HB}(Pt/A)$, where P = burning power, t = burning time, and A = area, at anaerobic (squares) and aerobic (circles) conditions at 4.2 K at 780 nm.

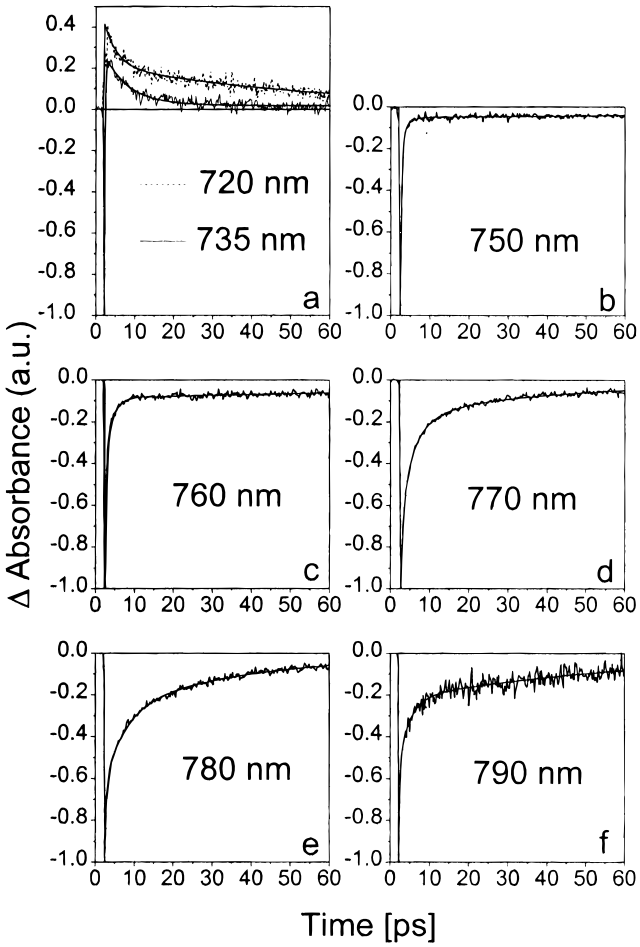

Figure 3. One-color isotropic absorption difference signals for *Chl. tepidum* for wavelengths from 720 to 790 nm at anaerobic conditions at 65 K, together with multiexponential fits (parameters listed in Table 1).

the absorption difference profiles differ significantly from those measured by Savikhin et al.[14] At the maximum of absorption into higher excitonic states of BChl *c* (750 nm) the overall decay is much faster than at room temperature. This difference becomes less dramatic with increasing wavelength, and at 780 nm, where the distribution of the LES has its maximum, the decay at 65 K is slower than at room temperature. At 790 nm

the decay at 65 K is again faster compared to profiles measured at 300 K. The fact that Savikhin et al.[14] used isolated chlorosomes (instead of whole cells studied in our case) and that the observed kinetics depend on growth conditions[23] cannot completely explain the observed differences.

At 720 nm only an increase of the transient absorption, due to ESA, is observed. At 735 nm a fast PB/SE is followed by ESA. Such behavior may be explained as due to the overlap of the ESA region of the long-wavelength absorbing spectral form of BChl *c* and the PB/SE region of short-wavelength BChl *c*. The existence of at least two spectral forms of BChl *c* is predicted, for instance, by the second derivative of asymmetric fluorescence spectra of BChl *c* exhibiting at least two negative maxima at ∼780 and ∼790 nm (not shown), similarly as for *Chl. limicola*.[13] The presence of different spectral forms (e.g., aggregates consisting of different BChl *c* homologues found in chlorosomes[25]) together with an appropriate spatial arrangement may facilitate downhill energy transfer from chlorosome to the FMO.

The initial PB/SE at 735 nm is very fast and can be fitted by a component with a 250 fs lifetime. A similar component was observed at all wavelengths above 735 nm. At 750 nm and 65 K, 90% of excitation decays with this short lifetime and about 7% with a lifetime of 1.7 ps. The decay curves measured at different temperatures were almost identical below 50 K and then again above 50 K at all wavelengths. Figure 4a compares the isotropic decays at 750 nm measured at 5 and 65 K. While at 50−65 K PB/SE dominates the $A(t)$ signal, at 5−50 K ESA is prevailing. This difference may be explained as due to a higher contribution of SE at 65 K when the LES may be populated directly by 750 nm light. This is manifested in the fluorescence spectrum (Figure 1) which shows that at 5 K there is negligible fluorescence emission at 750 nm, in contrast with the situation at 65 K.

