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Excitation-energy transport in the bacteriochlorophyll antenna systems of *Rhodospirillum rubrum* and *Rhodobacter sphaeroides*, studied by low-intensity picosecond absorption spectroscopy

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We have studied the energy-transfer dynamics in chromatophores of *Rhodospirillum rubrum* and *Rhodobacter sphaeroides* (formerly called *Rhodopseudomonas sphaeroides*) under conditions of closed and open reaction centers, using low-intensity picosecond infrared absorption recovery measurements. The possibilities of selective infrared excitation and probing as well as picosecond anisotropy decay measurements offered by this technique have allowed us to obtain a detailed picture of the transfer dynamics. Upon selective excitation of a bacteriochlorophyll (BChl)-protein complex (B800–850, B875 or B880) a very low initial value of the absorption anisotropy ($r(0) \approx 0.1$) is observed for the excited-state decays of BChl 850, BChl 875 and BChl 880. This is consistent with a very fast ($k \approx 3 \cdot 10^{12}$ s^{-1}) initial transfer of energy between similar bacteriochlorophyll molecules. We suggest that this fast transfer occurs within a minimum unit of 6–8 chromophores. Only direct excitation of BChl 800 shows highly polarized, very short-lived (1–2 ps) absorbance changes due to the excited state of BChl 800. On a slower time-scale, 5–10 ps, the energy then migrates between the units of 6–8 bacteriochlorophyll molecules. On a similar time-scale, 35–50 ps, an equilibration of the excitation density occurs between different pigment pools. Thus in *Rb. sphaeroides* there is an equilibration between B800–850 and B875 ($\tau = 37 \pm 4$ ps) and in *R. rubrum* there is a similar process between B880 and B896 ($\tau = 47 \pm 4$ ps). These slower processes are associated with a further decay of the anisotropy from the initial value of ≈ 0.1 to very low values (less than 0.01). Our results suggest that the protein-pigment complexes designated B800–850 and B880 are not spectrally homogeneous, but may consist of spectrally slightly different bacteriochlorophyll molecules.

Introduction

Upon excitation of the light-harvesting bacteriochlorophyll antenna of photosynthetic purple bacteria using relatively low intensity flashes a large fraction of the absorbed excitation is efficiently transferred to the reaction center [1,2]. If the reaction center is in a so called open state, i.e., the reaction center bacteriochlorophyll dimer (P) is reduced and the iron quinone electron acceptor (Q) is oxidized, then more than 90% of the absorbed excitations are converted into a charge separated state, P^+Q^- [3–6].

The light-harvesting system of the photosyn-

Abbreviations: BChl, bacteriochlorophyll; RC, reaction center; P, the reaction center bacteriochlorophyll dimer; Q, the iron quinone electron acceptor; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; PMS, *N*-methylphenazonium methosulphate.

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thetic purple bacterium *Rhodobacter sphaeroides* (formely called *Rhodopseudomonas sphaeroides*) contains two major pigment protein complexes, B800–850 and B875 [7–9]. The latter is supposed to interconnect the reaction centers in a 'lake'-like arrangement, consisting of at least 20 reaction centers and 1000 BChl 875 molecules [2,4,10–13], while B800–850 is thought to constitute the peripheral part of the antenna [11,14–16]. Both B800–850 and B875 can be obtained in purified form [8,9,17] and more or less detailed models concerning their pigment organization were proposed [2,14,18–22]. The B800–850 complex contains as a minimum unit four BChl 850 molecules and two BChl 800 molecules, among which rapid energy transfer takes place [20,21]. The arrangement of the BChl 850 transition dipoles is such that they lie in the same or in parallel planes, parallel to the membrane plane and form a circulary degenerate oscillator [18,21].

The B875 complex contains probably 6–8 BChl 875 molecules [22–24], the transition dipoles are again circularly degenerate [22] and the complex seems heterogeneous, i.e., about 10–20% of the pigments absorb at 10–15 nm longer wavelengths (BChl 896) [22].

The long-wavelength antenna of *R. rubrum* is also organized like a lake [10–12,25], containing at least 10 and probably to more than 20 reaction centers [12]. The B880 complex of *R. rubrum* shows several important spectroscopic properties similar to those of B875 of *Rb. sphaeroides* [22].

Fluorescence lifetime measurements have been quite numerous in these systems. Single-frequency phase modulation and single picosecond-pulse excitation indicate lifetimes in the 50–200 ps range when the traps are open [26–29]. More recently, multiple-frequency phase-modulation techniques were used to monitor the fluorescence [30–32] and the results indicated for *Rb. sphaeroides* a fluorescence lifetime of about 50 ps with open traps and 200–250 ps with closed traps (P^+). The 50 ps lifetime spectrum was ascribed to the excited state of BChl 875. the major fraction of B800–850 was supposed to transfer its excitation within a few ps to B875. Only a small part of unconnected BChl 850 molecules added a very long-lived phase (approx. 4 ns) to the emission decay [31]. These results were essentially confirmed using a synchroscan streak camera set-up; in that case the decay time with open traps was found to be somewhat slower, 60–70 ps [33,34].

Part of these results appears to be in contrast to steady-state fluorescence experiments, which suggest a relatively slow transfer rate from B800–850 to B875, followed by a slower decay of the equilibrated excitation density [35,36].

In *R. rubrum* chromatophores picosecond absorption measurements have suggested a decay time of about 60 ps for both open and closed traps [37,38]. No effect of closing the traps was observed, in contrast to the expected increase in lifetime parallelling the increase in emission yield [10,12]. More recently, Freiberg et al. [33] and Borisov et al. [34] obtained fluorescence lifetimes which ranged from 60 ps to 210 ps depending on the state of the traps. From excitation annihilation profiles the open trap excited state lifetime was estimated to be of the order of 90–100 ps [12]. Accurate absorption measurements employing single, 35 ps, 532 nm flashes showed that absorption and fluorescence yield measurements quantitatively reflected the same phenomena and gave about 80 ps for the BChl 880 lifetime in state (PQ) [57], which increased to 200–400 ps in (P^+Q^-) [39]. The authors of Ref. 39 could explain their absorption difference spectra observed upon the excitation of the B880 antenna by assuming that one BChl 880 molecule is bleached per absorbed excitation, while above five remaining BChl 880 molecule shift their ground-state absorption spectra to shorter wavelengths.

In the present work we shall report on the excited state decay times measured in chromatophores of *R. rubrum* and *Rb. sphaeroides*. We have, for the first time, directly monitored the excited state decays by selective infrared excitation and probing ($\lambda = 800$–900 nm) with low-intensity picosecond pulses ($5 \cdot 10^{11}$–$5 \cdot 10^{12}$ photons per cm^2 per pulse). Moreover, we have recorded the picosecond time-resolved polarisation changes associated with these excited states. To some extent the experimental results confirm the earlier fluorimetric results [31,34], but in addition significant differences are observed and their interpretation leads to a rather detailed picture of the energy-transfer processes in the antenna of *R. rubrum* and *Rb. sphaeroides*.

Materials and Methods

R. rubrum and *Rb. sphaeroides* were grown as described in Ref. 6. Chromatophores were prepared using a French press and were diluted in a buffer medium containing 250 mM Tricine/5 mM KH_2PO_4/5 mM $MgCl_2$ (pH 8).

