Ultrafast Energy Transfer Kinetics in Chlorosomes from *Chloroflexus aurantiacus*

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Received May 20, 1995

Transient absorption kinetics of bacteriochlorophylls in the chlorosomes from *Cf. aurantiacus* were measured at room temperature and 77 K using low- intensity near-infrared femtosecond pulses with a duration as short as 50 fs. For accurate determination of the isotropic and anisotropic decays, simultaneous two channel detection of both parallel and perpendicular polarizations in the one-color pump-probe setup was employed. A typical fast isotropic component was found within the Qy band of the BChl *c* absorption, which has a decay lifetime varying from 620 fs to 1.5 ps at room temperature, and between 400 fs and 1.6 ps at 77 K. Both exhibit a general trend of increasing decay time with detection wavelength. Very similar time constants were also observed for the corresponding single anisotropy decays. The origin of these fast decays is suggested to be due to either multiple excitonic relaxation or a sequential intra-chlorosomal energy transfer between different spectral forms of BChl *c* pigments. These spectral forms may be organized in such a way that there is a gradual decrease of polarization from the short-wavelength absorbing form to the long-wavelength absorbing one, accounting for the wavelength-dependent residual anisotropies with values of 0.34 at 720 nm, 0.30 at 730 nm and 0.22 between 734 - 760 nm. The 2 ps isotropic component resolved at 780 and 796 nm may be related to vibrational relaxation or to energy transfer among the baseplate BChl *a* molecules. Further energy transfer processes such as that from chlorosomal BChl *c* to the baseplate BChl *a*, are also discussed.

I. Introduction

The chlorosome is the major light-harvesting antenna in *Chloroflexus aurantiacus Cf. aurantiacus*). It typically contains 10 000 BChl *c* molecules with an absorption maximum at 740 nm[1]. The antenna BChl *c* in Chloroflexaceae are organized as rod-like elements with a diameter of 5.2 nm extending the full length of the chlorosomes[2,3]. In addition the chlorosomes contain a small amount of BChl *a* with an absorption maxima around 790-795 nm[4]. These BChl *a* molecules are found in the baseplate connecting the chlorosome to the cytoplasmic membrane where the reaction centers and core antenna components (B806-866) are situated[5].

The characteristic feature of the chlorosome architecture is proposed to be organized as BChl *c* oligomers. This means that its structural arrangement, spectral properties, as well as its energy transfer processes are mainly determined by the pigment-pigment interactions, in contrast to the pigment-protein interactions which are the predominant structural parameters in other photosynthetic antenna systems[6-11]. Support for this idea comes from the spectroscopic similarity between *in vitro* BChl *c* in organic solvents, isolated protein-free chlorosomes and the native chlorosomes *in vivo*[12,13]. A variety of evidence from linear dichroism[14-16], fluorescence polarization[17-19], as well as anisotropy measurements [20, Y.-Z. Ma *et al.*,, unpublished results], have revealed that there is a high degree of order among the BChl *c* molecules in the chlorosome structure. The average angle between the 740 nm transition dipole moment and the long axis of the rod elements of the chlorosome was estimated to

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be 15 - 25° [14, 18 - 20, Y.-Z. Ma *et al.*, unpublished results].

The energy transfer processes in chlorosomes from *Cf. aurantiacus* have been studied both in intact cells and isolated chlorosomes mainly by picosecond time-resolved fluorescence and transient absorption measurements[12,13,20,21–25]. The time constants were found to be about 8 - 16 ps for energy transfer from the chlorosomal BChl *c* to the baseplate BChl *a*, depending mainly on the kind of chlorosomes (isolated or intact)[12], and about 30 - 40 ps for the transfer from the baseplate BChl *a* to the B806-866 core antenna. An even shorter lifetime was resolved by Holzwarth *et al.*[26] using the single photon counting technique in combination with global analysis of the data. The obtained lifetime of 5 ± 1 ps was considered to be either the time for energy transfer between two BChl *c* pools or the relaxation time between two exciton states. With a 2 ps time resolution, Lin *et al.*[18] observed wavelength dependent decay behavior between 715 and 755 nm and a major lifetime of 3-4 ps. The same authors also resolved a wavelength dependent absorption anisotropy decay time, which changed from 7 ± 1 ps at 720 nm to 4 ± 1 ps at 740 nm. The observed wavelength dependent decay kinetics as well as the dynamic blue shift were explained by an exciton model for the chlorosomal BChl *c* organization based on modified linear J - aggregates. Very recently, Savikhin *et al.* observed an additional ultrafast equilibrium process which proceeded in about 100 fs at room temperature[27].

In this paper, we report transient absorption kinetic measurements performed on chlorosomes of *Cf. aurantiacus* at room temperature as well as at 77 K using femtosecond pulses with a typical duration of approximately 50 fs. A typical fast isotropic component was observed in the range of 720 - 760 nm, which has a lifetime varying from 620 fs to 1.50 ps at room temperature, whereas it changes in the range of from 400 fs to 1.6 ps at 77 K. Moreover, it also shows a similarity with the time constant observed in the corresponding anisotropy kinetics. Both these isotropic and anisotropy decays exhibit a general trend of increasing lifetime with detection wavelength in most cases at both room and low temperature. The origin of this fast component is interpreted as a result of either excitonic relaxation or a sequential energy transfer from the short- wavelength absorbing spectral form, with high degrees of polarization, to the long-wavelength absorbing ones, with a lower degree of polarization. Further energy transfer processes, including the transfer from the chlorosomal BChl *c* to the baseplate BChl *a*, as well as the fast relaxation within the baseplate BChl *a* molecules, are also discussed.

II. Materials and Methods

Cells of *Cf. aurantiacus* (strain Ok-70-fl) were grown as described previously[20]. Freshly thawed cells were resuspended in TA buffer: 10 mM Tris-HCℓ pH 8.0 containing 10 mM sodium ascorbate and disrupted by two passes through a French pressure cell at 20 000 psi in the presence of 2 mM PMSF. Unbroken cells and large debris were removed by centrifugation at 20 000 $\times g$ for 10 min at 4°C. Chlorosomes still attached to small membrane fragments were collected from the 20 000 $\times g$ supernatant by centrifugation at 180000 $\times g$ for 90 min, resuspended in a small volume of TA buffer, and diluted with 1 vol. of 87% (w/v) glycerol before storing at - 50°C. The sample was resuspended in 50 mM Tris-HCI (pH 8.0) to give a maximum absorbance of about 0.5 in a 1 mm cuvette for the femtosecond pump-probe measurements. A rotating cell of 1 mm pathlength was also used in some experiments to check for any possible influence of long-lived photoproducts in the measurement. Since it makes no observable difference whether the sample was static or rotating, we will therefore not specify the sample state for the kinetics reported in this paper. Low temperature experiments were performed using an Oxford Instruments cryostat. In order to obtain a good quality glass upon freezing, the sample was mixed to about 60% (v/v) glycerol and a maximum optical density of about 0.5 in a 1.0 mm glass cuvette was employed.

