Femtosecond Dynamics in Isolated LH2 Complexes of Various Species of Purple Bacteria[||]

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Polarized and magic angle two-color femtosecond spectroscopy was used to study B800-850 antenna complexes of the photosynthetic purple bacteria *Rhodopseudomonas acidophila* (*Rps. acidophila*), *Rhodobacter sphaeroides* (*Rb. sphaeroides*), and *Chromatium tepidum* (*Chr. tepidum*) and the B800-820 complex of *Chromatium vinosum* at room temperature. As was earlier found for *Chr. tepidum*, in the B800-850 complexes of *Rps. acidophila* and *Rb. sphaeroides* the bleaching signal of B850 was found to be several times larger than that of B800, indicating strong exciton interactions between the bacteriochlorophylls (BChls) in the B850 aggregate. Depolarization of the B850 excited state was found to occur within our time resolution of 80 fs. In all species, B800 to B850 or B820 transfer took place with a time constant of 0.7 to 0.9 ps. Depolarization studies indicated a transfer time of 1.5 ps between B800 molecules in *Rps. acidophila*. In *Chr. tepidum*, B800 depolarizes 2 to 4 times slower, dependent on the wavelength of excitation. Our results indicate that the double band structure of B800 of the latter organism is due to two separate pools of BChls, rather than dimeric exciton interaction. Upon excitation of the B800-820 complex of *Chr. vinosum* at 795 nm, the B820 absorbance difference spectrum shifted with time to the red by 20 nm, indicating that B820 is spectrally very heterogeneous. A 2 ps downhill energy transfer process within the B820 band is assigned to energy transfer between aggregated B800-820 complexes. Assuming that the B800-820 complex is similar to B800-850, we propose that the large spectral heterogeneity of B820 does not occur within individual B800-820 complexes.

Introduction

Compared to most other photosynthetic organisms, the antenna system of purple bacteria is relatively simple and consists of a core complex (LH1) and, in most species, one or more peripheral complexes (LH2). In bacteriochlorophyll (BChl) *a* containing purple bacteria, the LH2 complexes usually have two Q_y absorption bands, located near 800 and 850 or 820 nm,[1] and are referred to as B800-850 and B800-820, respectively.

An important step toward understanding the properties of the antenna complexes of purple bacteria was recently made by the elucidation of the three-dimensional crystal structure of such complexes by means of X-ray analysis. The B800-850 complex of *Rhodopseudomonas acidophila* (*Rps. acidophila*) was found to consist of a ring with 9-fold symmetry containing 18 BChls 850, 9 BChls 800, 9 carotenoids, and 9 α and β peptide subunits. The B850 molecules are close together, with an average distance of about 9 Å, whereas the distance between the B800 molecules and between the B800 and the B850 molecules is about twice as large.[2] A similar structure, but with 8-fold symmetry and differing in some important details, containing 16 B850 molecules and 8 B800 molecules was found for the B800-850 complex of *Rhodospirillum molischianum*.[3]

We have recently shown, by pump−probe time-resolved absorption measurements in the femtosecond region, that the oscillator strength of the B850 transition in the B800-850 complex of *Chromatium tepidum* (*Chr. tepidum*) is several times larger than of B800, and we explained this by exciton delocalization among the B850 molecules.[4] Similar conclusions were obtained for the LH1 complex of various species of purple bacteria, where it was found that the bleaching caused by excitations on the antenna BChls was several times larger than that due to the subsequent photooxidation of the primary electron donor.[5−7]

The present communication extends our studies to other species and antenna complexes: the B800-850 complex of *Rps. acidophila*, of which the 3-D crystal structure is known,[2] the B800-850 complex of *Rhodobacter sphaeroides* (*Rb. sphaeroides*), and the B800-820 complex of *Chromatium vinosum* (*Chr. vinosum*). It will be shown that the results are comparable to those obtained earlier with the B800-850 complex of *Chr. tepidum* and that may indicate extensive exciton interaction between the BChls 850 in these complexes.

Data will also be presented on the rates of energy transfer between B800 and B850 or B820. Time-resolved spectra and anisotropy measurements will be used to determine the rate of energy transfer between the B800 molecules in *Rps. acidophila*. Special attention will be given to the dynamics in the B800 band of *Chr. tepidum*, which unlike other species, shows a double-band structure.

Experimental Section

A mode-locked Ti:sapphire laser, home built according to the design of Asaki et al.,[8] was equipped with a cavity dumper as described by Pshenichnikov et al.[9] to provide a variable pulse repetition rate and an increased energy per pulse. The laser was pumped by typically 5.5 W of an Argon ion laser (Coherent-

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Innova). The output of the laser was centered at a wavelength of 820 nm with a bandwidth of 90 nm (fwhm). The experimental setup consisted of a pump−probe configuration with either parallel, perpendicular, or magic angle polarization of pump and probe beams. The isotropic spectra of (vide infra) Figures 6, 9, 10, and 12 were reconstructed from those measured at parallel and perpendicular polarization. The polarization was set with a dispersion compensated achromatic waveplate (CVI). The probe beam was obtained by splitting off a fraction of the excitation beam. For excitation, a bandpass filter was inserted in the pump beam to limit the excitation bandwidth. Both beams were precompensated for dispersion by double passing of two fused silica prisms in the optical path. This resulted in an instrumental response function of 80−120 fs (fwhm), depending on the type of band-pass filter used, which corresponds, assuming a $sech^2$ pulse shape, to near transform-limited pulses of 60−80 fs. The pulse repetition rate was set to 1 MHz, while the pump beam was modulated at 1 kHz by a mechanical chopper in the beam. The change in transmission of the probe beam was monitored by an integrating photodiode in combination with phase-sensitive detection referenced to the 1 kHz signal of the chopper. The probe wavelength was selected by a monochromator.