At 760 and 770 nm the $A(t)$ signal is dominated by PB/SE at all temperatures between 5 and 65 K. Figure 4b,c demonstrates that an increase of temperature from 5 to 65 K leads to slower overall decay, and hence there exists a tendency toward the yet slower decay observed at room temperature.[14] Again, such behavior can be understood in the context of an increase of fluorescence intensity at 750−770 nm between 5 and 65 K (Figure 1) due to a higher population probability of relatively long-lived LES, which in turn leads to slower decay at 65 K. However, the nature of temperature-dependent fluorescence change remains unclear. It arises from intrinsic conformation properties of aggregates or whole rods rather than from solvent-dependent effects.

At 780 nm, where the distribution of the LES has its maximum, the temperature behavior of the kinetics is opposite compared to shorter wavelengths: the overall decay is slower than at 300 K[14] and with an increase of temperature from 5 to 65 K becomes faster; i.e., again there is a tendency toward behavior observed at room temperature.

At 790 nm the kinetics become again faster compared to those measured at room temperature, and the amplitude of the fast component suddenly rises (Table 1). At this wavelength the absorption into higher excitonic states of BChl *a* in FMO already substantially contributes to the total absorption (Figure 1), and thus the fast component (interpreted as a weighted average of interstate relaxation from these excitonic states[16]) dominates the decay, similarly as in the case of BChl *c* at 750−760 nm (see section D).

D. Excited-State Relaxation and Energy Transfer at Anaerobic Conditions. The parameters of the optimized fits for selected wavelengths at 5 and 65 K are summarized in Table 1. Using the variance−covariance matrix approach, we calcu-