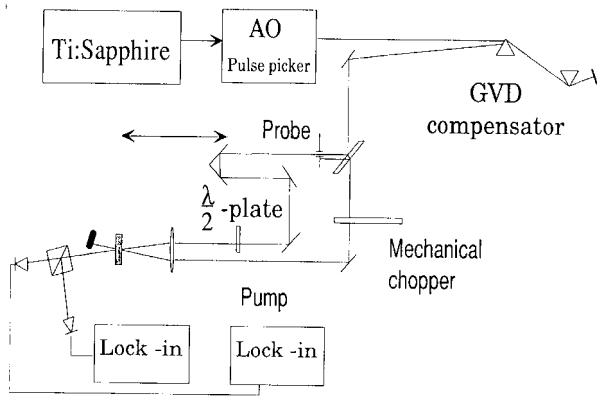

Figure 1. Schematic representation of the experimental setup.

The femtosecond absorption measurements were

performed using one - color pump - probe technique as shown in Fig. 1. A commercial Ti: sapphire femtosecond laser (Spectra Physics^{TM} Tsunami) with a optoacoustic pulse picker (Spectra Physics^{TM} 3980 model) was used to obtain short femtosecond pulses with the desired repetition rate (40 kHz for room temperature experiments and 80 kHz for the 77K ones). The maximum available pump power was less than 1.0 nJ/pulse with a beam diameter of about 40 μm in the beam overlap region. This corresponds to a maximum photon flux of 1.1×10^{13} photons·pulse^{-1}·cm^{-2}, which was further three- or tenfold attenuated with neutral density filters in order to minimize excitation annihilation effects. A dual SF10 prism pulse compressor installed after the pulse picker made it possible to obtain bandwidth limited pulses as short as 50 fs. After the prism compressor the light was directed into a standard noncollinear pump-probe setup with the following modifications for more accurate polarization measurements. The excitation beam was linearly polarized (vertically) and the probe beam was adjusted to contain a mixture of parallel and perpendicular polarizations. These two polarizations were split after the sample by a Glan prism and the intensities were measured by two infrared sensitive photodiodes and registered by separate lock-in amplifiers. In this way, the sample response for both polarization directions was measured simultaneously in a single scan, with significant reduction of the error in the obtained anisotropy values. To make an absolute calibration of ΔT/T in both polarization directions and to obtain the proper relationship between the parallel and perpendicular components in the probe beam, a mechanical chopper was placed in the probe arm and the signal in each channel was measured. The coefficients obtained for each excitation wavelength were used to divide the amplitude of the signal induced by the pump pulses, thus obtaining ΔT/T in absolute units during the measurements. The measured signals were processed with a personal computer. The pulse length and spectrum were monitored by a CAMAC-based system using a rapid scan autocorrelator and a grating spectrometer equipped with a CCD camera during the experiments. The calibrated signals with parallel and perpendicular polarizations were used to obtain the corresponding isotropic and anisotropic decays based on the following relations:

$$\Delta A_{iso}(t) = \Delta A_{\parallel}(t) + 2\Delta A_{\perp}(t) \quad (1)$$

$$r(t) = \frac{\Delta A_{\parallel}(t) - \Delta A_{\perp}(t)}{\Delta A_{\parallel}(t) + 2\Delta A_{\perp}(t)} \quad (2)$$

where $\Delta A_{\parallel}(t)$ and $\Delta A_{\perp}(t)$ are the transient absorbances with parallel and perpendicular polarization, respectively. The isotropic kinetic traces were analyzed using a multiexponential deconvolution procedure taking the pulse autocorrelation function into account. A similar deconvolution method was used to fit the anisotropy profiles with a single-exponential decay function.

The whole system was carefully calibrated before measurements by using the laser dye IR140 in a methanol solution. This system has a constant anisotropy of 0.4 within at least several tens of picoseconds as shown in Fig. 2.

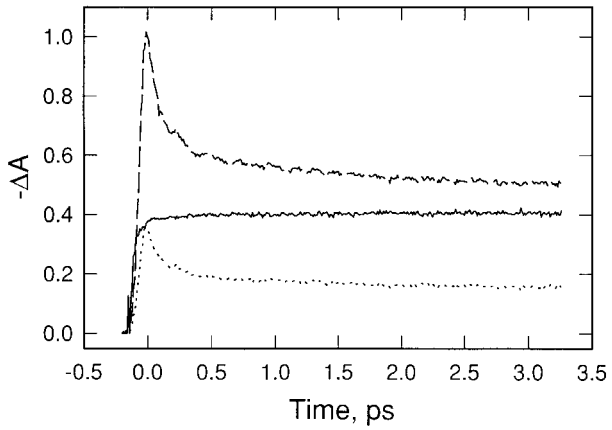

Figure 2. A single test scan using the laser dye IR140 in methanol solution at room temperature and 740 nm. The signals with parallel and perpendicular polarizations are shown as dashed and dotted lines, respectively. The solid line represents the calculated anisotropy.

III. Results

III.1 Steady-state absorption spectra

Fig. 3 shows the absorption spectra of chlorosome from *Cf. aurantiacus* at both room temperature and 77 K. The spectra have been normalized to the near infrared absorption maximum of BChl *c*, which was 739 nm at room temperature and 742 nm at 77 K. Besides

this slight red shift of the absorption maximum a narrowing of the absorption band was observed upon cooling. The less intense band with a maximum at 795 nm clearly observed in the expanded spectrum arises from absorption of BChl *a* baseplate pigments. Also shown in Fig. 3 are the spectra of two pulses centered at 760 or 780 nm

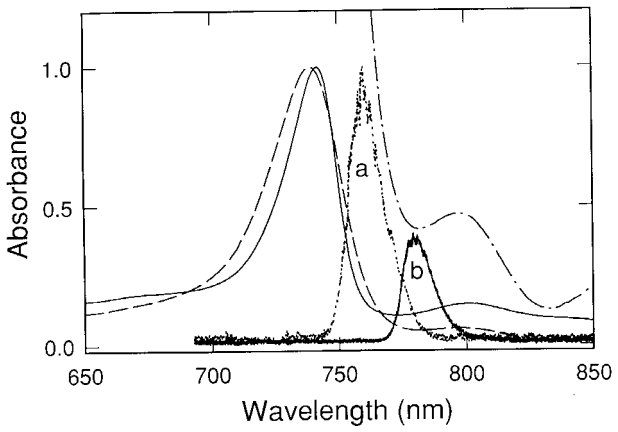

Figure 3. Steady-state absorption spectra of the chlorosomes from *Cf. aurantiacus* at room temperature (dash line) and 77 K (solid line). The spectra have been normalized at their near infrared absorption maxima. The dash-dot line shows the 10 fold expanded pattern of the red side of the room temperature absorption spectrum. Two spectra of the femtosecond pulses are also shown with a central wavelength at (a) 760 nm and (b) 780 nm, respectively. The relative intensities of these pulse spectra were modified for the sake of clarity.