Picosecond kinetics were measured by means of absorption recovery, as previously described elsewhere [40,41]. Some features of the measuring technique essential for the present measurements will, however, be described in detail. The picosecond pulse source was a mode-locked and cavity-dumped dye laser, synchronously pumped by a mode-locked argon ion laser. The cavity dumper was operated in the 80 kHz–4 MHz range, typically giving 5–10 ps long pulses (FWHM) of 1–2 nJ energy. By using the three infrared dyes pyridine 1, styryl 8 and styryl 9, continuously tunable picosecond pulses in the wavelength range 700–900 nm were produced. Since both pump and probe beams were generatedby the same picosecond dye laser, the wavelength of the two beams were always the same. The polarizations of pump and probe beams were controlled by a Soleil–Babinet compensator and prism polarizers, so that absorption recovery kinetics could be measured for any relative orientation of the pump and probe polarizations. Measurements with parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) polarization were used to follow the decay of induced anisotropy, $r(t) = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$, and measurements at the magic angle ($I_{54.7°}$) were used to obtain the isotropic decay, free of orientational effects. In order to obtain a correct anisotropy decay it is essential to perform precise adjustments of the polarization directions. To that end the anisotropy of the infrared absorbing cyanine dye 1,1′-diethyl-2,2′-tricarbocyanine ($\lambda_{max} = 820$ nm) in propanol was measured. The excited state lifetime of this dye is $\tau = 60 \pm 2$ ps. In propanol we expect the rotational relaxation time τ_r to be much longer than τ, on the order of 500 ps [42]. Thus for an optimized measuring system we expect $r(t)$ to be approximately constant and to be equal to 0.4 during the available measuring window of approx. τ ps. This is confirmed by the experimental traces and plotted $r(t)$ shown in Fig. 1.

The recorded kinetic curves were analyzed as a sum of exponentials and the lifetimes and amplitudes associated with each decay component were extracted by two different methods: (i) least-square fitting using a computer program and (ii) plotting the recorded decay curves on semilogarithmic paper. Both methods were found to yield the same results to within the experimental error. No deconvolution of the measured kinetics with the pulse shape was performed, since the measured lifetimes were substantially longer than the presently used pulses ($\Delta t = 5$–10 ps). Only the very fast depolarizing processes having lifetimes on the order of 1 ps or shorter are seriously affected by the pulse shape, but here a deconvolution procedure would hardly help to recover the lifetimes.

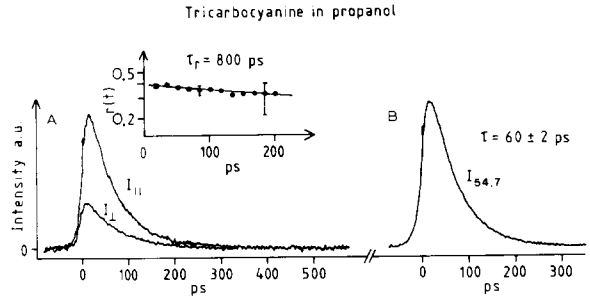


Fig. 1. Picosecond kinetics of the test dye 1,1′-diethyltricarbocyanine. (A) Anisotropy decay; (B) isotropic decay.

The pulse energy is another important parameter in the present studies. In antenna systems with a large number of connected chromophores singlet–singlet annihilation is known to quench the bacteriochlorophyll-excited states and thereby shorten the lifetime, if the excitation density is too high [12,39]. The maximum excitation density used in this work was $1 \cdot 10^{13}$ photons per cm^{-2} per pulse and by attenuating the excitation pulses with neutral density filters kinetics could be measured down to ca $1 \cdot 10^{12}$ photons per cm^{-2} pulse. Since samples of 0.3–0.5 absorbance were used, this excitation density corresponds to a minimum absorbed photon density of about $5 \cdot 10^{11}$ photons per cm^2 per pulse. These photon densities correspond to maximum and minimum excitation degrees of approx. 0.2% and approx. 0.02%, respectively.

In the kinetic curves of Figs. 1–5, a bleaching is displayed as a positive signal and an increased

absorption relative the initial state is shown as a negative signal. The measured 'signal intensity' represents the change of probe pulse intensity ($\Delta I/I$) caused by the excitation pulses. Since the degree of excitation is very low (less than 0.2%) the recorded $\Delta I/I$ signal can directly be used to obtain the variation of concentration (rather than $\log(\Delta I/I)$) with an error of less than 0.1%.

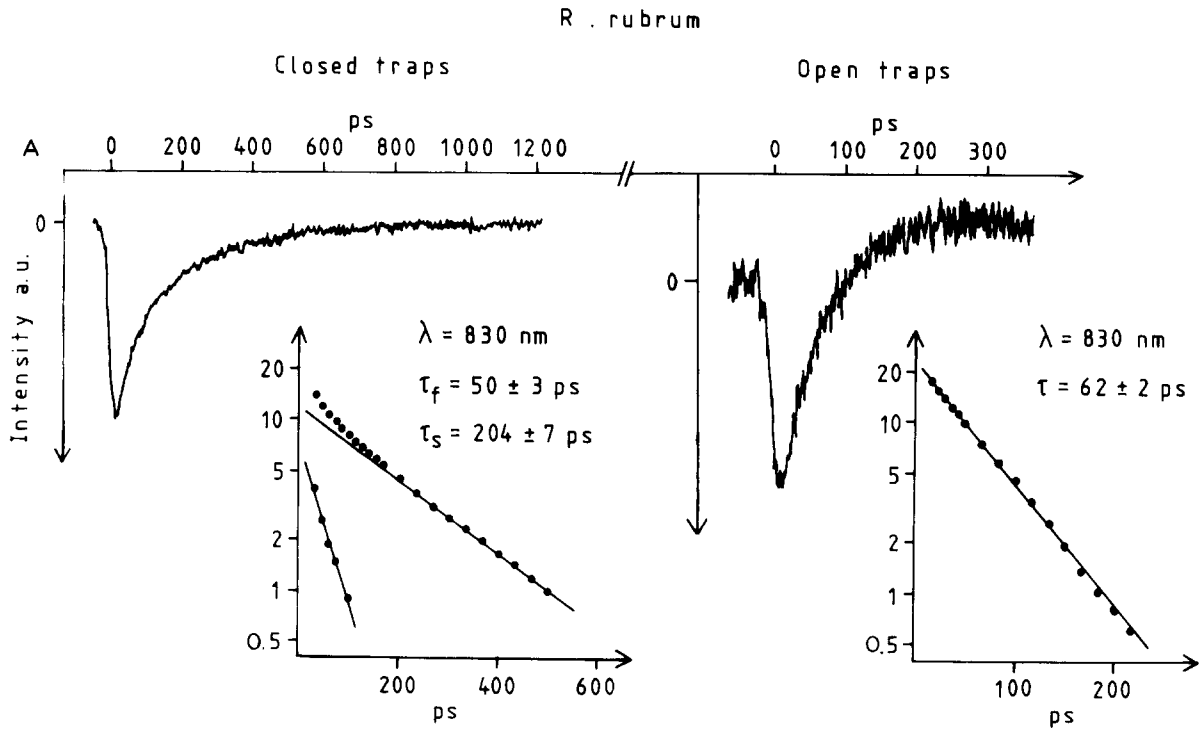
In order to ensure complete exchange of the sample between every excitation pulse a rotating sample cell consisting of two quartz plates separated by a 1 mm thick spacer was used. The rotating speed of this cell could be varied from 0 to 8000 r.p.m. corresponding to a variable flow speed of 0–20 ms^{-1} and recycling times greater than 12 ms. In this way a very high speed flow of the sample could be mimicked. This is particularly suitable for labile biological samples that might not stand a more conventional flow system. All measurements were performed at room temperature, 296 ± 1 K.