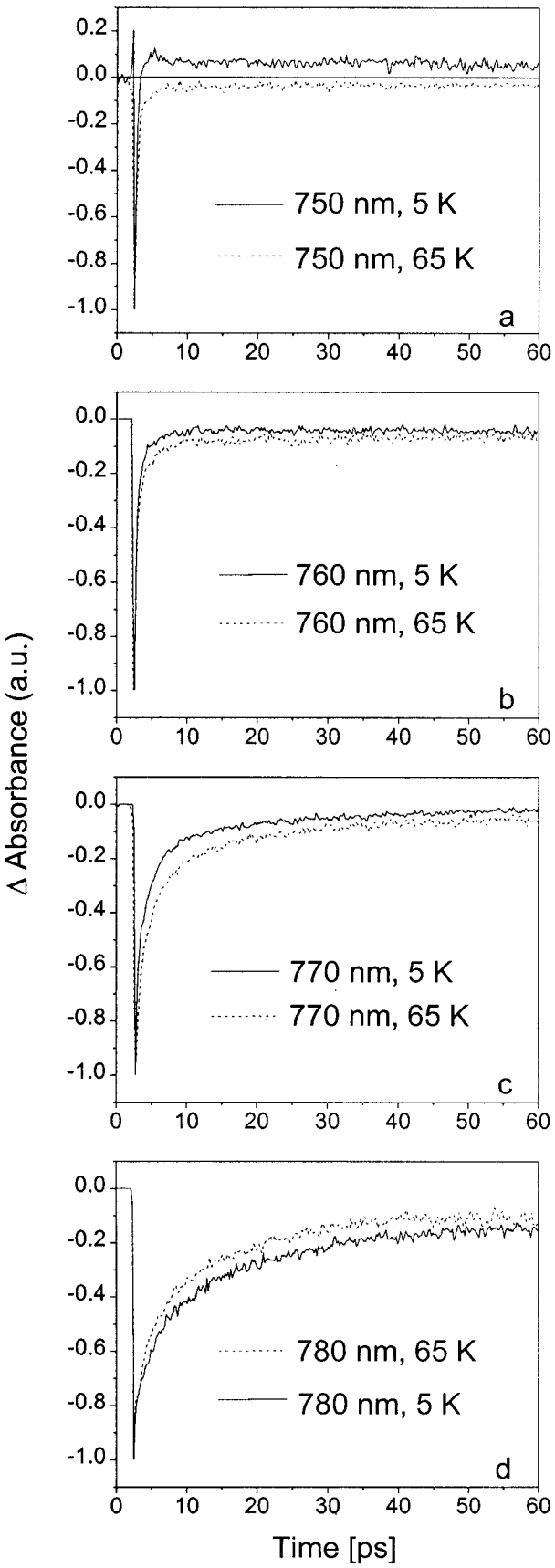

Figure 4. Effect of temperature on one-color isotropic absorption difference signals for *Chl. tepidum* for wavelengths from 750 to 780 nm measured at 5 K (solid line) and 65 K (dotted line); anaerobic conditions.

TABLE 1: Decay Lifetimes (τ_i) and Relative Amplitudes (α_i) of the One-Color Isotropic Absorption Difference Signals at 5 and 65 K at Anaerobic Conditions[a]

λ [nm]	T [K]	τ_1 [ps]	α_1	τ_2 [ps]	α_2	τ_3 [ps]	α_3	τ_4 [ps]	α_4	τ_5 [ps]	α_5
720	65					5.3	0.51	62	0.49		
735	65	0.25	−1.00			6.3	0.11			∼200	0.01
750	5	0.27	−1.00			6.0	0.05			∼200	0.05
	65	0.26	−0.90	1.7	−0.07					∼300	−0.03
760	5	0.27	−0.85	1.8	−0.13					∼300	−0.02
	65	0.25	−0.76	1.8	−0.19					∼300	−0.05
	65[b]	0.25	−0.76	1.7	−0.15	*5.4*	−0.05			∼300	−0.04
770	5	0.29	−0.50	1.8	−0.31	5.8	−0.11	36	−0.08		
	65	0.30	−0.35	1.7	−0.32	5.4	−0.20	40	−0.13		
780	5	0.25	−0.31			5.8	−0.38	39	−0.23	∼300	−0.08
	5[b]	0.28	−0.31	*1.8*	−0.08	5.9	−0.33	33	−0.20	∼300	−0.08
	65	0.26	−0.23			5.4	−0.42	38	−0.29	∼300	−0.06
	65[b]	0.25	−0.26	*1.7*	−0.13	5.4	−0.36	37	−0.25		
790	5	0.21	−0.70			5.7	−0.20	36	−0.02	∼300	−0.08
	65	0.25	−0.60			5.6	−0.19	38	−0.04	∼300	−0.07

[a] Lifetimes were determined with 10% error, except τ_1 at 750 nm, 5 K (due to presence of the coherent spike) and all lifetimes τ_5. [b] In these fits the parameter in italics was held fixed.

lated an error of the estimated lifetimes as 10%. To check whether the obtained lifetimes depends on the time window used, as was observed by Savikhin et al.,[14] we used the parameters of the best fits determined from a 60 ps window as initial values to fit decays obtained with 1 and 6 ps windows. The lifetimes shorter than the fitting window remain unchanged within the 10% error; however, the lifetimes longer than the used time window suffered with obvious loss of accuracy. The fastest decay component (200−300 fs) appearing at wavelengths between 735 and 780 nm is interpreted as a weighted average of interstate relaxation of higher excitonic levels of BChl *c* aggregates. As the aggregates of BChl *c* with essentially same spectral properties as those found in chlorosomes can be prepared artificially without the presence of any protein,[4] the spectral red shift between BChl *c* monomers and aggregates absorption maxima (∼1600 cm^{-1}) is the result of strong excitonic interactions between the molecules forming the aggregate. The whole aggregate acts as a supermolecule, and after the absorption of a light quantum the excitation is initially delocalized over a certain number of strongly coupled BChl *c* molecules. We can expect that the structure of the aggregates at room temperature enables the delocalization of excitation energy for sufficient time to allow energy transfer from excited excitonic levels to another BChl cluster. (See below the discussion about the 1.6 ps component.) It is known that the energetic disorder of pigment molecules leads to localization. To explain why the overall decay is faster at wavelengths dominated by absorption into higher excitonic states at low temperature (5−65 K), we assume that due to a decrease of ratio between strength of the dipole−dipole interaction and energetic disorder, the conditions favoring coherent sharing are modified. As a result, the initial delocalization is rapidly destroyed and followed by relaxation connected with a localization of excitation energy. The dynamic character of exciton localization was shown in a theoretical model by Kühn and Sundström.[26] For the excitonically coupled B850 antenna of purple bacteria, they determined an excitation to be initially delocalized over the whole aggregate at room temperature. With increasing time delay the exciton coherence domain shrinks to cover about four pigments in the asymptotic limit. Table 1 shows that the relative amplitude of the 200−300 fs component decreases with an increase of the wavelength (with the exception of 790 nm, where an overlap of BChl *c* and BChl *a* affects isotropic decays), and the distribution of this lifetime reproduces the spectrum of absorption into higher excitonic states. At a given wavelength, its relative amplitude decreases with an increase of temperature.