III.2 Isotropic transient absorption kinetics

The transient absorption kinetics for the chlorosomes from *Cf. aurantiacus* were measured at several wavelengths between 720 and 796 nm covering the region of the Q_y band of chlorosomal BChl *c* and the baseplate BChl *a* molecules. Fig. 4 shows an example of the kinetics with parallel and perpendicular polarizations measured at 743 nm and 77 K, together with the corresponding autocorrelation trace and pulse spectrum. Isotropic profiles were generated from the measured decays with parallel and perpendicular polarizations as described in the experimental section and a few typical traces obtained at room temperature are shown in Fig. 5. The isotropic decays are obviously wavelength dependent as observed by Lin *et. al.* using lower time- resolution[18]. At shorter wavelengths, around 720 nm, the signal is dominated by a strong excited-state absorption, which is represented as a negative signal in Fig. 5. At wavelengths above 730 nm a positive isotropic decay was observed containing contributions from ground-state photobleaching as well as stimulated emission.

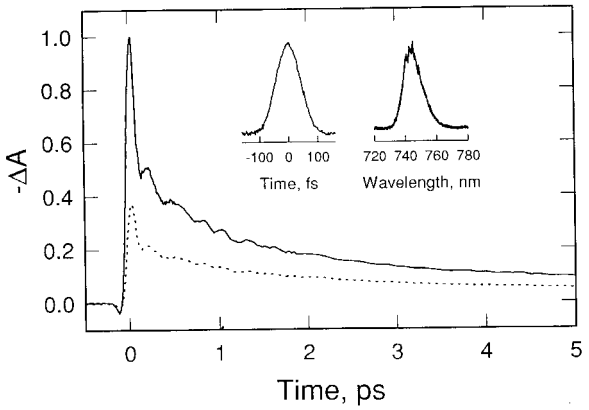

Figure 4. Measured transient absorption kinetics of the chlorosomes from *Cf. aurantiacus* at 743 nm and at 77 K with parallel (solid line) and perpendicular (dotted line) polarization. Inserted are the autocorrelation trace and the corresponding pulse spectrum.

The room temperature isotropic kinetics obtained can be well fitted by using a four-exponential decay function in a time-window of up to 100 ps, except at 796 nm, where a three exponential function was sufficient for a good fit. The resulting decay lifetimes and relative amplitudes are summarized in Table 1. The shortest decay component dominating the kinetics at most measuring wavelengths is of the order of several tens of femtoseconds. The second component has a lifetime varying from 620 fs to 1.50 ps, and exhibits a clear increase of lifetime with increasing detection wavelength in the range of 720 - 760 nm. The only exception from this pattern was observed at the shortest measuring wavelength, 720 nm, which represents excited-state absorption recovery instead of ground-state photobleaching. At 780 and 796 nm, a component with lifetime of about 2 ps was observed. The third component has a time constant in the range of 4.7 ps to 7.6 ps between 720 and 760 nm, and it increases to about 20 ps at 780 and 796 nm. Moreover, a slow component could be resolved with a lifetime of around 100 ps with clearly larger amplitude at 780 nm and 796 nm in comparison to the small contribution at shorter wavelengths.

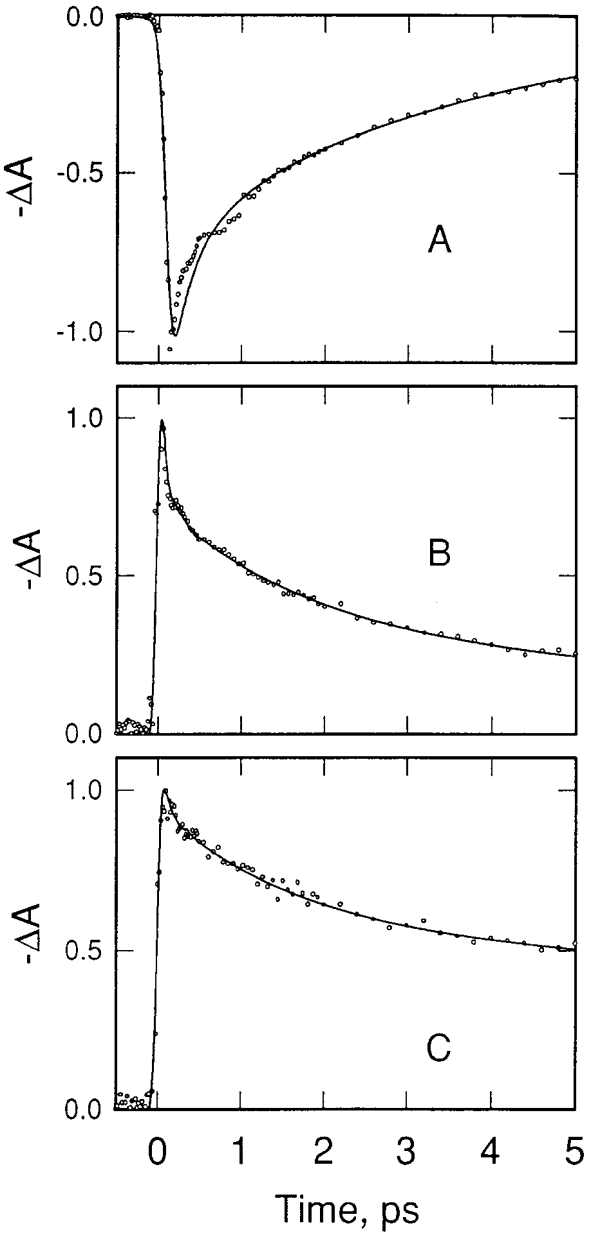

Figure 5. Isotropic transient absorption kinetics of the chlorosomes from *Cf. aurantiacus* at room temperature at 720 nm (A), 743 nm (B) and 796 nm (C). Continuous lines show the optimized fitting curves and the corresponding fitting results are given in Table 1.

Fig. 6 shows two isotropic decays at 730 and 796 nm, recorded at 77 K. The decay kinetics were analyzed by using three or four exponential and functions the fitting parameters are summarized in Table 2. In general, the lifetimec obtained at 77 K are only slightly longer than those determined at the same wavelengths at room temperature. The lifetime of the second component also increases with wavelength except at 720 nm. The change in the number of exponential components required for the satisfactory fitting of the low temperature isotropic decay at 760 nm, compared with the corresponding decay at room temperature, is probably due to the shorter time window (5 ps) used at 77 K.