These features of the laser and measurement system described above enabled us to measure, for the first time, absorption recovery kinetics of purple bacteria pigment systems at low excitation densities and selective infrared excitation and probing, at any wavelength within the absorption bands of the pigment.

Results

Rhodospirillum rubrum

The isotropic absorption kinetics were measured at several wavelengths over the B880 absorption band (see Table I) and the results are illustrated in Fig. 2 by the kinetics measured at three selected wavelengths; 830 (A), 850 (B), and 890 nm (C). Results for both open and closed reaction centers are shown. The condition of open traps was achieved by addition of 10 μM PMS and 1 mM sodium ascorbate to the sample in buffer and it was checked independently that all the reaction centers remained in the state (PQ) under our illumination conditions. If the sample was used in plain buffer the repetitive pulse illumination rapidly induced the steady state formation of the state (P^+Q) which was maintained throughout the course of the experiment. More-



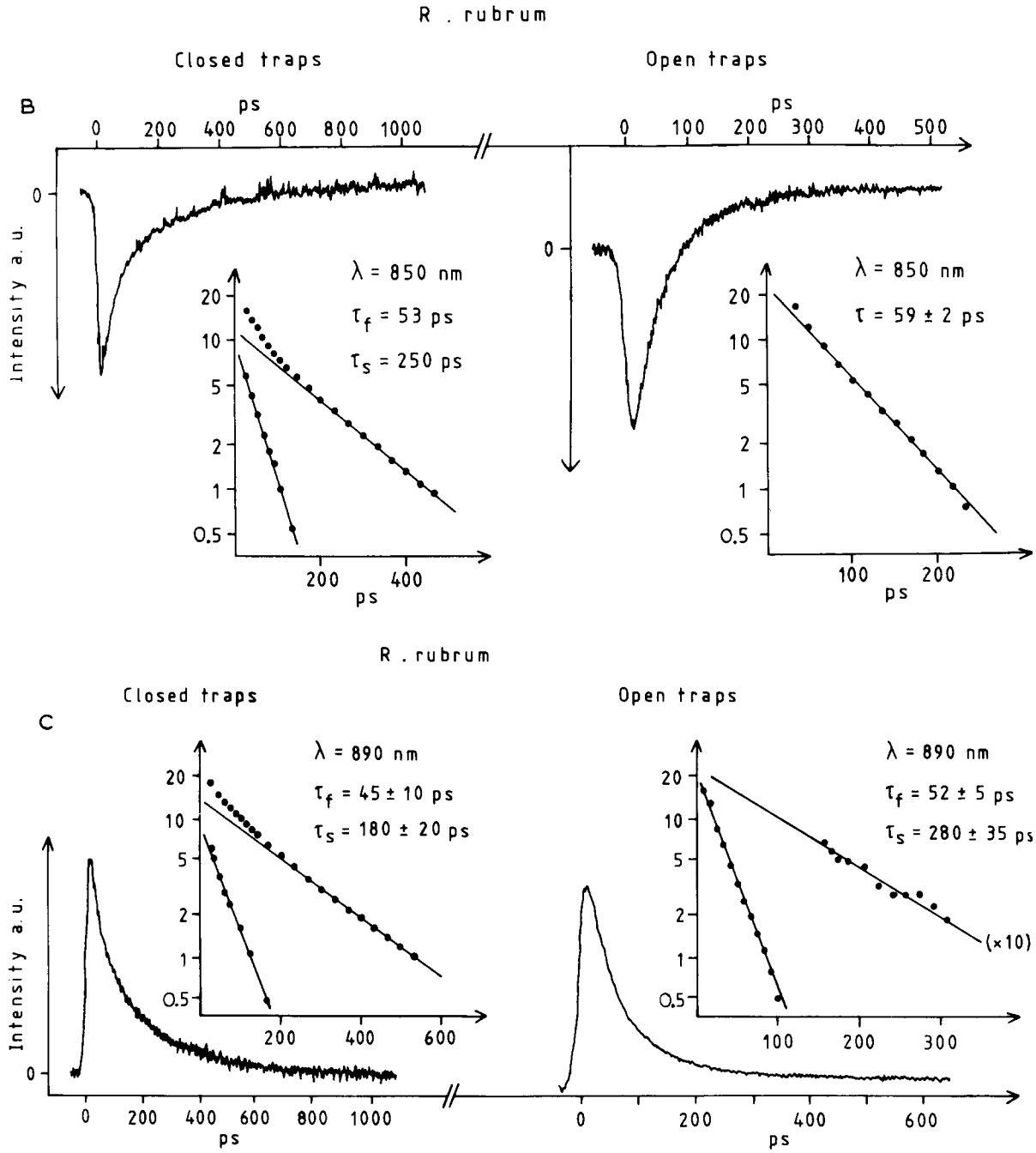


Fig. 2. Isotropic decays of *R. rubrum* chromatophores, open and closed traps at three selected wavelengths 830 nm (A), 850 nm (B) and 890 nm (C).

over, in some cases additional background illumination was added, but within the error of the measurement this had no effect on the decay observed with closed reaction centers. For both open and closed reaction centers the same kinetics were observed over an approx. 10-fold variation of

TABLE I

SUMMARY OF MEASURED LIFETIMES OF *RHODOSPIRILLUM RUBRUM* WILD-TYPE CHROMATOPHORES

λ	Closed reaction centers				Open reaction centers			
	τ_f	τ_s	R_f/R_s	number of measurements	τ_f	τ_s	R_f/R_s	number of measurements
830	50± 3	204± 7	0.6±0.04	4	62±2	–		6
848	–	208±15	(noisy)	3	68±2	–		4
850	53	251		1	59±2			5
853	50± 8	222±21	0.4±0.2	4	71	–		1
870					54	(close to λ_{iso})	–	2
872	B875 isosbestic wavelength							
880	41± 5	175±30	1.2±0.4	6	48±4	242±30	10±2	4
890	45±10	178±21	0.6±0.05	4	52±5	277±35	11±2	6
895	44	195	0.6	1				
900	44± 8	202±15	0.3±0.03	3				
Average	47± 4	204±22			59±8	260±20		

the laser pulse intensity ($5 \cdot 10^{12}$–$5 \cdot 10^{11}$ photons per cm^2 per pulse absorbed). The maximum single pulse intensity at which these experiments were all performed was about 20% of the pulse intensity required to observe the onset of excitation annihilation [12,13].

The picosecond absorption changes as shown in Fig. 2 follow the spectral characteristics of the B880-excited state as reported by Nuys et al. [39]. At 830 and 850 nm an absorption increase is observed due to absorption of B880*, while at 890 nm a strong bleaching occurs. In the experiments reported here the isosbestic point for the changes due to B880* was found to be at 872 nm.

When the reaction centers are all in the open state (PQ) the decay of B880* is dominated by a component having a lifetime $\tau_f = 59 \pm 8$ ps over the whole absorption band. The decay is monophasic in the blue side of the spectrum (830–870 nm) but acquires a low intensity (approx. 10%) of a slower component, $\tau_s = 260 \pm 20$ ps, in the red part of the spectrum (880–900 nm). The formation of a stable long-lived product after the decay of the B880-excited state is evident from the kinetic traces of open reaction centers in Fig. 2. This remaining very long-lived absorption change is characteristic of the state (P^+Q^-).