B800-850 complexes of *Chr. tepidum* and B800-820 complexes of *Chr. vinosum* were prepared as described earlier.[10,11] B800-850 complexes of *Rps. acidophila* (strain 10050) were prepared by incubating chromatophores in a 10 mM Tris/2% LDAO buffer (pH = 8) and subsequent purification of the proteins on a Poros column. The B800-850 complex of *Rb. sphaeroides* was isolated in the same way from a M(Y)210 mutant of this species, which has a mutation in its reaction center and a native antenna system.

The samples were diluted to an absorbance of about 0.3/mm at their absorption maxima and were contained in a rotating optical cell spinning at about 3000 rpm, with a sample thickness of 1.5 mm, providing a linear velocity of 16 m/s of the sample through the focus of the laser beam (30 μm spot diameter at focus). This velocity was amply sufficient to assure a fresh sample volume for each excitation pulse up to a pulse repetition rate of 1 MHz. The excitation density corresponded to less than 0.01 photon/BChl to avoid annihilation in B850 or B820, except for the depolarization measurements of B850 and the measurements on *Chr. tepidum* where it was approximately 0.04 photon/BChl. All measurements were performed at room temperature.

Results and Discussion

Absorption Spectra. Figure 1 shows the absorption spectra of the four LH complexes studied. The spectra are similar to those published earlier[10−14] with bands at 801 and 858 nm for the B800-850 complex of *Rps. acidophila*, at 799 and 847 nm for the corresponding complex of *Rb. sphaeroides*, 800 and 850 nm for the B800-850 complex of *Chr. tepidum*, and 800 and 815 nm for the B800-820 complex from *Chr. vinosum*.

Rps. acidophila and Rb. sphaeroides. Time-resolved absorbance difference spectra of the *Rps. acidophila* complex upon excitation at 810 nm are shown in Figure 2. Within the response time of the apparatus, a bleaching band developed near 810 nm, due to excitation of B800. A significant signal was also observed near 870 nm, which may be due to direct excitation of B850. At increasing delays after the excitation, the bleaching near 800 nm reversed again, and simultaneously the bleaching near 870 nm increased, together with a positive band near 850 nm. These long-wavelength bands may be ascribed to ground state depletion and stimulated emission and excited state absorption of B850, respectively, and thus the spectra directly demonstrate the occurrence of rapid energy transfer from B800 to B850. The kinetics of both the decay at 810 nm and the development of the bleaching at 870 nm (Figure 3) could be fitted with a time constant for energy transfer of 0.9 ps, in agreement with our earlier measurements on the LH2 complex of *Chr. tepidum*[4] and with numbers obtained by others on LH2 complexes of various other species.[15−17]

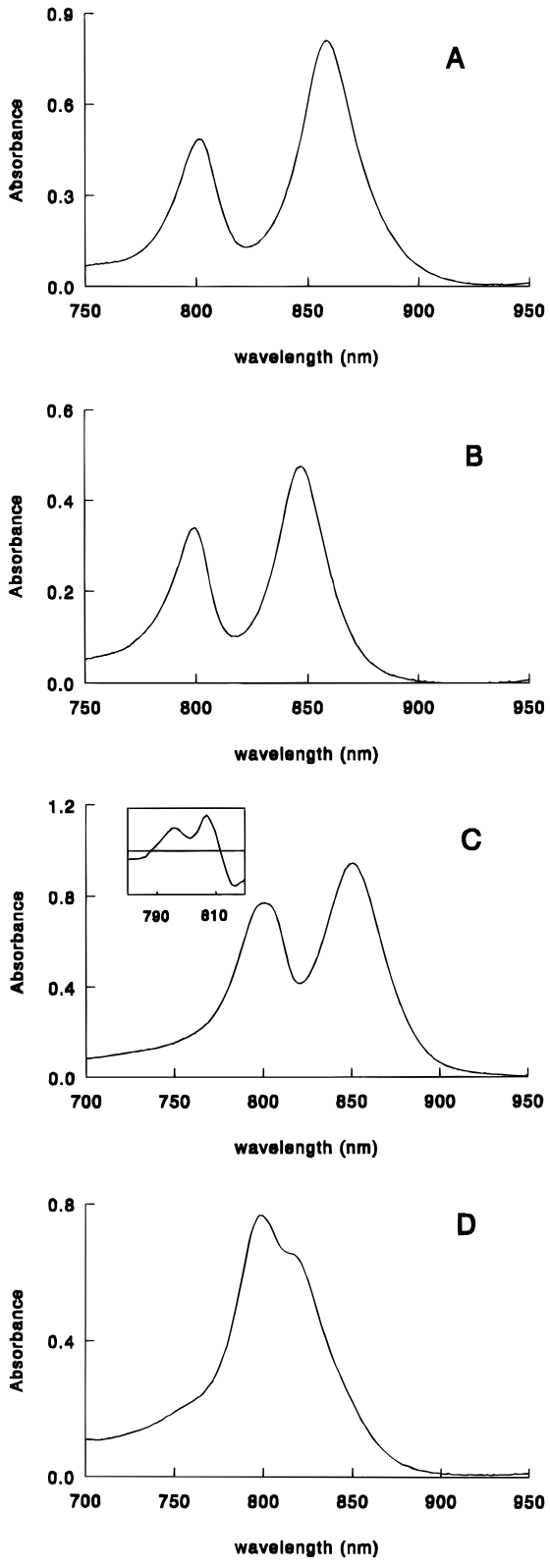

Figure 1. Absorption spectra of (A) the B800-850 complex of *Rps. acidophila*, (B) the B800-850 complex of *Rb. sphaeroides*, (C) the B800-850 complex of *Chr. tepidum*, and (D) the B800-820 complex of *Chr. vinosum*. The inset of Figure 1C shows the inverted second derivative in the 800 nm region.