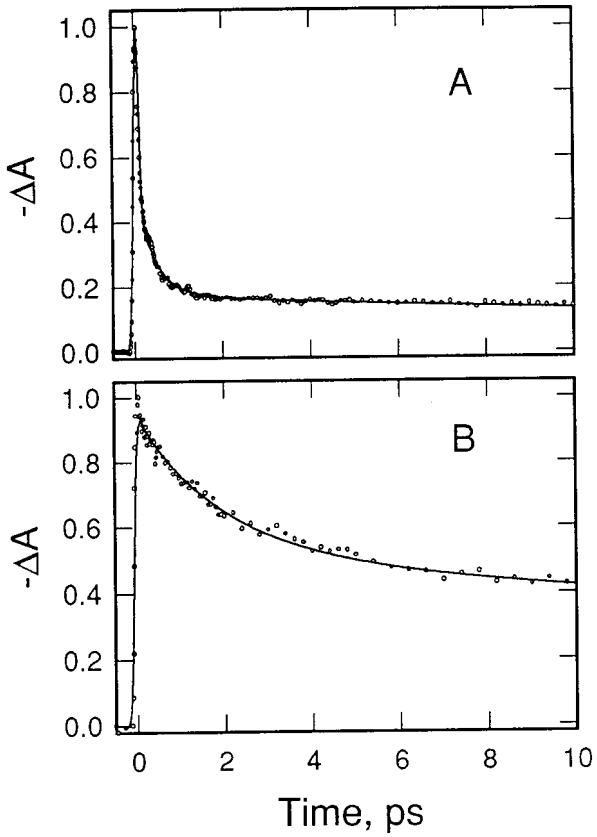

Figure 6. Isotropic transient absorption kinetics of the chlorosomes from *Cf. aurantiacus* at 730 nm (A) and 796 nm (B) obtained at 77 K. The solid lines show the optimized multi-exponential decay fitting curves with parameters summarized in Table 2.

III.3 Time-resolved anisotropy

Time-resolved anisotropy kinetics at room temperature are also strongly wavelength dependent. The measured profiles could be well fitted to a single-exponential decay function and the fitting parameters are summarized in Table 3. A few examples of the anisotropic kinetics are shown in Fig. 7. In the main part of the BChl *c* Qy band the anisotropy decays very rapidly from the theoretical upper limit of 0.4, or slightly lower, to a wavelength dependent constant residual value in less than 1.4 ps. Two similar features can be recognized by comparison with Tables 1 and 2. Firstly, the

Table 1. Lifetimes and relative amplitudes for the isotropic kinetics of excited bacteriochloroplylls in chlorosomes from *Chloroflexus aurantiacus* at room temperature.

λ(nm)	τ_1(fs)	τ_2(ps)	τ_3(ps)	τ_4(ps)	A_1	A_2	A_3	A_4
720	14	1.56	4.7	109	-0.61	-0.16	-0.21	-0.02
730	46	0.62	7.6	122	0.55	0.23	0.15	0.07
734	33	0.92	5.3	91	0.58	0.15	0.21	0.06
743	26	1.45	6.6	104	0.41	0.21	0.34	0.04
760	10	1.50	5.9	139	0.94	0.03	0.03	0.002
780	27	2.46	22	141	0.57	0.15	0.13	0.15
796		2.25	23	118		0.45	0.15	0.40

Table 2. Lifetimes and relative amplitudes for the isotropic kinetics of excited bacteriochloroplylls in chlorosomes from *Chloroflexus aurantiacus* at 77 K.

λ(nm)	τ_1(fs)	τ_2(ps)	τ_3(ps)	τ_4(ps)	A_1	A_2	A_3	A_4
720	52	1.56	5.7	94	-0.25	-0.49	-0.22	-0.04
730	10	0.40	7.9	116	0.80	0.13	0.02	0.05
743	59	1.01	7.6	194	0.59	0.27	0.13	0.01
760	44	1.63	10.3		0.42	0.23	0.35	
796		1.86	20	150		0.43	0.26	0.31

general trend of an increasing decay time with wavelength also holds for the anisotropic kinetics. Secondly, there is a good consistency between the anisotropy decay times and the second isotropic decay component. Moreover, the residual constant anisotropy at longer times decreases as the detection wavelength increases. In the blue wing of the Qy band of the BChl *c* absorption, the residual anisotropy changes from 0.34 at 720 nm to 0.30 at 730 nm. While, in the central part of the band from 734 nm to 760 nm, a relative low but constant value of 0.22 was obtained. The latter value is in excellent agreement with our previous measurements of steady - state fluorescence anisotropy upon excitation at 464 nm in the Soret band [Y.-Z. Ma *et al.*, unpublished results], as well as the residual value of 0.20 ± 0.05 obtained using picosecond time-resolved one-color pump-probe measurements at 750 nm in isolated chlorosome[20], with all preparations obtained from *Cf. aurantiacus*. Good agreement was again obtained at 750 nm and room temperature for the chlorosomes from *Cb. limicola*, which also show a residual anisotropy of 0.20[28]. Our results are also in good agreement with the constant anisotropy of 0.20 determined by picosecond one-color pump-probe technique for the isolated chlorosomes from *Cb. vibrioforme* at 754 nm and room temperature by van Noort *et al.*[29], as well as that determined by fluorescence polarization in the whole cells of the same species at 6K [30].

In contrast to the very fast anisotropy decays observed at room temperature at wavelengths shorter than 760 nm, the anisotropy decays measured at 780 nn and 796 nm are rather slow. Analysis of these kinetics shows that both of them could be well fitted by a single exponential function in a measuring range up to 100 ps. At both wavelengths, the decay is from a value slightly smaller than the theoretical maximum anisotropy of 0.4 to a residual constant value of 0.14 within 6 - 8 ps. No corresponding component was observed in the isotropic decays. It is interesting to note that the residual anisotropy values and the corresponding decay times, obtained at 780 nm and 796 nm, are consistent within experimental error with our previous result of 0.13 ± 0.02 and 7.6 ± 1.7 ps, measured with picosecond time resolution at 800 nm[20]. A closely similar value of 0.12 was also found at 800 nm for the chlorosomes from the same bacteria in recent femtosecond pump-probe experiments at room temperature[27].