With the reaction centers all in the closed state the excited-state decay is much slower. A best fit to the experimental traces is obtained if the isotropic decay is described by a sum of two exponentials, a fast phase with a time constant $\tau_f = 47 \pm 4$ ps and a slow phase with a time constant $\tau_s = 200 \pm 20$ ps. The amplitude ratio R_f/R_s of fast and slow phase is of the order of 0.6–1 over the whole spectrum, except for the red wing, $\lambda >$ 895 nm, where it drops to approx. 0.3. It was established that the fast decay phase does not reflect a fraction of open traps, because the kinetics were independent of excitation light intensity over a 20-fold intensity range. Moreover, the difference in the fast lifetime τ_f between the conditions of open and closed reaction centers is large enough to be significant. The ratio of the integrated areas under the decay curves with the closed and open traps is about 2.4 if the contribution of P^+ is accounted for in the latter case. This number is very close to the ratio of the fluorescence yields with closed and open traps [2,12]. Under closed reaction center conditions we also measured the anisotropy decay $r(t)$ at several different wavelengths. Fig. 3 shows the decay of anisotropy for *R. rubrum* measured at 880 nm. The initial anisotropy is low, about 0.1 and $r(t)$ decays to zero, or to a very low value of $r < 0.02$, with a time constant of about 40 ps. Very similar results were obtained at other wavelengths throughout the spectrum; an average value of the anisotropy decay time of $\tau_r = 40 \pm 10$ ps was observed for all wavelengths (see Table III).

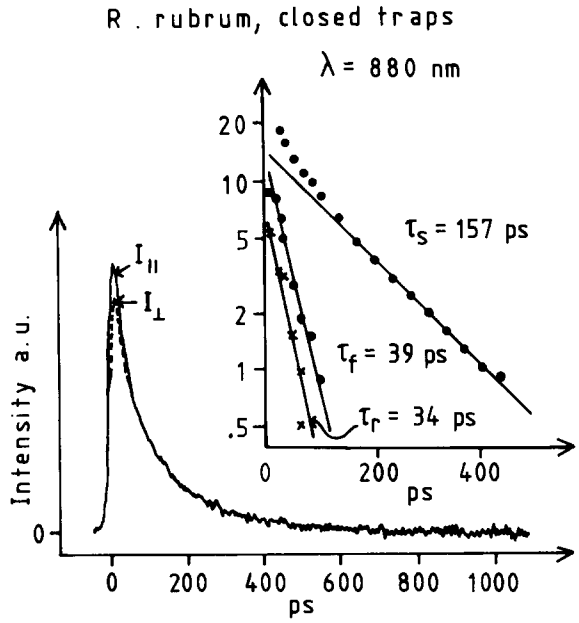


Fig. 3. Anisotropy decay of *R. rubrum*, closed traps, as measured at 880 nm. A plot of $(I_{||} + 2I_\perp)$ is also shown for comparison with the isotropic decays of Fig. 2.

Rhodobacter sphaeroides

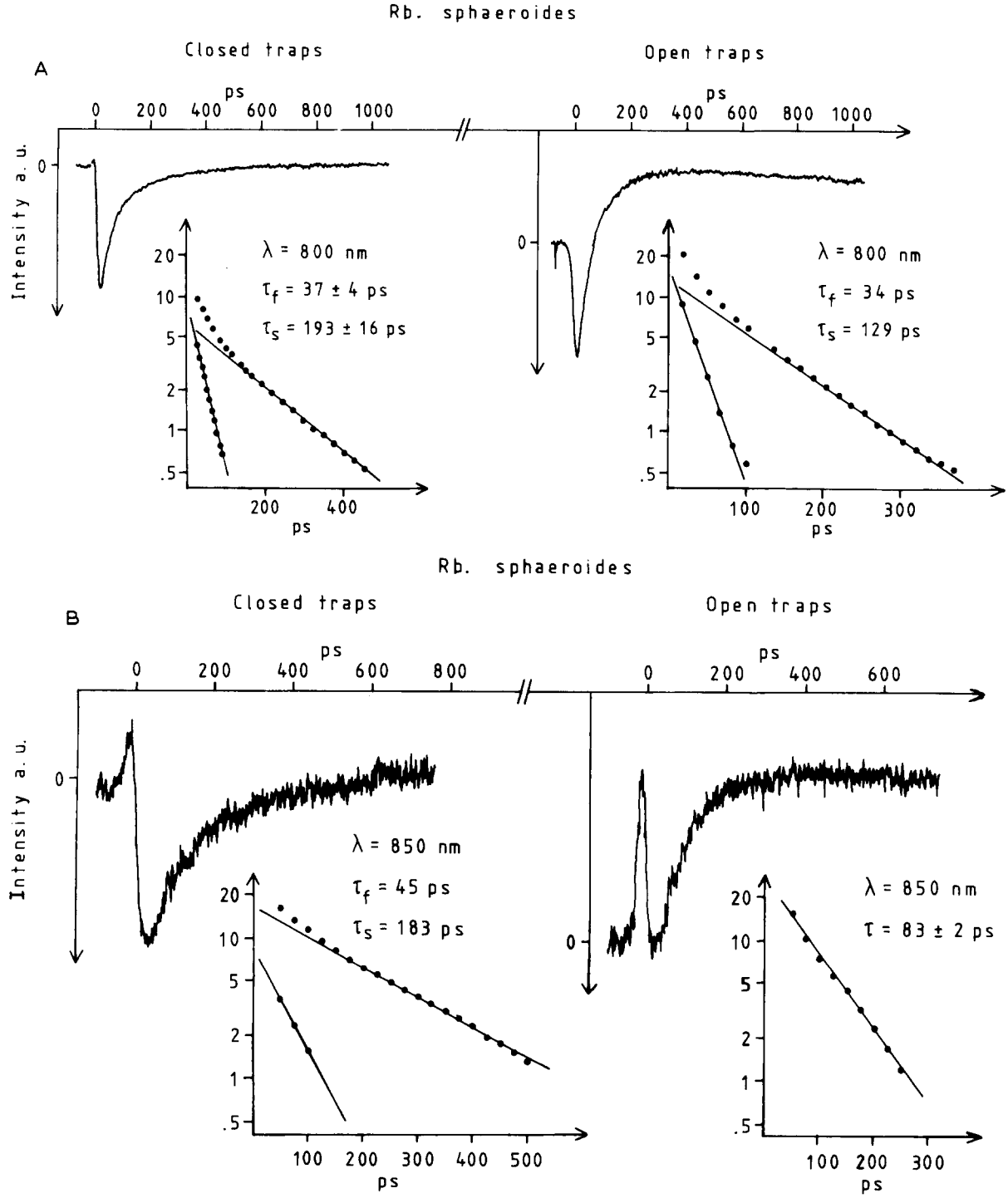
In *Rb. sphaeroides* wild-type chromatophores the decay of the bacteriochlorophyll-excited states is more complex than in *R. rubrum* due to the presence of the additional light-harvesting antenna pigment B800–850. In Fig. 4 we show the isotropic absorption kinetics at 800 (A), 850 (B) and 890 (C) nm, either with all traps open or all traps closed. In addition, plots of $I_{||} + 2I_\perp$, which are equivalent to an isotropic measurement, are shown for closed traps at 800, 827 and 880 nm in Fig. 5. Upon excitation and detection at 800 nm the BChl 800* that is formed in the pulse induces a small initial bleaching which, already during the pulse, is followed by an absorption increase most likely due to BChl 850*. The initial bleaching is much more pronounced in the measurements with excitation and probing beams polarized parallel (see below and Fig. 5). The lifetime of BChl 800* is difficult to obtain with the present used pulselength of approx. 10 ps, but from the shape of the decay we estimate it to be on the order of 1–2 ps. The increase in absorbance at 800 nm, attributed to BChl 850*, following the initial very rapidly decaying bleaching, agrees with the spectra determined with a 35 ps pulse (A.M. Nuys, unpublished results) and corresponds to the increase in absorption observed at the blue side of the B880 absorption band in *R. rubrum*.