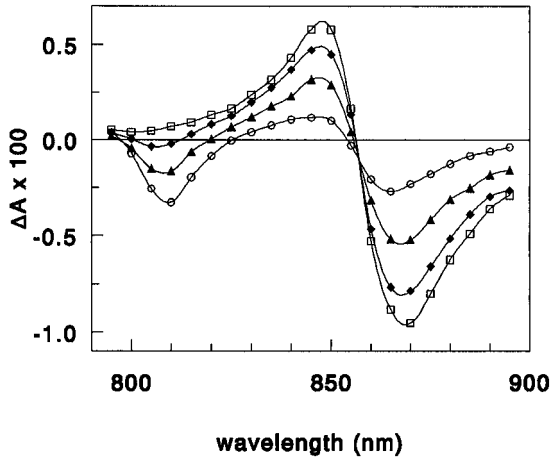

Figure 2. Isotropic time-resolved absorbance difference spectra in the B800-850 complex of *Rps. acidophila* upon excitation at 810 nm at delays 0.1 ps (circles), 0.5 ps (triangles), 1.4 ps (diamonds), and 4.5 ps (squares).

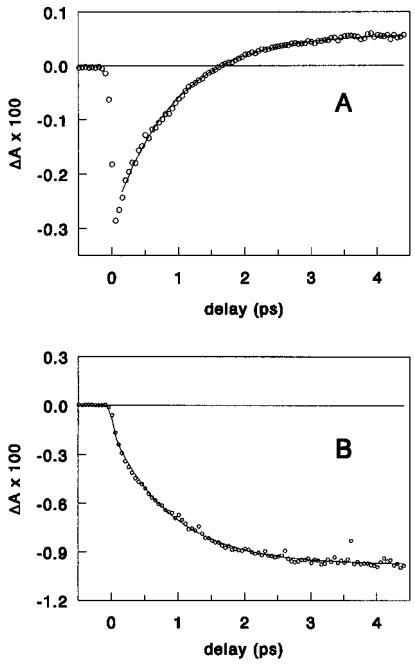

Figure 3. Kinetics of absorbance changes in the B800-850 complex of *Rps. acidophila* upon excitation at 810 nm at (A) 810 nm and (B) 870 nm with pump and probe polarized at the magic angle. Both kinetic traces are fitted with a single exponential function with a time constant of 0.9 ps. The first 150 fs of the trace at 810 nm are ignored to avoid contributions from the coherent coupling artifact.

Comparison of the integrated area of the bleaching near 810 nm with that near 870 nm after energy transfer has been completed shows that the latter is 3.5 times larger than the former. Since the BChls 800 are far apart in the complex and may be assumed to be monomers, this may indicate extensive exciton interaction between the BChls 850, as in *Chr. tepidum*.[4] However, an estimate of the oscillator strength of B850 is hampered by the relatively strong excited state absorption near 850 nm. The possibility of a simultaneous blue shift of the absorption bands of neighboring BChls affecting the difference spectrum[18] should also be taken into account.

Our recent low-temperature measurements (to be published) have demonstrated that, at 10 K, the lowest state in B850 has an oscillator strength of 2−3 BChls. The low-temperature measurements also showed that the ground state bleaching part of the B850 signal was larger than the stimulated emission contribution, which indicates that the observed ground state bleaching may not correspond to the oscillator strength of the

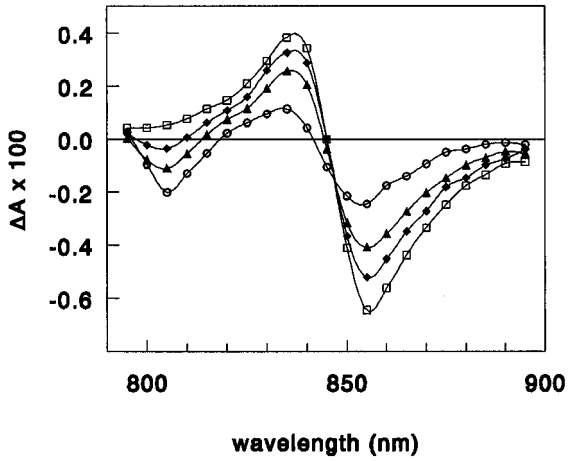

Figure 4. Isotropic time-resolved difference spectra in the B800-850 complex of *Rb. sphaeroides* upon excitation at 810 nm at delays 0.1 ps (circles), 0.4 ps (triangles), 0.9 ps (diamonds), and 4.4 ps (squares).

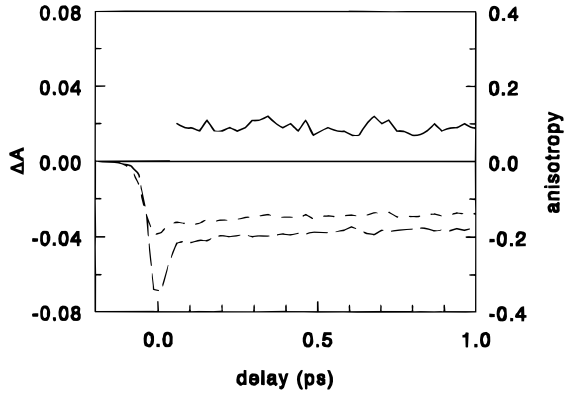

Figure 5. Kinetics in the B800-850 complex of *Rps. acidophila* upon excitation at 860 nm and probed at 870 nm with pump and probe polarized parallel (long-dashed line) or perpendicular (short-dashed line). The resulting anisotropy is drawn with a solid line.

thermalized, emitting state. It seems likely that a band shift as suggested by Nuijs et al.[18] occurs. We cannot exclude that a similar situation applies to our present room temperature data. The above considerations make clear that it is very difficult to make quantitative assignments to the entire difference spectrum of B850, and one should be very careful when trying to draw rigid conclusion from them.