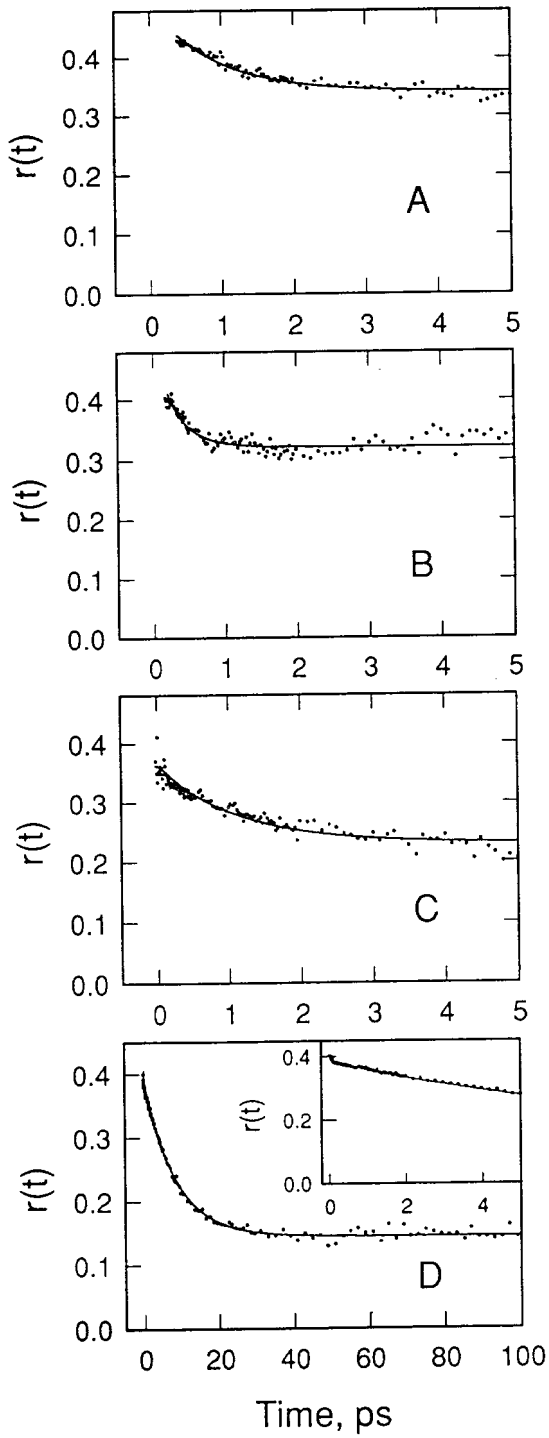

Figure 7. Time-resolved anisotropies of the chlorosomes from *Cf. aurantiacus* at room temperature at 720 nm (A), 730 nm (B), 760 nm (C) and 796 nm (D). The continuous lines show the optimized single - exponential deconvolution fitting curves taking into account the corresponding auto-correlation functions. Insert in (D) shows the short time fitting result at the same wavelength. The fitting parameters are summarized in Table 3.

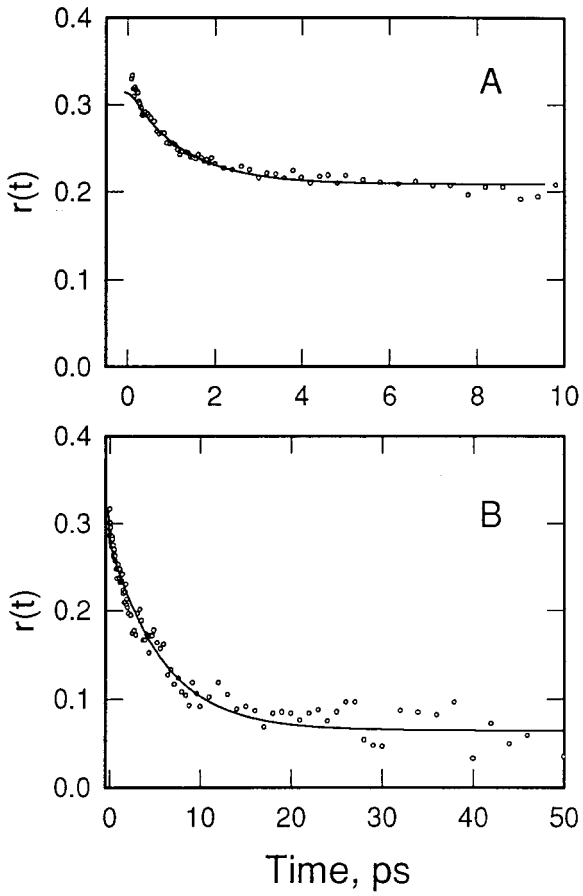

Figure 8. Time-resolved anisotropies of the chlorosomes from *Cf. aurantiacus* at 743 and 796 nm obtained at 77 K. The solid lines show the optimized single exponential decay fitted with the parameters summarized in Table 4.

The anisotropy decays at 77 K can also be well fitted with a single-exponential decay and the fitting parameters are listed in Table 4. The anisotropy decays in the region of 720 - 760 nm also show time constants comparable to one components of the corresponding isotropic kinetics, except at 730 nm. The reason for the obvious deviation from the general trend at this particular wavelength, which is very close to the isosbestic point of the transient absorption spectrum, is not clear. Moreover, a clear trend of increasing lifetime with wavelength was again observed at the other detection wavelengths. It should be noted that considerable difterences exist between some of the results obtained at 77 K and the corresponding results obtained at room temperature. In general, a lower initial anisotropy was obtained at most wavelengths. At 720 nm, both the initial and residual anisotropies are much lower than at room temperature. At 796 nm, the anisotropy decays from a initial value of 0.31 to an even lower residual

anisotropy than that at room temperature in approximately 6 ps. There is corresponding isotropic decay component.

Table 3. Summary of the anisotropy decays of chlorosomes from *Chloroflexus aurantiacus* at room temperature.

λ(nm)	t(ps)	r(0)	r(∞)
720	1.11	$\sim$0.4	0.34[a]
730	0.37	0.37	0.30[a]
734	0.99	0.35	0.22[a]
743	1.35	0.36	0.22[a]
760	1.32	0.37	0.22[a]
780	6.15	0.38	0.14[b]
796	8.09	0.39	0.14[b]

[a] Determined after 8 ps; [b] Determined after 100 ps.

Table 4. Summary of anisotropy decays of chlorosomes from *Chloroflexus aurantiacus* at K.

λ(nm)	t(ps)	r(0)	r(∞)
720	0.85	0.20	0.09[a]
730	2.82	$>$0.40	0.30[a]
743	1.25	0.33	0.21[a]
760	2.46	0.31	0.25[b]
796	5.70	0.31	0.06[c]

[a] Determined after 5.0 ps; [b] Determined after 10.0 ps; [c] Determined after 50.0 ps;

IV. Discussion

IV.1 Optical inhomogeneity of the chlorosomal absorption band

Several lines of experimental evidence indicate that the ground state absorption of chlorosomes is optically inhomogeneous [15, 16, 23, 26, 31, Y.-Z. Ma *et al.*, unpublished results]. Such an inhomogeneity could of course be induced in several ways such as (1) the existence of several distinct spectral forms of BChl *c*, (2) inhomogeneous broadening of a single spectral form in the BChl *c* absorption band and (3) the involvement of optical transitions to different excitonic states. However, hole-burning experiments have indicated that the inhomogeneous broadening makes only a minor contribution to the spectra[32–35]. Therefore, for the multiple spectral form model, we may simply conclude that at least three spectral forms of BChl *c* must exist in the chlorosomes, and each of them differs in the degree of polarizations, in view of the wavelength dependent residual anisotropies observed in our present measurements. If instead the excitonic model is appropriate, however, the situation will become more complicated. We leave this point for the next section.