With all traps closed, the decay of BChl 850* is strongly biphasic (see Fig. 4A) and characterized by a fast phase with a lifetime $\tau_f = 37 \pm 4$ ps and a slow phase having a lifetime $\tau = 200 \pm 25$ ps (see Table II). An 'isosbestic point' at 848 nm is observed for the absorption changes due to BChl 850. For excitation wavelengths larger than 890 nm the B875 is selectively excited. As can be seen from Table II the same lifetimes $\tau_f \approx 35$ ps and $\tau_m \approx 200$ ps are still detected as in the blue part of the spectrum. However, the amplitude ratio R_f/R_s between the fast and slow components has changed from a value close to 1.5 in the 800–870 nm region to a value of approx. 0.5 at 890–900 nm. This behaviour is expected if the fast phase represents an equilibration of the excitation density over the B800–850 and B875 pigment pools, and the slow phase reflects the subsequent simultaneous decay of the equilibrated densities of B800–850 and B875. This point is discussed further below.

The decay kinetics at 850 nm, very close to the 'isosbestic point' at 848 nm for absorption changes in BChl 850, reveals an interesting feature around $t = 0$. Superimposed on the excited state absorption signal due to directly excited BChl 875, a very shortlived bleaching, with a lifetime of at most a few picoseconds, is visible. This resembles the signal observed at 800 nm for the BChl800 → BChl850 transfer. The very short lifetime tells us that it most likely corresponds to energy transfer between closely spaced and strongly coupled chromophores. We suggest that it represents transfer between slightly different BChl 850 chromophores, or possibly transfer from B800–850 to B875 for the parts of the two complexes that are in very close contact with each other, i.e., the contact areas between B800–850 and B875.

The decay of anisotropy with all traps closed was recorded at several wavelengths (see Table III). At 800 nm (Fig. 5A) the initial absorption changes due to BChl 800* are strongly polarized parallel to the polarization of the excitation beam. The highly polarized state decays within the lifetime of BChl 800* (1–2 ps) and the remaining

absorption changes due to BChl 850* are only weakly polarized, $r < 0.1$. The absorption changes resulting from direct excitation of BChl 850 (830–860 nm) or BChl 875 (approx. 890 nm) are also weakly polarized (see Fig. 5B); the initial anisotropy is approx. 0.1 and decays to zero or to



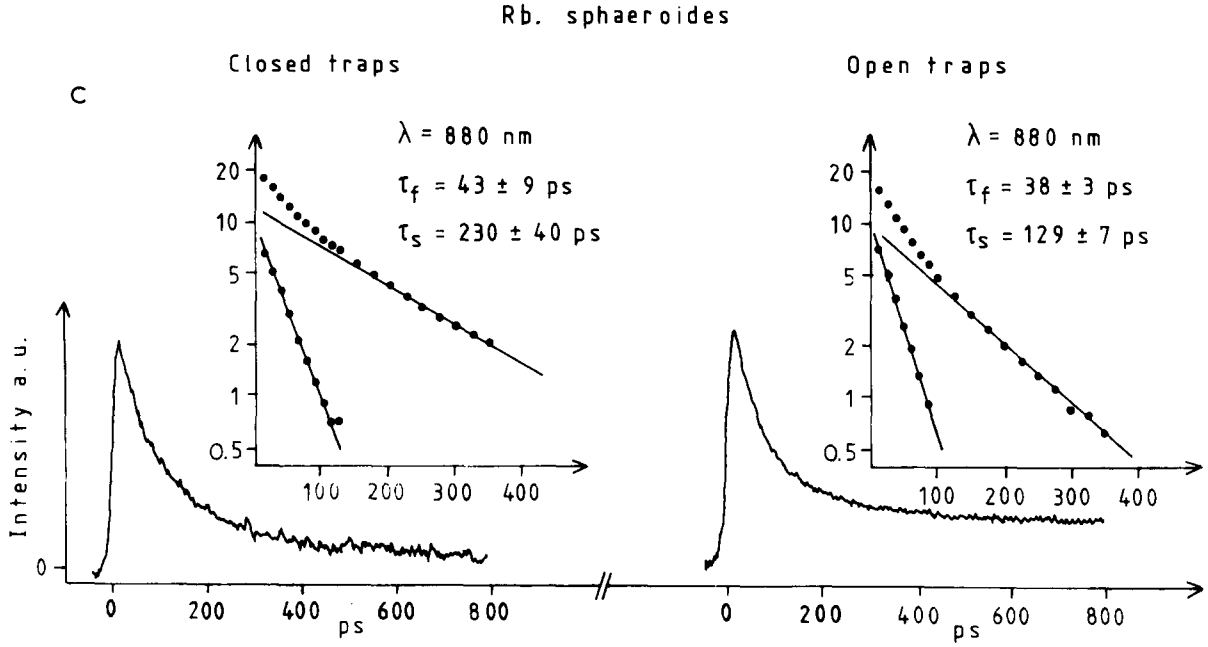


Fig. 4. Isotropic decay of *Rb. sphaeroides* chromatophores, open and closed traps at three selected wavelengths 800 nm (A), 850 nm (B) and 880 nm (C).

a very low value (less than 0.01) with a characteristic decay time $\tau_r \approx 40$ ps. These results suggest that there exists a very fast depolarizing process, with a lifetime far below our time resolution ($\tau < 1$ ps), followed by a slower depolarization occurring on the same timescale as the equilibration process

