Energy transfer within the isolated B875 light-harvesting pigment-protein complex of *Rhodobacter sphaeroides* at 77 K studied by picosecond absorption spectroscopy

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The energy transfer dynamics at 77 K within detergent solubilized and purified preparations of the B875 pigment-protein complex of *Rhodobacter sphaeroides* have been investigated by picosecond absorption spectroscopy. Isotropic absorption recovery and decay of induced absorption anisotropy provide clear evidence that B875 is inhomogeneous in these preparations. We interpret the results as fast ($\tau = 15 \pm 5$ ps) energy transfer from the major BChl 875 pigments to a minor pigment pool, B896. The excited state of B896 decays with a time constant of 650 ± 50 ps. We suggest that B896 is intrinsic to B875 complexes and exists in a highly organized state, close to the reaction center. In the intact membrane, B896 may concentrate excitation energy in the vicinity of the reaction center special pair, thereby increasing the efficiency of the final energy transfer step.

Antenna bacteriochlorophyll-protein complex; Bacterial photosynthesis; Energy transfer; (*Rhodobacter sphaeriodes*)

1. INTRODUCTION

The presence of a special pigment component connecting the long wavelength antenna B875/B880 with the reaction center of purple bacteria has been proposed [1–6]. Fluorescence polarization at 4 K of the isolated B875 complex [7] was interpreted in terms of the existence of such an antenna pigment. Picosecond fluorescence streak-camera measurements of *Rhodospirillum rubrum* at 77 K displayed non-exponential fluorescence decay, which was also attributed to inhomogeneity of the B880 antenna [8]. Recent picosecond absorption recovery measurements on chromatophore preparations of *Rs. rubrum* and *Rhodobacter sphaeroides* at room temperature [9] and at 77 K [10] yielded conclusive evidence for the existence of a highly red-shifted antenna component, designated as B896 [7], in both these species.

The absorption recovery results suggested a B875/B880 ⟶ B896 transfer time of 15–20 ps, and a strong wavelength dependence of the induced absorption anisotropy. Hence, very low anisotropy (the absorption anisotropy is defined as:

$$r = \frac{\Delta A_{\parallel} - \Delta A_{\perp}}{\Delta A_{\parallel} + 2\Delta A_{\perp}}$$

[9]), $r \leq 0.1$, was observed for excitation and probing in the main antenna absorption band, whereas strongly increased anisotropy with r values of up to 0.25, was found in the red wing of the absorption band (i.e., for $\lambda > 890$ nm). This increase of absorption anisotropy was interpreted to be a consequence of selective excitation of B896, in

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which only limited energy transfer among identical bacteriochlorophyll *a* (BChl) 896 molecules occurs. Alternatively, a highly ordered structure of the B896 pigment could allow for energy transfer among BChl 896 molecules without causing a detectable depolarization. Still another possibility is to consider the B896 excited state as a long-lived exciton state.

The absorption recovery results cited above were all obtained with membrane preparations containing the intact light-harvesting apparatus which in *R. sphaeroides* consists of peripheral B800-850 and core B875 antenna pigment-protein complexes. Here picosecond absorption recovery was measured on isolated and purified preparations of B875 which has simplified the kinetic analyses. The results confirm the proposed energy transfer dynamics and pathways and can be explained by assuming that B896 is an intrinsic component of B875 complexes.

2. MATERIALS AND METHODS

The B875 pigment-protein complex was isolated from chromatophores of *R. sphaeroides* NCIB8253 and purified by lithium dodecyl sulfate-polyacrylamide gel electrophoresis at 4°C [11]. Molar ratios of B800-850/B875 were determined as in [12,13] and bands with values ≤ 0.15 were pooled. Detergent solubilization results in a broadening on the blue side of the near-IR absorption band but the red side appears unchanged [13]. Concentrated solutions of the B875 complex were diluted with glycerol to yield an absorbance of ~0.3/mm at 875 nm. This produced a sample solution with a glycerol/water ratio of approx. 3:1 which was optically clear when cooled to 77 K.

Absorption recovery measurements with infrared (800–900 nm) pump and probe pulses were performed as previously described [9,10], by using 10-ps long pulses generated in a sync-pumped and cavity-dumped dye laser system operating at 800 kHz. Pump and probe beams were polarized by using prism polarizers and a Soleil-Babinet compensator. This permitted measurement of isotropic decays as well as the decay of induced anisotropy, at any wavelength covered by the laser system. Lifetimes and amplitudes of the decay components were obtained by using a non-linear least-square fitting program. In order to obtain reliable lifetimes for very short-lived components ($\tau < 20$ ps), deconvolution of the measured kinetics was performed with a Gaussian pulse having the width of a laser pulse autocorrelation function. This was especially important in decays where the fast component was present as a risetime, i.e., in the wavelength region around 880 nm (see fig.1C). This procedure was tested with a dye molecule which undergoes fast relaxation and the deconvoluted lifetimes (10-ps pulses) were in agreement with direct measurements using visible, 300-fs long pulses (Bergström, H. et al., submitted).