Very similar results were obtained with the B800-850 complex from *Rb. sphaeroides*. Difference spectra are shown in Figure 4. The kinetics at 810 and 860 nm yielded a time constant of 0.7 ps for energy transfer from B800 to B850 in this complex (not shown). Pullerits et al.[19] have used the absorbance difference spectrum of B850 to calculate the delocalization length on the B850 aggregate. We note, however, that the ΔA spectrum of excited B850 that we measured, shown as the last spectrum in Figure 4, differs quite significantly from that published by Pullerits and co-workers, as the position of the isosbestic point and the extent of excited state absorption are clearly different. The origin of these differences is not clear, but it is obvious that they would have a serious effect on delocalization length calculations. We also performed these measurements with an amplified dye laser setup with continuum generation and optical multichannel analyzer detection, which gave results (not shown) that were identical to those of Figure 4.

The depolarization at 870 nm in the *Rps. acidophila* LH2 complex upon excitation at 860 nm is shown in Figure 5. During the first 80 fs, the signals were dominated by the coherent response of the sample, but after that the anisotropy

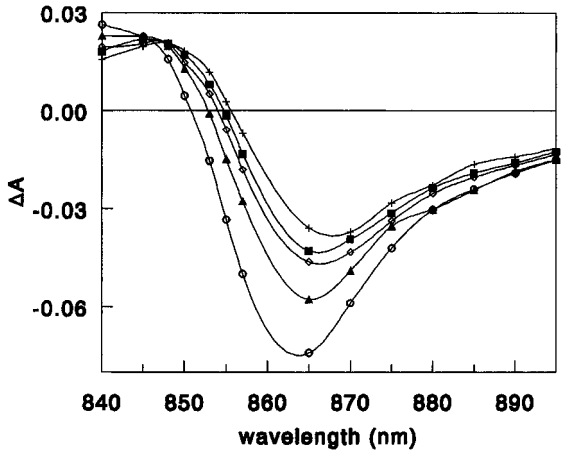

Figure 6. Time-resolved isotropic spectra in the B800-850 complex of *Rps. acidophila* upon excitation at 860 nm at delays 0 fs (circles), 60 fs (triangles), 160 fs (diamonds), 430 fs (squares), and 4 ps (crosses).

remained constant, with a value of 0.08, close to the value of 0.1 for a circularly degenerate oscillator. These measurements show that depolarization of the B850 excited state proceeds very fast, within 80 fs. Due to the many coherent contributions around zero delay,[20] it is very hard to obtain unambiguous information about very fast processes with pump−probe measurements. Therefore, we did not try to determine the anisotropy at shorter times than 80 fs. Our results generally agree with those performed with the *Rb. sphaeroides* LH2 complex.[17,19] In a similar experiment with the *Rb. sphaeroides* complex, we found a minor decay component of 300 fs as well (not shown). Employing fluorescence−up-conversion, Jimenez et al.[17] found a depolarization time of 50 fs together with a minor 500 fs decay component. Pullerits et al. found a minor decay component in their anisotropy of 2 ps.[19]

Our results indicate that the thermalized B850 excited state is delocalized over several pigments, so depolarization should be described by exciton theory,[21] rather than by energy transfer between monomers. A hopping model between the $\alpha\beta$-BChl dimers, as proposed by Jimenez et al.,[17] would only be appropriate if there is enough difference in coupling between BChls within one $\alpha\beta$-subunit and that between adjacent BChls of different $\alpha\beta$ subunits. Recent calculations indicate that these couplings are comparable.[22]

Figure 6 shows the time-resolved spectra in the B850 band of *Rps. acidophila* upon excitation at 860 nm. We observe that, after the coherent response of the sample, the difference spectrum shifts to the red by about 3 nm with a time constant of 250 fs. This shift reflects thermalization of the excited state on the B850 aggregate[4] and is considerably less than that observed by Visser et al. for the LH1 complex in membranes.[23] Although in the measurements by Visser et al. some excess vibrational energy may have been present due to the excitation wavelength (600 nm), the smaller shift in LH2 suggests that LH2 is spectrally less heterogeneous than LH1, which is also reflected by the widths of the absorption bands of the complexes.

Depolarization measurements were performed in the B800 band of the LH2 complex of *Rps. acidophila* to acquire information about energy transfer among the B800 molecules. Figure 7 shows the results obtained upon excitation at 795 nm. The kinetics at 810 nm with probe and pump polarized parallel and perpendicular are plotted, together with the anisotropy calculated from these kinetics. The anisotropy had an initial value of 0.35, which is close to the value of 0.4 expected for excitations localized on monomers. At a delay of a few picoseconds, energy transfer to B850 has taken place and the signal is due to excited state absorption of B850. Now the

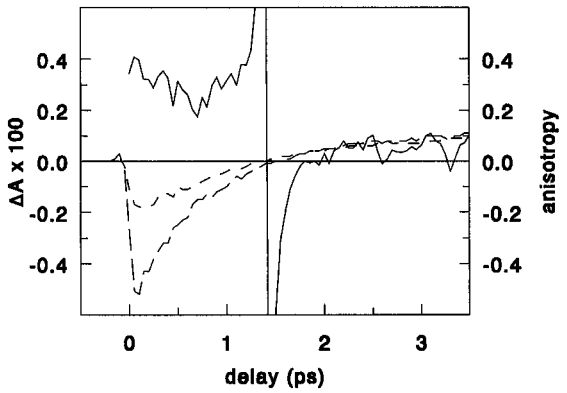

Figure 7. Kinetics in the B800-850 complex of *Rps. acidophila* upon excitation at 795 nm and probed at 810 nm with pump and probe polarized parallel (long-dashed line) or perpendicular (short-dashed line). The resulting anisotropy is drawn with a solid line.