The presence of multiple spectral forms of BChl *c* is supported by the observation of Matsuura *et al.*[15]. By deconvolution of the linear dichroism and circular dichroism spectra measured on aligned chlorosomes, these authors found that there are two major forms of BChl *c* with peaks at 727 and 744 nm, respectively, as well as a minor one at 766 nm. Further evidence comes from recent hole-burning experiments[35] and from picosecond time-resolved absorbance difference spectra[29]. In the former work, different hole widths at different burning wavelengths in the chlorosomal Qy band of *Cb. limicola* were observed, while in the latter work, a red shifted maximum as compared to the absorption spectrum was reported.

The excitonic model of the energy transfer in the chlorosomes was proposed in view of a possible strong electronic coupling between BChl *c* pigments due to their aggregation. A theoretical interpretation based on a modified linear J - aggregate model was proposed for the observed bipolar transient absorption difference spectra, dynamic spectral shift as well as the wavelength dependent values of residual anisotropy in isolated chlorosomes using picosecond time resolution[18]. In a recent femtosecond pump-probe measurement at room temperature, the authors claimed that the internal energy transfer within the BChl *c* antenna is also of an excitonic nature[27].

The obvious wavelength-dependence of the residual anisotropies in the spectral range of 720 - 760 nm is interesting. The gradual decrease of these values with wavelength indicates that the lowest excitonic state or the longest absorbing spectral form of BChl *c* has a transition dipole orientation that makes a smaller angle with respect to that of the baseplate BChl *a* pigments. Therefore, it may favor the energy transfer from the chlorosomal BChl *c* to the baseplate BChl *a*.

It should be noted that the wavelength dependence of the residual anisotropy was also observed in previous picosecond pump-probe experiments, however, an increase in anisotropy with wavelength was reported[18]. The reason for this obvious discrepancy is unclear. One possibility is that different light intensities were used to grow the cells, which has an unknown but explicit influence on the organization of the BChl *c* molecules in the chlorosomes [Y.- Z. Ma *et al.*, unpublished results].

IV.2 The role of excitonic states

It has been suggested that the BChl *c* molecules may be organized as aggregates, and an effective aggregate size of 13 BChl *c* molecules for chlorosome from *Cf. aurantiacus in vivo* was estimated in an EPR study[6].

Annihilation experiments, further, indicated a domain size of about 20 BChl *c* molecules[20]. Strong electronic coupling between BChl *c* pigments in aggregates will result in formation of discrete exciton levels for a finite linear aggregate. The energies, transition dipole moments and oscillator strengths of these excitonic levels is highly dependent on the coupling strength as well as the geometry of the aggregate[36,37]. Considering the excitonic mechanism to be the origin of the ultrafast decay components observed, two candidates, the shortest (τ_1) and the second isotropic components (τ_2), may be related.

If we consider the shortest isotropic component resolved in the spectral range of the BChl *c* absorption between 720 - 760 nm. The existence of such a component at 780 nm might result from a minor contribution from BChl *c* molecules due to the broad pulse spectrum (see Fig. 3). This fast component could represent the coherent coupling artifact which persists in one color pump-probe experiments. However, the lack of such a component at 796 nm at both room temperature and 77 K under similar experimental conditions indicates the presence of an additional ultrafast dynamic process. One possibility is due to the excitonic relaxation, and the excitonic states involved may be formed by strong electronic coupling within a finite linear aggregate (for instance, a single rod element) with chromophores having parallel transition dipole moment, since there is no corresponding anisotropy relaxation component. However, it should be pointed out that the observed slightly lower initial anisotropy value than the theoretical limit of 0.4, especially at cryogenic temperature, implies that there might be an unlesolved even fast anisotropy relaxation.

If instead the second isotropic component originates from excitonic relaxation, we would have a more complex scheme of excitonic states in view of the similarity of decay lifetime between the isotropic and anisotropy kinetics as well as their wavelength dependence. Such an excitonic relaxation may proceed though a multiple of non-degenerate excitonic levels, in which a number, if not all, of the excitonic levels are optically allowed. The transition dipole moment orientations of these optically allowed excitonic levels should change in such a way that their angles, with respect to the symmetry axis of the linear aggregates, gradually decrease from the higher excitonic state to the lower one. Variation of excitation wavelengths, furthermore, will result in a different occupation of the excitonic levels involved due to coherent excitation by the broadband femtosecond pulses. This occupation difference, as well as the weak exciton-phonon coupling as observed by hole burning experiments[32,33], could together contribute to the wavelength dependence of the decay kinetics. It should be also noted that such a complicated structure of excitonic states further requires that the BChl *c* chromophores involved must have a more complicated geometrical arrangement in the aggregates than in the linear J- aggregates. Further arguments also suggest that there may be more that one independent set of excitonic levels, formed due to the presence of non-identical pigment in different aggregates, and the lowest excitonic level of each set may have a transition moment orientation that corresponds to the residual anisotropies observed at the different wavelengths. This assumption, however, also implies the existence of more than one spectral form of BChl *c* pigments and thus energy transfer between these spectral forms should also be taken into consideration (see following discussion). Clearly more structural information about the rod elements and their package in the chlorosomes is requiled to identify the excitonic mechanism as well as the detailed scheme.

IV.3 Energy transfer

Besides the excitonic mechanism, an alternative origin of the second isotropic component could be energy transfer between at least three different spectral forms of BChl *c* in the chlorosome. The similarity of decay lifetime of the isotropic and corresponding anisotropy kinetics can be interpreted as energy transfer between two spectral forms of BChl *c*, each of these forms has a different transition dipole moment orientation. Moreover, the observed general trend of increasing lifetime with wavelength further indicates a sequential intrachlorosomal energy transfer. Such a sequential energy transfer proceeds from the short-wavelength absorbing spectral form (a single rod element, for instance), with high degrees of polarization, to the long-wavelength absorbing ones, with a lower degree of polarization, in order to account for the wavelength dependent residual anisotropies. This energy transfer model is supported by hole burning[35] and picosecond transient absorption results[29]. However, the recent transient absorbance difference spectra measured with femtosecond resolution[27] exhibit no time evolution during the first 2 ps and thus seems to contradict the above model. As claimed by these authors, this measurement might suffer from singlet-singlet annihilation induced by a high pump intensity[27]. If so, upon excitation in the blue side of the BChl *c* Qy band, a fast energy transfer from short wavelength absorbing spectral forms to the longer ones, or excitonic relaxation, will result in a highly populated low- energy spectral form of BChl *c* or excitonic state and therefore efficient excitation annihilation could occur. Hence, no red-shifted transient absorption bleaching spectrum would appear.