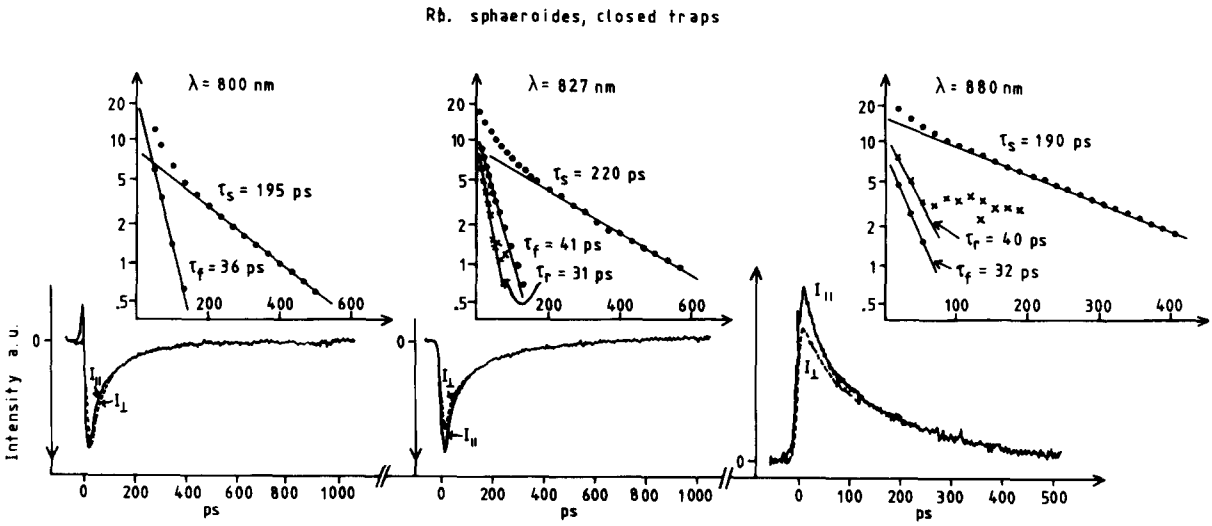


Fig. 5. Anisotropy decay of *Rb. sphaeroides*, closed traps, as measured at 800, 827 and 880 nm. Plots of $I_{\parallel} + 2I_{\perp}$ are shown in the upper panels for comparison with the isotropic decays of Fig. 4. Note that the $I_{\parallel}$ and $I_{\perp}$ traces at 800 nm have been slightly shifted relative to each other, in order to make them both clearly distinguishable.

TABLE II

SUMMARY OF MEASURED LIFETIMES OF *RHODOBACTER SPHAEROIDES* WILD-TYPE CHROMATOPHORES

λ	Closed reaction centers				Open reaction centers			
	τ_f	τ_s	R_f/R_s	number of measurements	τ_f	τ_s	R_f/R_s	number of measurements
798					34	129	1.6	1
800	37±4	193±16	2.0±0.7	13				
810	39	174	1.6	1				
815					38	93	0.3	1
827	39	216	1.2	2				
834	38	192	1.1	1				
845					38	110	0.5	1
848	B850 isosbestic wavelength							
850	45	183	–	2	83±2		–	4
855	34	–	–					
860	31±7	214±60	3.6±0.9	6	24±2	110±16	2.4±0.6	3
870	31	163	2	1	25	105	1.5	1
880	43±9	229±40	0.8±0.2	6	38±3	129± 7	1.2±0.1	4
890	34	239	0.7	1	26	104	1.0	2
900	36	180	0.3	2	35	115	1.3	2
Average	37±4	198±25			32±6	112±12		

between the B850 and B875 pigment pools.

The decay with all traps open is also markedly biphasic with a fast phase of about 30 ps and a slow phase close to 110 ps (see Fig. 4B). The amplitude ratio R_f/R_s of fast and slow phase is between 1 and 2 at all wavelengths, except around 850 nm which is close to the 'isosbestic point' for the absorption changes due to B800–850 (see Table II). Anisotropy decays were also measured under the conditions of open traps, and the results were very similar to those obtained under closed-traps conditions.

TABLE III

MEASURED PICOSECOND ABSORPTION ANISOTROPY IN CHROMATOPHORES OF *RHODOSPIRILLUM RUBRUM* AND *RHODOBACTER SPHAEROIDES*, UNDER CLOSED REACTION CENTER CONDITIONS

$r(0)$ is the anisotropy extrapolated to $t = 0$. $r(\infty)$ is the anisotropy at long times, $t \geq 100$ ps.

λ (nm)	τ_r (ps)	$r(0)$	$r(\infty)$
R. rubrum			
853	45±15	0.15	< 0.01
880	40± 5	0.06	< 0.01
890	38	0.10	≈ 0.04
900	34	0.06	< 0.01
Average	40±10	0.09±0.04	< 0.01
Rb. sphaeroides			
800	< 1	high, > 0.1	< 0.01
827	40	0.11	≈ 0.02
834	60	0.10	< 0.01
880	30	0.10	≈ 0.05
Average (827–880 nm)	40±15	0.1±0.02	< 0.01

Discussion

From this work it will be evident that picosecond absorption spectroscopy utilizing low-intensity, continuously tunable infrared pulses of high repetition rate is a powerful technique for the study of isotropic kinetics and anisotropy decay of bacteriochlorophyll-excited states in chromatophores of purple bacteria. In the following section we shall first compare the observed decay times with those obtained from time-resolved fluorescence spectroscopy and earlier picosecond absorption measurements. Next we shall discuss the anisotropy decays and finally combine the results into a scheme for energy transfer and hopping in these membranes.

Excited-state lifetimes and fluorescence yields

Chromatophores of Rhodospirillum rubrum

Recently, Freiberg et al. [33] and Borisov et al [34] have reported fluorescence lifetimes for *R rubrum* cells and chromatophores. With all the reaction centers active the lifetime of the fluorescence was about 60 ps, and increased to about 210 ps with P oxidized. According to these authors [33,34], the decays were monophasic, even at intermediate oxidation levels of P, and did not depend on the excitation wavelength. In general, the isotropic decay times for *R. rubrum* reported in the present work and summarized in Table I are in good agreement with the data of Refs. 33 and 34. This shows that excited-state decays measured either via low-intensity absorption or via fluorescence detection report essentially the same phenomena. Earlier absorption measurements employing high-intensity single 35 ps pulses have indicated B880* lifetimes of approx. 60 ps [37,38] or approx. 80 ps [39] with all traps open and approx. 60 ps [37,42] or 200–400 ps [39] with all the traps closed. Apart from the extremely fast decay observed in Ref. 42 with the traps closed, which most probably seems to be due to excitation annihilation [12,39], these decay times correspond reasonably well to those obtained in our work.

We start to discuss the measured decays of *R. rubrum* with open traps. In correspondence with earlier work [12,33,34,37,38] we attribute the 60 ps decay time to the overall trapping rate of the excitation energy at the active reaction centers. It corresponds to a fluorescence yield of about 0.5%, which is very close to that experimentally observed [3,6]. The analysis of kinetic traces having very good signal to noise ratios, obtained at 880–890 nm revealed the presence of a weak decay phase with about 10% of the initial total amplitude, and a decay time close to 250 ps. It seems highly likely that this phase represents the fraction of B880* that decays in equilibrium with P^+I^-. The decay time of 250 ps would then reflect the oxidation of I^- by Q_A. A very similar time constant was given by Nuys et al. [57] and is in close agreement with that found in isolated reaction centers [48,49]. The amplitude ratio (approx. 1 : 10) corresponds to a ΔG of 0.06 eV between B875* and P^+I^-. This number is very close to values given elsewhere [50,51], if the entropy of the excitation over all the antenna bacteriochlorophyll molecules is taken into account. Although the noise in the traces and the large background signal due to $[P^+Q^-]$ at the other wavelengths did not allow such an accurate analysis, a comparison of these traces with those obtained in the wavelength interval 880–890 nm suggested that they also may contain this weak 250 ps component. We are currently investigating the kinetics upon excitation and probing in the 760–820 nm region of *R. rubrum*. Such experiments are expected to yield more information concerning these processes.