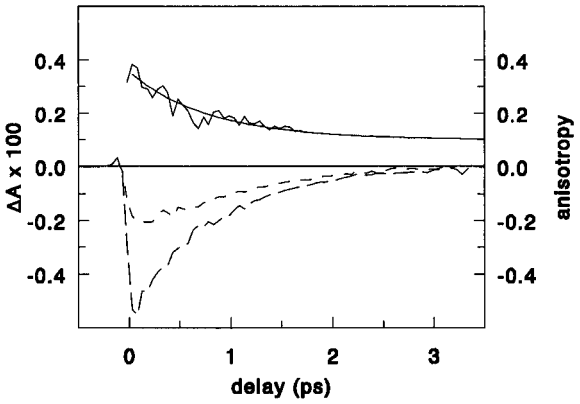

Figure 8. Kinetics in the B800-850 complex of *Rps. acidophila* upon excitation at 795 nm and probed at 810 nm, from which the contribution by B850 excited state absorption has been subtracted, with pump and probe polarized parallel (long-dashed line) or perpendicular (short-dashed line). See text for details. The resulting anisotropy (solid line) is fitted with a single-exponential decay of 0.8 ps.

anisotropy had a value of 0.07, in agreement with the B850 depolarization measurements shown in Figure 5. Due to mixing of the bleaching signal of B800 and excited state absorption of B850, the anisotropy shows an anomalous behavior at intermediate delays. To find the depolarization of excited B800, we therefore had to subtract the contribution by B850 excited state absorption from the kinetics. An induced absorption with a rise time of 0.9 ps and the amplitude of the long-lived end level was subtracted from the kinetics, hereby implicitly assuming that excitations become depolarized as soon as they reach B850, which is justified by the above depolarization measurements on the B850 band (Figure 5). The result is shown in Figure 8. The anisotropy had an initial value of 0.35 and then decayed in about 1 ps to a lower value. Depolarization of B800 thus occurs in the same time range as energy transfer to B850, which implies that, after about 1.5 ps, the population of excited B800 is so low that the anisotropy values derived from the corrected kinetics are not reliable any more. Consequently, the final value of the anisotropy cannot be determined. However, from the crystal structure of LH2[2] we conclude that the final value should be 0.1, and if we fix this number in the fitting procedure, and allow a fitting window from 0 to 1.5 ps, we find a depolarization time of 0.8 ps. We obtained similar results at other probe wavelengths and with excitation at 810 nm, as summarized in Table 1. One-color measurements on the B800 band in membranes of *Rb. sphaeroides* and *Rps. palustris* have yielded comparable depolarization times.[15]

With the crystal structure at hand, we can calculate the energy transfer rate between the B800 molecules. The relation between

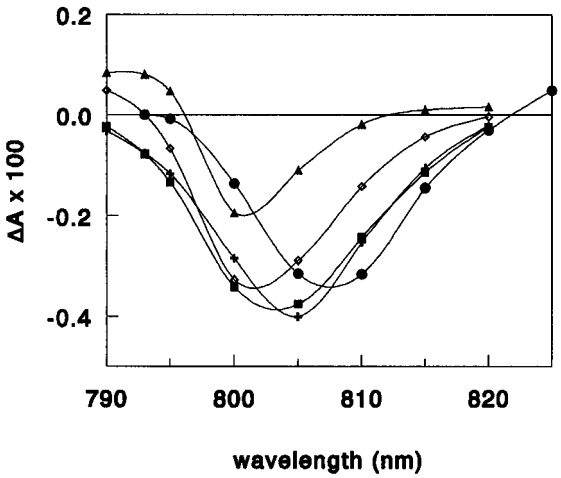

Figure 9. Time-resolved isotropic spectra of the B800-850 complex of *Rps. acidophila* upon excitation at 795 nm at delays 0 fs (triangles), 50 fs (diamonds), 100 fs (squares), and 150 fs (crosses). The circles represent the difference spectrum upon excitation at 810 nm at a delay of 0.3 ps.

TABLE 1: Anisotropies at Zero Delay $r(0)$ and Time Constants τ of Anisotropy Decay for Various Excitation and Probe Wavelengths in the B800-850 Complexes of *Rps. acidophila* and *Chr. tepidum*. See Text for Details

	Rps. acidophila		*Chr. tepidum*	
probe (nm)	$r(0)$	τ (ps)	$r(0)$	τ (ps)
		795 nm Excitation		
800	0.32	0.84	0.37	1.6
805	0.35	0.95	0.35	1.8
810	0.35	0.79	0.34	1.7
		810 nm Excitation		
805	0.36	0.96	0.38	3.3
810	0.34	1.34	0.40	3.0
815	0.31	0.83	0.37	3.8

depolarization time and pairwise hopping time for a symmetric ring is given by the relation[17]

$$\tau_{dep} = \frac{\tau_{hop}}{4(1 - \cos^2 \Theta)}$$

with τ_{dep} the depolarization time, τ_{hop} the hopping time, and Θ the angle between the dipole moments of neighboring pigments. Substituting 0.9 ps for the depolarization time and 40° for Θ (see ref 2), we find a hopping time of 1.5 ps. Comparing this result with the transfer time to B850, which is 0.9 ps, and taking into account that each B800 molecule has two neighbors, we conclude that, on average, excitations on the B800 ring are transferred 1.2 times to a neighboring B800 molecule before they are transferred to B850.