The third isotropic decay component that is resolved between 720 nm and 760 nm has a lifetime of about 4.7 - 7.6 ps at room temperature and a slightly longer lifetime, about 5.7 - 10.4 ps, at 77 K, with no corresponding anisotropic component. This decay may arise trom the subsequent energy transfer from the long wavelength absorbing spectral form or the lowest excitonic state of BChl *c* to the baseplate BChl *a* molecules. The change of the decay lifetime with wavelength, moreover, might indicate an inhomogeneous nature for the energy transfer from BChl *c* to the baseplate BChl *a* pigments. It may correspond to the inhomogeneity in the organization of the BChl *c* and/or baseplate BChl *a* pigments. This is consistent with the observation of a wavelength dependent energy transfer efficiency in the spectral range of the BChl *c* Qy band[38]. This is also supported by previous time - resolved fluorescence measurements on isolated chlorosomes[12] as well as by our single photon counting experiment on the same preparation [Y.-Z. Ma *et al.*, unpublished results]. In the latter work, we resolved a 7 ps component with positive amplitude in the spectral range of the chlorosomal BChl *c* emission and negative amplitude above 800 nm using global analysis. Moreover, the slow isotropic decay components have lifetimes of roughly 100 ps, with a low amplitude at < 760 nm and a considerably higher amplitude at 780 and 796 nm. This component can be related to the excitation energy trapping process by some unknown internal traps, in the spectral range of the BChl *c* Qy band, as suggested before[30], or by the reaction centers as observed at 780 nm and 796 nm. The latter assignment is made based on the fact that the sample used in the present measurements contains membrane-bound chlorosomes and the laser was operated at a low repetition rate and thus, the reaction centers might be in both photochemically active and inactive states. Photochemical state dependent trapping processes by reaction centers were found to have lifetimes of 70 - 90 ps for open and 180 - 200 ps for closed reaction centers, respectively[25].

As shown in Table 1-4, the kinetics observed at 780 nm and 796 nm turn out to be of a different nature in comparison to that obtained at shorter wavelengths, since there are no components with similar lifetimes in the isotropic and corresponding anisotropic decays, respectively. However, a similarity between the kinetics at 780 and 796 nm was observed at room temperature, such as, for example, the decay lifetimes and the residual anisotropies. Therefore, it is reasonable to assume that they both are mainly related to the baseplate BChl *a* molecules. One intermediate isotropic decay component at 780 nm and 796 nm was found to have a lifetime of about 20 ps at both room temperature and 77 K. This decay probably is a result of the energy transfer from the baseplate to the membrane. The decay lifetime is comparable to the result of previous pump- probe measurement using isolated chlorosomes at 800 nm within experimental error[20]. However, it is shorter than the

time constants obtained in time- resolved fluorescence experiments[12,21,25]. Such a difference might be at least partly related to the type of chlorosome sample used.

The origin of the 2 ps isotropic component resolved at these two longer wavelengths is not clear. It could arise from vibrational relaxation, according to observations of BChl *a* monomer relaxation processes in organic solvents[39,40], or excitation energy transfer between proximal BChl *a* molecules in the baseplate with somewhat different spectra. The anisotropic decays obtained at the corresponding wavelengths, moreover, may represent a depolarization process within the baseplate due to energy transfer between the BChl *a* molecules with non-parallel transition dipole moments. Since the anisotropic kinetics have time constants of about 6 ps and 8 ps, respectively, both are approximately three or four times longer than the 2 ps isotropic component. This difference, however, may be interpreted as a result of energy transfer between spectrally different BChl *a* molecules with approximately the same transition dipole moment orientation within the baseplate. Therefore, our results imply that the organization of the baseplate BChl *a* molecules have a local order with respect to their transition dipoles. In this regard, it is noted that a cluster organization containing similar amount of molecules has been suggested for the baseplate of the *Cf. aurantiacus* by excitation annihilation measurements[24].

Finally, we want to point out that we have observed the similar damped oscillatory behavior as was observed by Savikhin *et al.*[27]. These oscillations turned out to be even more pronounced at 77 K than at room temperature, as shown in Fig. 4 and Fig. 5. The presence of oscillations causes some difficulty in optimizing the fits of the observed decays, especially during the first few picoseconds. An analysis of these oscillations in chlorosomes will be published elsewhere.

V. Summary

1. The optical inhomogeneity of the Qy band of the bacteriochlorophyll *c* absorption in the chlorosomes from *Cf. aurantiacus* could be due to the presence of either an excitonic manifold or at least three distinct spectral forms of BChl *c*. These spectral forms may be organized in such a way as to cause a gradual decrease of polarization from the short-wavelength to the long - wavelength absorbing chromophores. This organization pattern corresponds to the observed residual anisotropies of 0.34 at 720 nm, 0.30 at 730 nm and 0.22 between 734 and 760 nm. This organization may favor energy transfer.

2. A fast isotropic component with a characteristic lifetime between 620 fs and 1.5 ps at room temperature and lifetimes from 400 fs to 1.6 ps at 77 K, were resolved in the 720 - 760 nm spectral range. The general trend is an increasing lifetime with detection wavelength. Very similar time constants were observed in the corresponding single exponential anisotropy decays at both room and cryogenic temperature. This fast decay may originate from either a multiple excitonic relaxation or from a sequential excitation energy transfer between distinct spectral forms of BChl *c* pigments, whose transition moment orientation differs from each other. In order to account for the features of these decay lifetimes observed, a complicated scheme of excitonic levels is expected.

3. The subsequent energy transfer from the chlorosomal BChl *c* to the baseplate BChl *a* molecules takes place within 5 - 8 ps at room temperature and between 6 and 10 ps at 77 K, and also exhibits some inhomogeneity. The slowest isotropic component with a lifetime of around a 100 ps might be related to excitation trapping by internal traps in the BChl *c* antenna or by reaction centers.

4. The 2 ps isotropic component resolved at 780 and 796 nm may represent a vibrational relaxation or energy transfer processes in the baseplate BChl *a* pigments. In the case of energy transfer, it can only proceed between BChl *a* molecules with dissimilar spectra, but having similar transition dipole moments, to account for the much shorter isotropic time constant, compared to those of the corresponding anisotropic kinetics. This also implies that the BChl *a* molecules have some local order.

Acknowledgments

This research was supported by the Swedish and Danish Natural Science Research Councils and the Swedish Institute. We thank Mrs. Eva Wikström for technical assistance and Dr A. N. Macpherson for critical reading of the manuscript.