We shall next turn to the case of closed reaction centers. Here we do not have any contribution to the measured recovery signals from absorbance changes in the reaction centers. This fact and the much longer lifetime make it possible to study the energy transport in the antenna pigments in great detail. The 200 ps decay with all the traps closed is much faster than the expected decay time for free bacteriochlorophyll (approx. 2 ns [52]) and moreover even faster than that observed in reaction centerless preparations [28,32, 53]. For instance, in the isolated B800–850 complex the main decay component has a lifetime of approx. 900 ps (results not shown). Therefore, we assume that quenching due to the presence of P^+ accounts for a major part of the 200 ps decay. The additional decay phase of about 50 ps obtained with all traps closed was not observed in earlier studies and may be due to energy transfer within the B880 pigment protein complex. Energy transfer from B880 to B896 [22] might be a good candidate. Assuming a 6 : 1 ratio of the number of BChl 880 to BChl 896 chromophores and unchanged relative spectral positions at room temperature and 4K [22] there should be a 0.6 : 1 amplitude ratio, R_f/R_s, of the fast to slow decay component when the B880 is selective excited (i.e., $\lambda < 880$ nm). The experimental results of Table I seem to verify this prediction, since an average value $R_f/R_s = 0.7 \pm 0.3$ of this ratio is observed over the wavelength region 830–890 nm.

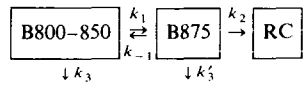
Chromatophores of Rhodobacter sphaeroides

For chromatophores of *Rb. sphaeroides* Sebban et al. [31,32] reported decay times of 50 ps and 200 ps with open and closed traps, respectively. Frei-

berg et al. [33] and Borisov et al. [34] obtained 60–70 ps for open and 180 ps for closed traps. We find that with all traps open the decay of the excited state absorption changes are biphasic at all wavelengths, where a reasonable high signal-to-noise ratio was obtained. The fast phase is about 30 ps, while the slow phase is of the order of 100–120 ps, and the amplitudes of both phases are of the same order of magnitude. If we ignore the biphasic character of these decays they would correspond to a 1/e decay time of about 60–70 ps, very close to the results cited above [31–34].

Sebban et al. [31] claimed that the 200 ps decay for the closed traps was due only to B875*; the main emission of B800–850 was supposed to be extremely fast. Similarly, Borisov et al. [34] do not observe a clear decay component associated to the transfer B800–850 → B875, and they suggest that the transfer time is at most a few picoseconds. In contrast to this conclusion our results clearly show that there is a phase of about 35 ps in the decay of the B800–850- and B875-excited states. We propose that this phase represents the time it takes to equilibrate the excitation densities in B800–850 and B875, and this is equal to the reciprocal of the sum of the rate constants for forward (B800–850 → B875) and backward (B875 → B800–850) transfer. The fast decay phase is observed both upon excitation of the main B800–850 absorption bands and upon excitation of the B875 band, as is expected in the presence of an equilibrating process. Together with the amplitude ratio R_f/R_s of the fast and slow decay components at 800–870 nm of 1.5 : 1 (see Table II) this implies that the forward and backward transfer times are about 60 and 90 ps, respectively. We can compare these values with an estimation by Zankel [35,44] from the relative B850 and B875 emission yields with open and closed traps. Taking 60 ps for the average trapping time constant upon direct excitation of B875, it follows that the time constants of forward and backward transfer are 60 and 120 ps, respectively, in reasonable agreement with the values estimated directly from our experimental results. It is also interesting to compare the measured ratio of forward and backward transfer rates with that calculated from the knowledge of antenna size and location of absorption maxima. Taking a 3 : 1 ratio for the number of BChl 850 and BChl 875, and absorption maxima at 850 and 875 nm, an R_f/R_s ratio of 1.7 is predicted for selective excitation of BChl 850. The corresponding value for BChl 875 excitation ($\lambda = 890$–900 nm) is 0.6. As can be seen from table II both these values compare favourably with the measured ratios. The relatively slow rate of transfer from B800–850 to B875 is in good agreement with the significant yield of the B800–850 emission, even at low temperatures [36], where the transfer from B800–850 to B875 is irreversible.

After having obtained this picture of the energy transport between the B800–850 and B875 pigment complexes under closed reaction center conditions, it is now easy to explain the observations for open reaction centers. In fact, the existence of an equilibrating process between the two pigment pools and a trapping process from one of the pools predicts a biexponential decay of the B800–850- and B875-excited states at all wavelengths. This is best seen from the following kinetic scheme



which is characterized by the two compound rate constants

$$k_{eq} = \frac{1}{\tau_{eq}} = \tfrac{1}{2}\Big((k_1 + k_{-1} + k_2 + k_3 + k_3') + \sqrt{(k_1 - k_{-1} - k_2 + k_3 - k_3')^2 + 4k_1k_{-1}}\Big)$$

$$k_{trap} = \frac{1}{\tau_{trap}} = \tfrac{1}{2}\Big((k_1 + k_{-1} + k_2 + k_3 + k_3') - \sqrt{(k_1 - k_{-1} - k_2 + k_3 - k_3')^2 + 4k_1k_{-1}}\Big)$$

and an equilibrium constant

$$K = \frac{k_1}{k_{-1}}$$

Using the values of k_1, k_{-1} and k_2 as obtained for closed reaction centers of *Rp. sphaeroides* (k_1, k_{-1}) and open reaction centers of *R. rubrum* (k_2) and assuming $k_3 \approx k_3' \gg k_2$ we obtained values of τ_{eq} and τ_{trap}, which are somewhat depen-

dent on the equilibrium constant K. Thus, for a value of the equilibrium constant in the range 1–2 (as suggested by Table II) we obtained $\tau_{eq} = 27$–28 ps and $\tau_{trap} = 117$–168 ps. The choice of overall trapping time $k_2^{-1} = 60$ ps, as measured for *R. rubrum* with open traps, neglects the possibility of B880 and B875 having substantially different energy-transfer dynamics. In addition, this simplifying assumption does not take into account complications of the kinetics which could be caused by a possible back-transfer from P^+I^- (the existence of which was suggested in the decays of *R. rubrum*, Fig. 2C open traps). However, the relatively good agreement between the lifetimes predicted by this simple model and the measured fast and slow decay components of *Rb. sphaeroides* with open traps suggests that the model describes the essential features of the equilibrium and trapping processes in the BChl antennas of *Rb. sphaeroides*. Hence, we can see from these results that the fast relaxation time, associated with the equilibration of the excitation density, essentially remains constant when going from closed to open reaction centers. However, the equilibration process between B800–850 and B875 produces an apparent increase in the time constant describing the trapping process (from 60 ps to approx. 120 ps).

In the experiments of Sebban et al. [31] and Borisov et al. [34] with open and closed traps, the kind of biphasic decays reported here were not observed. A possible reason for this could be that when using excitation wavelengths of 514.5 nm [31] and 385 nm [34] a distribution of the excitation density close to that expected for equilibrium is directly generated. In that case no fast decay component would be observed.

Finally, from the BChl 800 fluorescence yield it was calculated that the transfer rate from BChl 800 to BChl 850 is of the order of $3 \cdot 10^{11}$ s^{-1} at low temperature [20,21] and probably higher at room temperature. This number compares reasonably well with the estimated transfer time of 1–2 ps obtained in this work.