Figure 9 shows the time-resolved isotropic spectra in the 800 nm region upon excitation at 795 nm. During the pulse, the spectrum shifts to the red from 801 to 805 nm. After that, the spectrum stayed in place and decayed into a broad absorbance increase due to energy transfer to B850 (not shown). The shifting is much faster than the depolarization, indicating that it is not related to B800-B800 energy transfer. A dynamic Stokes shift is a more likely explanation for his phenomenon.[24,25] Moreover, coherent contributions to the transient absorption signals[20] may be mixed in at these early delays. The line with the solid circles shows the difference spectrum with excitation at 810 nm at a delay of 0.3 ps. At later delays, the spectra excited at 810 nm had the same spectral position (not shown). They were red-shifted by 3 nm with respect to the corresponding one excited at 795 nm. This phenomenon is most likely related to inhomogeneous broadening of the B800 band: as a result of the relatively slow B800-B800 energy transfer determined above, excitations made in the blue edge of the inhomogeneous distribution will not be able to reach the red-most pigments on the B800 ring before they are transferred to B850.

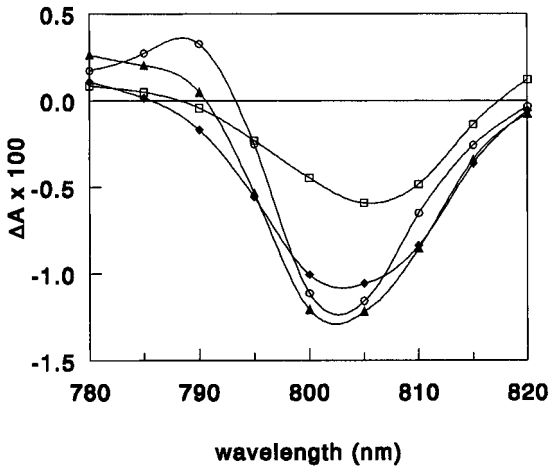

Figure 10. Time-resolved isotropic spectra of the B800-850 complex of *Chr. tepidum* upon excitation at 795 nm at delays 30 fs (circles), 90 fs (triangles), 180 fs (diamonds), and 700 fs (squares).

Our results on the B800 dynamics support most of the conclusions drawn in the photon echo paper on the LH2 complex of *Rb. sphaeroides* by Joo and coworkers.[26] They proposed from three-pulse photon echo measurements that only a limited B800-B800 transfer would take place. A Förster energy transfer calculation yielded a B800-B800 transfer time of 1.2 ps,[26] which agrees well with the outcome of this study. Three-pulse photon echo peak shift measurements indicated the occurrence of "static" inhomogeneity in B800 at physiological temperature, i.e., inhomogeneity on a time scale longer than the lifetime of B800, in agreement with our results upon selective excitation of the blue or the red edge of the B800 band.

Chr. tepidum. Figure 1C shows the absorption spectrum of the B800-850 complex of *Chr. tepidum*. The inset shows the second derivative of the B800 band. It is clearly composed of two bands, peaking at 796 and 806 nm. We shall call the two spectral species B796 and B806, respectively. To examine the nature of these two bands, we performed the same polarized two-color experiments as with *Rps. acidophila*, described above, with excitation at 795 and 810 nm. Judging from the transmission characteristics of the applied interference filters and the positions and widths of the B796 and B806 bands, we estimate that with 795 nm excitation we obtained a 60% selectivity for B796, and with 810 nm, a 65% selectivity for B806.

Figure 10 shows the time-resolved isotropic spectra upon excitation at 795 nm. A bleaching develops around 800 nm which we attribute to excited B796 and a fraction of excited B806. During the first few hundreds of femtoseconds, the spectrum shifts by a few nanometers to the red. The bleaching then decayed into a broad absorbance increase (not shown, see ref 4), which we assign to excited B850. In Figure 11 the time-resolved anisotropy, corrected in the same way as we did with *Rps. acidophila*, is plotted. The probe wavelength was 805 nm. We observe that the initial value of the anisotropy is 0.35. If we again fix the final value of the anisotropy to 0.1, we find a decay time of 1.8 ps.

Figure 12 shows the time-resolved isotropic spectra upon excitation at 810 nm. A bleaching develops around 810 nm which broadens and shifts by a few nanometers to the blue in the course of a few hundreds of femtoseconds. Compared to the spectra with 795 nm excitation, they are 4 nm shifted to the red throughout their lifetime.This implies that no complete spectral equilibration between B796 and B806 takes place.

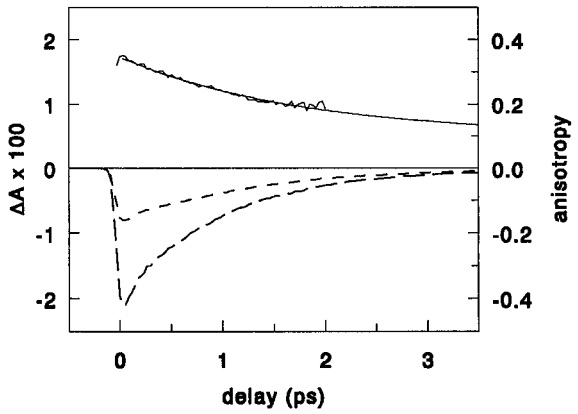

Figure 11. Kinetics in the B800-850 complex of *Chr. tepidum* upon excitation at 795 nm and probed at 805 nm, from which the contribution by B850 excited state absorption has been subtracted, with pump and probe polarized parallel (long-dashed line) or perpendicular (short-dashed line). See text for details. The resulting anisotropy (solid line) is fitted with a single exponential decay of 1.8 ps.