The second component observed at 750−780 nm is similar to the 1−2 ps component observed frequently at room temperature,[14] which was interpreted as perhaps corresponding to a single energy-transfer step between BChl *c* aggregates. If this interpretation is also appropriate at low temperatures, then such a process must occur from higher excitonic states, because from hole burning it is known that the lifetime of the LES is more than 3 times longer. Further, the spectral distribution of this component (Table 1) is shifted to the blue with respect to that of the LES and therefore supports our interpretation. At room temperature the energetic gap between the exciton levels becomes comparable to thermal energy (kT > 200 cm^{-1} at 300 K while kT = 3 cm^{-1} at 4.2 K); hence, the higher excitonic states may be thermally populated from the LES during its lifetime, and energy transfer may dominantly occur from these states. The 200−300 fs component has been attributed to relaxation of higher excitonic levels. In our opinion, there is no contradiction if one of the excitonic levels possesses a 1.6 ps lifetime, since particular levels may be expected to have considerably different (shorter and longer) lifetimes than the averaged value, as was observed in the excitonically coupled FMO protein complex, where the lifetimes of particular excitonic levels were estimated ranging between 30 fs and 2.5 ps.[27] Moreover, the distributions of 200−300 fs and 1.7−1.8 ps components are spectrally separated (Table 1), and the 1.7−1.8 ps may be an excited-state lifetime of a level, which does not contributes to the 200−300 fs component.

The 5.6−5.9 ps component has similar lifetime and spectrum (within the studied wavelengths) as those of the BChl *c* LES determined from hole burning. We believe that this lifetime is determined by energy transfer between BChl *c* clusters (aggregates, rods) occurring from the LES, probably by the Förster energy-transfer mechanism. As the temperature increases up to 65 K, the process becomes slightly faster (5.4 ps).

At wavelengths above 770 nm a process characterized by a lifetime of 30−40 ps contributes to the overall decays. Components with a similar lifetime were often observed for green bacteria containing BChl *c*,[9,14,28] and they were usually interpreted as due to the BChl *c*-to-BChl *a* energy transfer.

In addition, to fit the nonzero absorbance change at the end of the 60 ps time window, it was necessary to use a slow minor component of 200−300 ps lifetime at almost all wavelengths. Because of the short scan range, we could not determine the

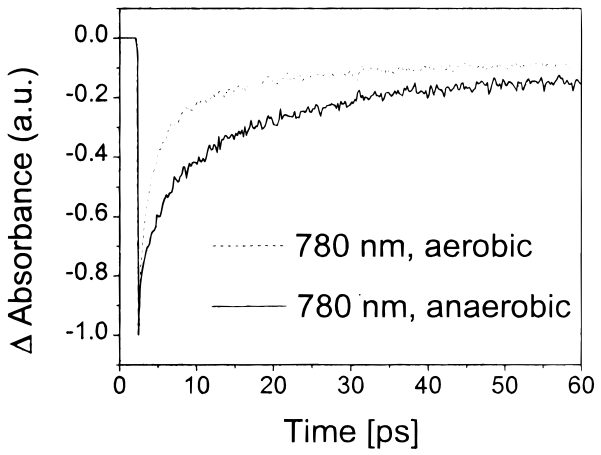

Figure 5. Effect of redox conditions on one-color isotropic absorption difference signals for *Chl. tepidum* at 780 nm measured at anaerobic (solid line) and aerobic (dotted line) conditions at 5 K.

lifetime with sufficient accuracy; however, it resembles the 200−300 ps components earlier observed[28,29] under saturating light conditions or at low temperatures when the RC's are in the closed state.