Time dependence of absorption anisotropy

In all experiments the observed absorption anisotropy even extrapolated to zero time is small and positive ($r(0) \approx 0.1$). Only upon direct excitation of BChl 800 can highly polarized absorption changes due to BChl 800* be observed which decay within the transfer time from BChl 800 to BChl 850 (1–2 ps). Highly polarized initial decays ($\tau = 1$–10 ps) with $r(0)$ approaching 0.4 have previously also been observed for phycobilisomes of blue-green bacteria [55] and the isolated chlorophyll *a/b* protein of Photosystem II [56]. It suggests that the primary process following absorption of light has been resolved. From the fact that, in most cases, even the initial absorption anisotropy is low and independent of the excitation/detection wavelength we may conclude that both BChl 850 and BChl 875 in *Rb. sphaeroides* and BChl 880 in *R. rubrum* have the Q_y transitions of the bacteriochlorophyll molecules organized like a circularly degenerate oscillator [14,18,21,22]. Fast energy transfer evidently takes place among the bacteriochlorophyll molecules and the excited state becomes depolarized (to a value of $r \approx 0.1$) within the time resolution of our experiments. From our experiments it also follows that this very fast depolarization process must be faster than approx. 1 ps, but it cannot be decided whether the process involves a coherent exciton state initially delocalized over a few bacteriochlorophyll molecules, or involves a Förster type of transfer among nearest neighbours (for a discussion of transfer mechanisms, see Ref. 44).

After the initial rapid depolarization a slower decay of the anisotropy, having a decay-time of 40 ± 10 ps, is observed in both *R. rubrum* and B800–850 and B875 of *Rb. sphaeroides*. If the fast initial depolarization represents energy transfer within a minimum B800–850, B875 or B880 unit containing 6–8 bacteriochlorophylls [23], the subsequent process may indicate the slower transfer between different units. The fact that the anisotropy decay times are not significantly different from those needed to trap the excitation is not surprising because for both processes a similar number of jumps between different units would be required.

A schematic model for excitation energy transfer and trapping in R. rubrum and Rb. sphaeroides

The results of this work obtained with picosecond absorption spectroscopy suggest a scheme for

energy transfer in the long-wavelength antenna of photosynthetic bacteria that in many respects is different from the models based on a random walk of the excitation on a (more or less) regular array of pigment molecules [45–47]. If in *R. rubrum* we take 6 BChl 880 molecules per B880 antenna complex, then the excitation is delocalized among these six BChl 880 molecules within 1 ps. Then, a random walk occurs among 8–9 of such complexes and assuming that trapping occurs at a rate of $3 \cdot 10^{11}$ s^{-1} [48,54] we can calculate that the average jumping time from one B880 to another is of the order of 20 ps. This corresponds to a distance between the edges of the B880 complexes of about 20–25 Å.

The rapid delocalization within a B880 complex containing 6 BChl 880 molecules implies that the transfer rate between pairs of BChl 880 molecules in a complex is about $3 \cdot 10^{12}$ s^{-1}. Assuming the transfer to be incoherent hopping a maximum distance of 14 Å between individual BChl 880 molecules within a complex is required. Very similar considerations apply for the B875 antenna of *Rb. sphaeroides.*

It is not clear to what extent the analysis of singlet–singlet annihilation experiments in photosynthetic bacteria [12,47] is affected by assuming a structure of the long-wavelength antenna bacteriochlorophyll molecules as discussed above. We note that the model as described in Ref. 47 is not easily extended to include such an irregular structure and a computer simulation of the excitation-transfer process is required. Earlier Monte-Carlo simulations have indicated that a certain degree of irregularity of the lattice did not affect the calculated 'effective' rate constants of trapping and annihilation as deduced from the annihilation curve. Of course, domain sizes are not influenced by any assumptions about the lattice structure.

It is beyond the purpose of the present work to reanalyze in detail the excitation annihilation data. However, it is worthwhile to discuss the conclusions of Bakker et al. [12] in relation to those suggested in this work. It was calculated by Bakker et al. [12] that the rate of energy transfer between two neighbouring BChl 880 molecules was close to 10^{12} s^{-1}. This led to a trapping time of about 90 ps. A remarkable property of the transfer and trapping process was the fact that the excitation could escape from an open trap with a relatively high probability ($\eta \approx 0.7$–0.9). The model presented in this work assumes very fast transfer $k \approx 3 \cdot 10^{12}$ s^{-1} between individual BChl 880 molecules in a B880 complex. Transfer between different B880 complexes is supposed to be slower ($k \approx 5 \cdot 10^{10}$ s^{-1}). One might conclude that now B880 → B880 transfer has become the rate-limiting process, in contrast to the idea that trapping at the reaction center is rate limiting.

All these considerations neglect the inhomogeneity of the long-wavelength antenna of purple bacteria. Our results, especially those with the traps closed, show that equilibration between BChl 880 and the long wavelength antenna pigment, BChl 986, most likely takes place with a time constant very close to that observed for trapping. How this will influence the results at room temperature is hard to estimate, but preliminary computer simulations indicate that no special arrangement of BChl 896 has to be assumed to explain the experimental results. However, in view of the time required for excitation transfer to B896 as compared to the trapping time with open reaction centers, it seems possible that B896 surrounds the reaction center.

We shall apply the picosecond absorption technique also at low temperatures to these systems and hope to obtain a more detailed picture concerning the role of B896.

In *Rb. sphaeroides* the effective transfer time from B800–850 to B875 is about 40 ps. The number of B800–850 complexes coupled together to transfer their excitation to B875 is unknown, but recent excitation annihilation experiments at 4 K indicated that this number corresponds to about 30 connected BChl 850 molecules (Vos, M., personal communication). Assuming these to be organized in B800–850 complexes, each containing six BChl 800–850 molecules, and assuming BChl 875 to act as a perfect trap the transfer rate between two B800–850 complexes is about 1–$2 \cdot 10^{11}$ s^{-1}.

We finally remark that the transfer time from BChl 800 to BChl 850 of about 1–2 ps corresponds to a maximum distance in the B800–850 complex of 22 Å between BChl 800 and at least one of the BChl 850 molecules. From our experiment it is not clear to what extent rapid energy

transfer between different BChl 800 molecules precedes the transfer to BChl 850, but the high anisotropy of BChl 800* suggests that no strongly depolarizing transfer process occurs. Distances of the order of 20 Å for the separation of spectrally slightly different BChl 850 molecules are also suggested by the very fast transients with picosecond–subpicosecond lifetimes observed upon excitation of BChl 850 of *Rb. sphaeroides* around its 'isosbestic point'.

Conclusions

We have studied the energy-transfer dynamics in chromatophores of *R. rubrum* and *Rb. sphaeroides*, under conditions of closed and open reaction centers, using low-intensity picosecond infrared absorption recovery measurements. Our results are in overall agreement with earlier time-resolved fluorescence measurements, but our possibilities of selective infrared excitation and probing as well as picosecond anisotropy decay measurements have given a more detailed picture of the transfer dynamics. Upon selective excitation of a bacteriochlorophyll-protein complex (B800–850, B875, B880) there is a very low initial value of the absorption anisotropy ($r(0) \approx 0.1$) for the excited state decays of BChl 850, BChl 875 and BChl 880. This is consistent with a very fast ($k \approx 3 \cdot 10^{12}$ s^{-1}) initial transfer of energy between similar bacteriochlorophyll molecules within a minimum unit of 6–8 chromophores. Only direct excitation of BChl 800 shows highly polarized, very shortlived (approx. 1 ps) decays, indicating only limited BChl 800–BChl 800 transfer during the lifetime of B800*. On a slower time-scale (5–20 ps) the energy then migrates between the minimum units. On a similar time-scale, 40–50 ps, an equilibration of the excitation density occurs between different pigment pools. Thus, in *Rb. sphaeroides* there is an equilibration between B850 and B875 and in *R. rubrum* there is a similar process between B880 and B896. These slower processes are associated with a further decay of the anisotropy from the initial value of 0.1 to very low values less than 0.01.

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