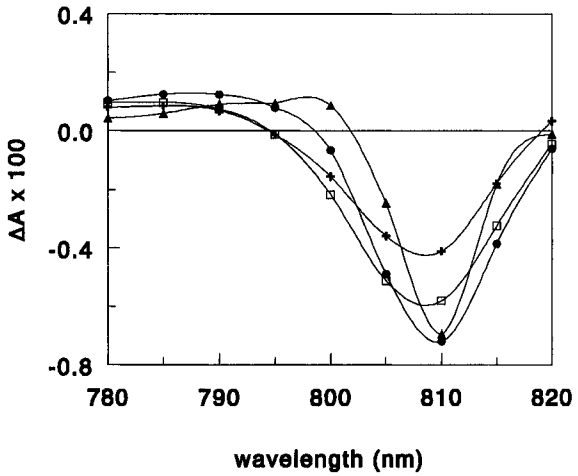

Figure 12. Time-resolved isotropic spectra of the B800-850 complex of *Chr. tepidum* upon excitation at 810 nm at delays 60 fs (triangles), 150 fs (circles), 300 fs (squares), and 600 fs (crosses).

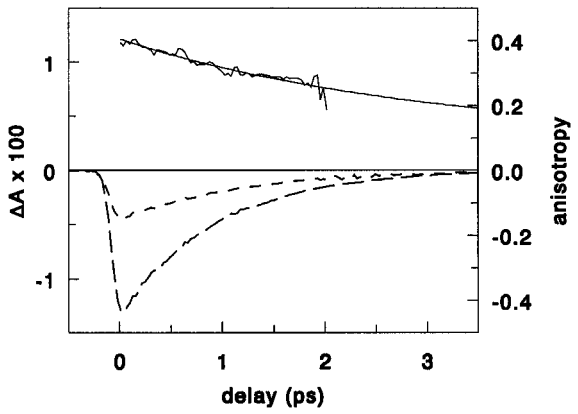

Figure 13. Kinetics in the B800-850 complex of *Chr. tepidum* upon excitation at 810 nm and probed at 810 nm, from which the contribution by B850 excited state absorption has been subtracted, with pump and probe polarized parallel (long-dashed line) or perpendicular (short-dashed line). See text for details. The resulting anisotropy (solid line) is fitted with a single exponential decay of 3.0 ps.

Figure 13 shows the corrected time-resolved anisotropy at 810 nm, which starts at 0.4 and decays with a time constant of about 3 ps to the fixed end value of 0.1. It should be noted that this rather long depolarization time was obtained using a fitting window of 2 ps. This means that this time constant is not very accurate, but it appears to be longer than that upon excitation at 795 nm. The results obtained at other probe wavelengths and with excitation at 795 nm are summarized in Table 1.

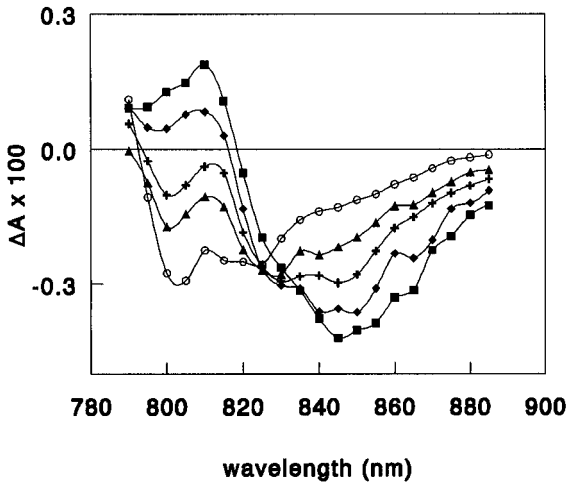

Figure 14. Time-resolved isotropic spectra in the B800-820 complex of *Chr. vinosum* upon excitation at 795 nm at delays 80 fs (circles), 230 fs (triangles), 500 fs (crosses), 1.5 ps (diamonds), and 4.5 ps (squares).

The time-resolved spectra of *Chr. tepidum* are quite similar to those obtained with *Rps. acidophila*. The depolarization in *Chr. tepidum*, however, seems to be slower, especially upon excitation in the red edge of the B800 band. We can interpret these results in a very straightforward manner by assuming that B796 and B806 represent two distinct pigment groups bound to different sites at the α,β polypeptides. As judged by the depolarizations of excited B795 and B806, limited energy transfer within B796 or to B806 occurs upon excitation at 795 nm. When exciting at 810 nm, an excitation virtually remains localized on a B806 molecule until it is transferred to B850. We need only to assume a slightly larger distance between the pigments, or an unfavorable mutual orientation of the pigments, to explain this slow B800-B800 energy transfer rate with respect to *Rps. acidophila*.

In principle it is possible that B806 and B796 occur in separate B800-850 complexes, but the 1:1 stoichiometry in the absorption spectrum strongly suggests that they occur within the same B800-850 complex in a well-defined manner. One could for instance envisage that B796 and B806 molecules are alternatingly bound to the $\alpha\beta$-subunits in a ring structure, which would explain the slow depolarizations we measured for B806.

The above proposed picture contrasts with the excitonic dimer model we applied previously,[4] which was based on steady-state spectroscopic data only.[10] If B796 and B806 were the upper and lower exciton components of a BChl dimer, excitation of the upper band would be followed by a rapid equilibration with the lower band through the Davydov exciton scattering mechanism, resulting in a fast depolarization at the pump wavelength, and a very specific polarization behavior at the other exciton component's wavelength.[27] None of these phenomena is seen, so we may safely dismiss this model. Still, the origin of the conservative circular dichroism signal around 800 nm[10] remains obscure.

Chr. vinosum. In Figure 1D the absorption spectrum of the B800-820 complex of *Chromatium vinosum* is plotted. It shows a maximum at 800 nm and a shoulder at 815 nm. More than do B800-850 complexes of other species, it exhibits a relatively long tail towards the low-energy side, indicating the presence of long-wavelength spectral subspecies.