Figure 6 shows a proposed energetic scheme of the main low-temperature relaxation processes in chlorosomes at anaerobic conditions. Neither hole burning nor one-color decays allow a definite identification of the acceptor of the transferred energy. The 1.8 ps component may thus dominate in different energy-transfer steps (e.g., energy transfer between aggregates within one rod) over the 5.8 ps lifetime (e.g., energy transfer between rods). The proposed interpretation is based on the idea of the rods containing tightly packed aggregates where the energy transfer within the rod is preferred over the rod-to-rod hoping due to assumed weaker interaction between clusters located in neighboring rods compared to adjacent aggregates within one rod. To verify the proposed models, a calculation of the mathematical model is important. For this reason, performing additional experiments is necessary, including study of two-color decays, transition absorption spectra, and anisotropy decays. Such experiments are planned in the near future.

E. Excited-State Relaxation and Energy Transfer at Aerobic Conditions. An effect of aerobic conditions was studied by both hole burning and time-resolved spectroscopy. In hole-burning experiments the shortening of the LES lifetime to 2.65 ps was observed at aerobic conditions. This shortening is attributed to quenching of the BChl *c* excitation by a mechanism that is believed to protect low potential electron acceptors in RC's against superoxide-induced damage when oxygen is present.[8] In time-resolved experiments the effect of aerobic conditions was most pronounced at 780 nm (Figure 5), which suggests that the lifetime of the LES is most affected. Indeed, the dominating decay component fits well with the lifetime of the LES obtained from hole burning (2.5−2.6 ps, Table 2). The second component (14−16 ps) is similar to the

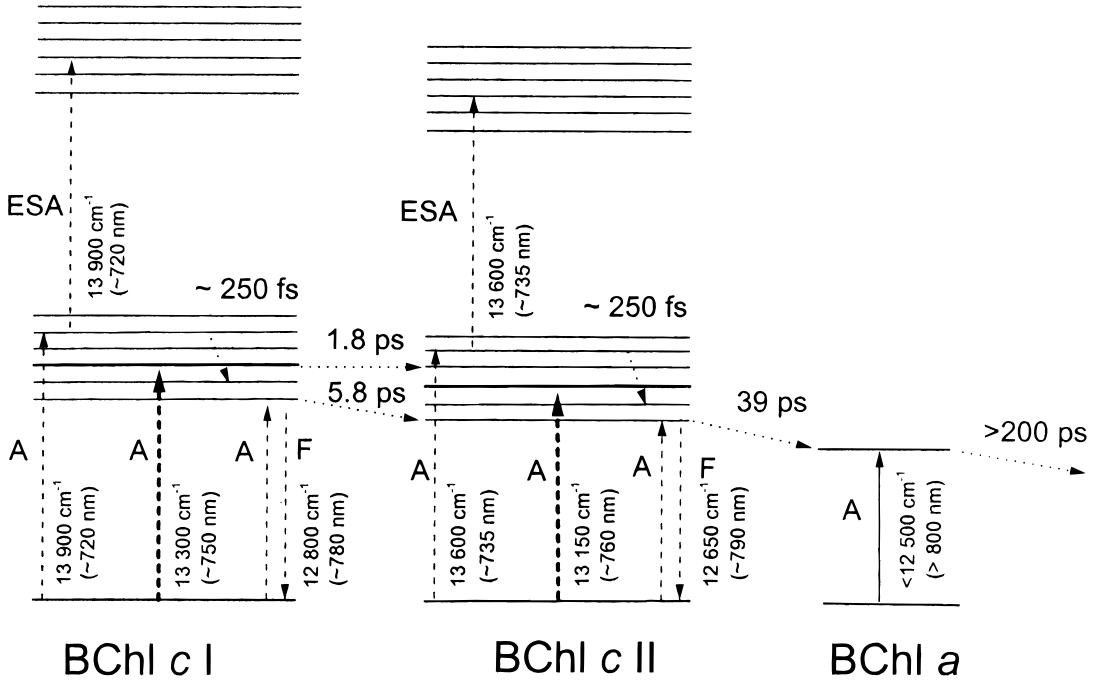

Figure 6. Proposed energetic diagram of main transitions in chlorosomes at anaerobic conditions. The width of lines represents intensity. A = absorption, F = fluorescence.