References

1. J. M. Olson. Biochim. Biophys. Acta **594**, 33 (1980).
2. L. A. Staehelin. J. R. Golecki, R. C. Fuller and G. Drews, Arch. Microbiol. **119**, 269 (1978).
3. S. G. Sprague, L. A. Staehelin, M. J. Dibartolomeis and R. C. Fuller, J. Bacteriol. **147**, 1021 (1981).
4. R. G. Feick and R. C. Fuller, Biochemistry **23**, 3693 (1984).
5. R. E. Blankenship and R. C. Fuller, In *Encyclopedia of Plant Physiology,* New Series, Vol. 19, Photosynthesis III (L. A. Staehelin and C. J. Arntzen eds.), pp 390-399, Springer, Berlin (1986).
6. K. M. Smith, L. A. Kehres and J. Fajer. J. Am. Chem. Soc. **105**, 1387 (1983).
7. J. M. Olson, P. D. Gerola, G. H. van Brakel, R. F. Meiburg and H. Vasmel, In *Antenna and Reaction Centers of Photosynthetic Bacteria* (Michel - Beyerle, M. E., ed.), Springer - Verlag: West Berlin, pp 67 - 73 (1985).
8. D. L. Worcester, T. J. Michalski and J. J. Katz, Proc. Natl. Acad. Sci. U. S. A. **83**, 3791 (1986).
9. D. C. Brune, T. Nozawa and R. E. Blankenship, Biochemistry **26**, 8644 (1987).
10. R. van Grondelle, J. P. Dekker, T. Gillbro and V. Sundström, Biochim. Biophys. Acta **1187**, 1 (1994).
11. R. E. Blankenship, J. M. Olson and M. Miller, In *Anoxygenic Photosynthetic Bacteria* (R. E. Blankenship, M. T. Madigan and C. E. Bauer, eds.), Kluwer Academic Publishers, The Netherlands, 399-435, (1995).
12. T. P. Causgrove, D. C. Brune, J. Wang, B. P. Wittmershaus and R. E. Blankenship, Photosynth. Res. **26**, 39 (1990).
13. M. G. Müller, K. Griebenow and A. R. Holzwarth. In *Current Research in Photosynthesis* (M. Baltscheffsky, ed.), Vol. Il, pp 177- 180, Kluwer Academic Publishers, Dordrecht, The Netherlands (1990).
14. H. van Amerongen, H. Vasmel and R. van Grondelle, Biophys. J. **54**, 65 (1988).
15. K. Matsuura, M. Hirota, K. Shimada and M. Mimuro, Photochem. Photobiol. **57**, 92(1993).
16. K. Griebenow, A. R. Holzwarth, F. van Mourik and R. van Grondelle, Biochim. Biophys. Acta **1058**, 194 (1991).
17. Z. G. Fetisova, A. M. Freiberg and K. E. Timpmann, Nature **334**, 633 (1988).
18. S. Lin, H. van Amerongen and W. S. Struve, Biochim. Biophys. Acta **1060**, 13 (1991).
19. H. van Amerongen, B. van Haeringen, M. van Gurp and R. van Grondelle, Biophys. J. **59**, 992 (1991).
20. M. Miller, R. P. Cox and T. Gillbro, Biochim. Biophys. Acta **1057**, 187 (1991).
21. M. Mimuro, T. Nozawa, N. Tamai, K. Shimada, I. Yamazaki, S. Lin, R. S. Kno, B. P. Wittmershaus, D. C. Brune and R. E. Blankenship, J. Phys. Chem. **93**, 7503 (1989).
22. D. C. Brune, G. H. King, A. Infosino, T. Stehler, M. L. W. Thewalt and R. E. Blankenship, Biochemistry **26**, 8652 (1987).
23. M. Mimuro, H. Hirota, K. Shimada, Y. Nishimura, I. Yamazaki and K. Matsuura, In *Research in Photosynthesis* (M. Murata, ed.), Vol. I, pp 17-24 Kluwer Academic Publishers, Dordrecht, (1992).
24. M. Vos, A. M. Nuijs, R. van Grondelle, R. J. van Dorssen, P. D. Gerola and J. Amesz, Biochim. Biophys. Acta **291**, 275 (1987).
25. M. G. Müller, K. Griebenow and A. R. Holzwarth, Biochim. Biophys. Acta **1144**, 161 (1993).
26. A. R. Holzwarth, M. G. Müller and K. J. Griebenow, J. Photochem. Photobiol. Biol. **5,** 457 (1990).
27. S. Savikhin, Y. Zhu, S. Lin, R. E. Blankenship and W. S. Struve, J. Phys. Chem. **98**, 10322 (1994).
28. T. Gillbro, Å. Sandström, V. Sundström and J. M. Olson, In *Green Photosynthetic Bacteria* (J. M. Olson, J. G. Ormerod, J. Amesz, E. Stackebrandt and H. G.Trüper, eds.), pp. 91-96, Plenum Press, New York (1988).
29. P. I. van Noort, C. Francke, N. Schoumans, S. C. M. Otte, T. I. Aartsma and J. Amesz, Photosynth. Res. **41**, 193 (1994).
30. S. C. M. Otte, J. van der Heiden, N. Pfennig and J. Amesz, Photosynth. Res. **28**, 77 (1991).
31. P. Hildebrandt, K. Griebenow, A. R. Holzwarth and K. Schaffner, Z. Naturforsch. **46C**, 228 (1991).

32. Z. G. Fetisova and K. Mauring, FEBS Lett. **307**, 371 (1992).
33. Z. G. Fetisova and K. Mauring, FEBS Lett. **323**, 159 (1993).
34. Z. G. Fetisova, K. Mauring and A. S. Taisova, Photosynth. Res. **41**, 205 (1993).
35. J. Pšenčk, M. Vácha, F. Adamec, M. Ambrož, J. Dian, J. Boček, and J. Hála, Photosynth. Res. **42**, 1 (1994).
36. R. G. Alden, S. H. Lin and R. E. Blankenship, J. Lumin. **51**, 51 (1992).
37. E. G. McRae and M. Kasha, In *Physical Processes in Radiation Biology*, pp 23 - 41, Academic Press, New York (1964).
38. R. J. van Dorssen and J. Amesz, Photosynth. Res., **15**, 177 (1988).
39. S. Savikhin and W. S. Struve, Biophys. J. **67**, 2002 (1994).
40. M. Becker, V. Nagarajan and W. W. Parson, Biochemistry. **113**, 6840 (1991).
    Note: Change all term "Y.-Z. Ma et al, unpublished results" to a reference No. 41, as,
41. Y.-Z. Ma, R. P. Cox, T. Gillbro and M. Miller, Photosynth. Res. in press (1996).