Figure 14 displays the time-resolved spectra upon excitation at 795 nm. At early delays, two bleaching bands at 805 nm and 830 nm are seen, which we assign to excited B800 and a

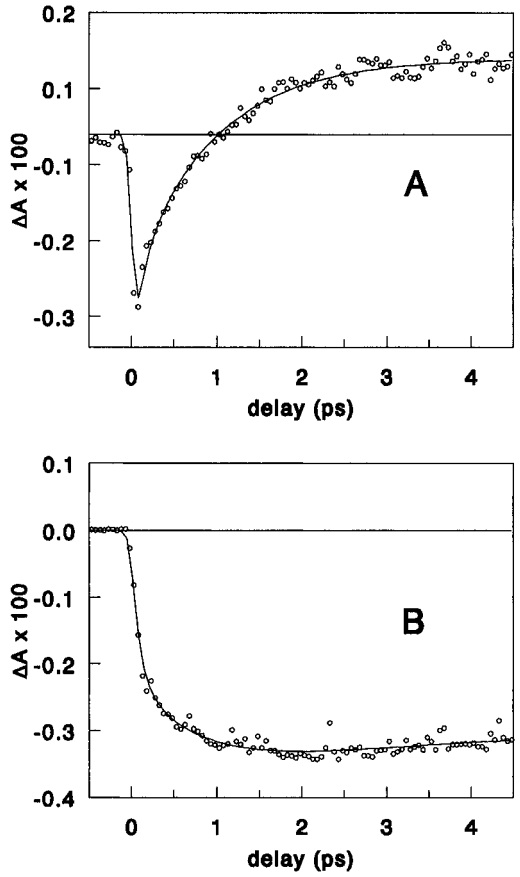

Figure 15. Kinetics of absorbance changes in the B800-820 complex of *Chr. vinosum* upon excitation at 795 nm at (A) 805 nm and (B) 835 nm with pump and probe polarized under the magic angle. The kinetics were fitted with the four components mentioned in the text.

significant fraction of directly excited B820, respectively. With increasing delays, the bleaching at 805 nm disappears and is replaced by an induced absorption. The bleaching at 830 increases, broadens, and shifts by 20 nm to 850 nm. These time-resolved spectra look quite unusual compared with those of the B800-850 complexes studied (see above and ref 4), where a shift of only about 5 nm is observed. Moreover, the overall shape is different, and the width of the bleaching, 40 nm, is larger.

The kinetic trace at 805 nm plotted in Figure 15A, however, is quite similar to the kinetic traces observed in B800-850 complexes. It shows a bleaching that decays with a main time constant of 0.9 ps to an absorbance increase, indicating energy transfer from B800 to B820. In addition, a pronounced 100 fs component is present in the signal, which is probably affected by the coherent response of the sample.[20] The kinetics at 835 nm (Figure 15B) show a prompt component due to direct excitation of B820, and a rise time of 0.9 ps. It also exhibits a decay component of about 2 ps, which indicates that there is an additional dynamic process taking place in the complex.

We fitted the measurements over the entire wavelength range with the time constants 0.1, 0.9, and 2 ps and a long-lived end level. The resulting decay-associated spectra are shown in Figure 16. The 0.9 ps component is negative around 805 nm and positive at the long-wavelength side, so we can directly assign it to energy transfer from B800 to B820. The 100 fs component shows the typical shape of a coherent artifact around 800 nm.[20] Above 820 nm, it is positive and represents a 100 fs rise time; therefore, apart from possible coherent contributions, it indicates direct excitation of B820.

The shape of the 2 ps component is interesting: it is negative around 820 nm and positive around 860 nm, which demonstrates

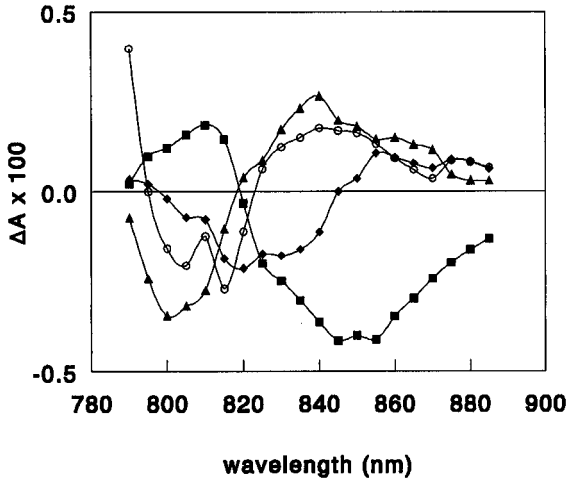

Figure 16. Decay-associated spectra derived from the time-resolved spectra measured on the B800-820 complex of *Chr. vinosum* upon excitation at 795 nm. Circles denote the 100 fs component, triangles the 0.9 ps component, the diamonds the 2 ps component, and the squares the long-lived end level.

that it represents downhill energy transfer within the B820 band. This could be explained by assuming that there are low-energy states present in individual B800-820 complexes, be it by exciton interaction or inhomogeneous broadening, to which energy is transferred in about 2 ps. However, in the light of current antenna models for LH2 complexes, this is hard to believe, because the supposedly close proximity of the B820 pigments will lead to energy transfer that is much faster than 2 ps, as was shown above for the B800-850 complex of *Rps. acidophila*. A perhaps more plausible explanation is provided if we assume that the spectral heterogeneity of B800-820 only occurs among different complexes and that they form aggregates in the detergent solution, so that downhill energy transfer can take place between different B800-820 complexes. The time scale for this process is expected to be similar to that in chromatophores, which we, in fact, have measured to be a few ps.[28] It is known that *Chr. vinosum* adapts its antenna composition to environmental conditions and may synthesize more than 10 different antenna polypeptides,[1] so the same mechanism might induce a variability in the B800-820 complexes.

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