TABLE 2: Comparison of Excited State Lifetimes for *Chl. tepidum* Determined from Persistent Spectra Hole Burning (T_1) with Decay Lifetimes (τ_i) and Relative Amplitudes (α_i) of the One-Color Isotropic Absorption Difference Signals at Anaerobic and Aerobic Conditions at 5 and 65 K at 780 nm[a]

conditions	δ_{HB} [cm^{-1}]	T_1 [ps]	τ_1 [ps]	α_1	τ_2 [ps]	α_2	τ_3 [ps]	α_3	τ_4 [ps]	α_4
anaerobic										
5 K	1.85	5.75	0.25	−0.31	5.8	−0.38	39	−0.23	∼300	−0.08
65 K			0.26	−0.23	5.4	−0.42	38	−0.29	∼300	−0.06
aerobic										
5 K	4.0	2.65	0.22	−0.38	2.6	−0.40	16	−0.14	∼200	−0.08
65 K			0.22	−0.35	2.5	−0.32	14	−0.27	∼200	−0.06

[a] Lifetimes were determined with 10% error, except τ_5.

lifetimes previously observed at high redox potentials[8,10] and is probably connected with quenching of BChl *c*-to-BChl *a* energy transfer. (No component of the 30−40 ps lifetime was obtained when oxygen was present.) This suggests that quenching operates on at least two levels. Two different quinones found in *Chl. tepidum*, menaquinone-7 and chlorobiumquinone, are assumed to be involved in the processes.[11]

Conclusions

In this report we argue that the main absorption of BChl *c* at around 750 nm is due to the absorption into higher excitonic states of the aggregates, while the LES is distributed around 780 nm and its absorption is relatively weak. In this respect, the results are qualitatively similar to those obtained for the B850 complex of purple bacteria[21] and are of significance in understanding the aggregate structure. The lifetime ($\geq$5.75 ps) and spectral distribution of the LES determined in hole-burning experiments have their counterparts in time-resolved experiments (Table 1). This simplified the interpretation of isotropic decays: because of its lifetime and a distribution, the observed 1.7−1.8 ps component reflects relaxation from excitonic levels, rather than from the LES. Both ~5.8 and 1.7−1.8 ps processes are ascribed to energy transfer between BChl *c* clusters, however, in different energy-transfer steps. It was shown that the higher probability of the 1.7−1.8 ps process at room temperature[14] may be explained by the thermal population of higher excitonic levels from the LES. The observed lifetimes of 30−40 and 200−300 ps fit well with results of earlier works and were attributed to BChl *c*-to-BChl *a* energy transfer and to energy transfer toward closed RC's, respectively. (The RC's are in a closed state below 77 K.[29]) The isotropic decays at 735 nm are explained by the presence of at least two different spectral forms of BChl *c*, supporting thus the concept of spectral inhomogeneity in chlorosomes of green sulfur bacteria. The main changes of isotropic decays between 5 and 65 K occur at ~50 K and are connected with the temperature dependence of the population probability of relatively long-lived LES, manifested in corresponding changes of fluorescence. The second important factor, which leads to faster isotropic decays at wavelengths corresponding to absorption into higher excitonic states (compared to kinetics measured at room temperature[14]), is a high contribution of fast (200−300 fs) interstate relaxation from higher excitonic levels at low temperatures. Presumably the conditions enabling coherent sharing of excitation over several molecules of the aggregate in early times are destroyed due to an increase of energetic disorder at low temperatures. Interstate relaxation takes place instead. At aerobic conditions the excitation is quenched on at least two different levels with lifetimes of 2.5−2.7 and 14−16 ps, preventing BChl *c*-to-BChl *c* and BChl *c*-to-BChl *a* ET, respectively. The quenching is a result of a protecting mechanism in which two different quinones are involved.[11]

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