3. RESULTS AND DISCUSSION

Polarized and isotropic absorption recovery kinetics were measured at several wavelengths throughout the near-IR absorption band of B875 at 77 K. Representative curves of the isotropic kinetics are shown in fig.1. At most wavelengths within the B875 absorption band, the kinetics are well described by a double exponential decay with time constants $\tau_f = 15 \pm 5$ and $\tau_s = 650 \pm 50$ ps. For wavelengths above 900 nm, the decay was dominated by the slow component and closely approached a single exponential fit. At slightly shorter wavelengths, 890–895 nm, the fast component acquired substantial intensity (fig.1A). At 886 nm (fig.1B), the fast component accounted for all the intensity of the decay curve, since the slow component has an isosbestic point at this wavelength (see below). At 880 nm (fig.1C), the fast component still appeared as a bleaching, whereas the slow component is characterized by an intense absorption. The presence of the fast component as a risetime of the induced absorption at this wavelength is clearly seen in the high-resolution scan shown as the insert of fig.1C. In order to recover this fast risetime, deconvolution of the measured curve with the response function was necessary. In the wavelength interval 850–870 nm (fig.1D), both decay components appear with positive amplitude, i.e., increased absorption.

From the amplitude variation of the two components which characterize the decay curves of fig.1, it is clear that the fast and slow components represent species having different absorbance difference spectra. The slow component has an isosbestic point at 886 nm and an intense bleaching in the region 895–900 nm, while the fast component has an isosbestic point at about 875 nm with intense bleaching in the region 880–885 nm but almost no bleaching around 895 nm. It is clear that these kinetics and absorbance differences cannot be due entirely to BChl 875, but in addition, reflect a fast energy transfer from BChl 875 to a red-shifted pigment. Previous work [7–10] has strongly suggested the presence of such a pigment, designated B896 [7]. Thus, we assign the fast component, $\tau_f = 15 \pm 5$ ps, to energy transfer from BChl 875 to BChl 896 and the slow decay, $\tau_s = 650 \pm 50$ ps, to deactivation of BChl 896*, decoupled

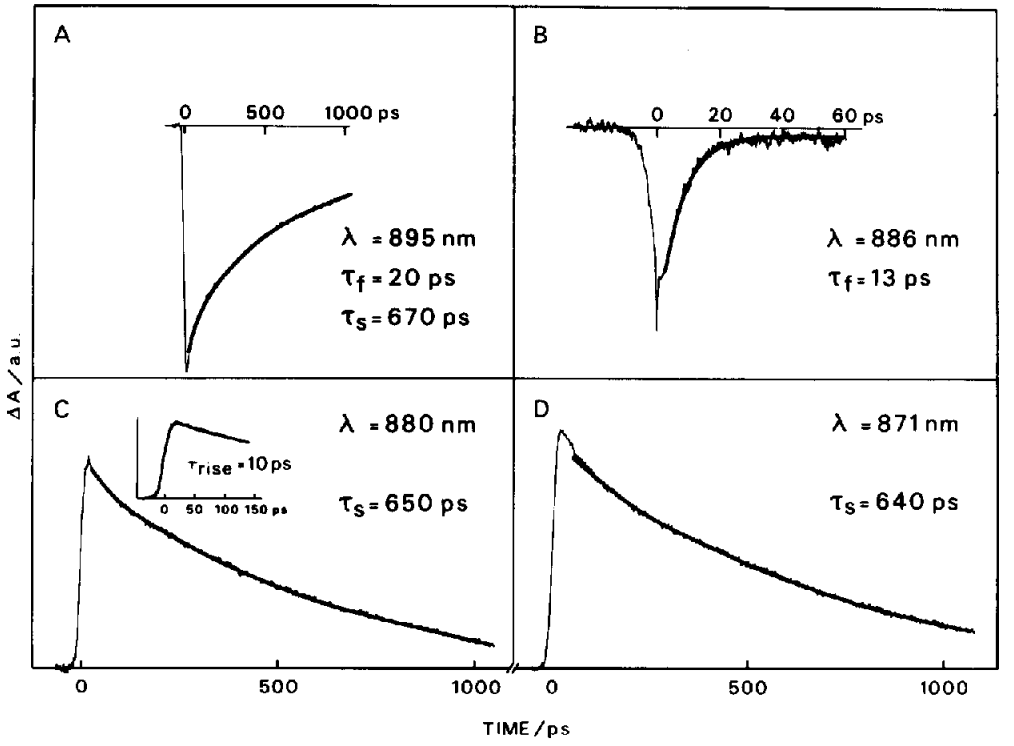

Fig.1. Isotropic kinetics of B875 at 77 K. (A) λ = 895 nm; (B) λ = 886 nm; (C) λ = 880 nm, the insert is the same decay but measured with slower delay scan speed to resolve the rising part of the curve; (D) λ = 871 nm. The solid lines represent best fits to experimental curves.

completely from its energy acceptor, the reaction centers.

Measuring the decay of induced anisotropy yields additional information about energy transfer among identical chromophores [9,10]. The time dependence of anisotropy was measured at several wavelengths as indicated in fig.2 and table 1. The wavelength dependence of anisotropy is seen to follow the same pattern as that observed previously in chromatophores [10]. Only at wavelengths above 905 nm can B896 be excited selectively. This was manifested by a high and almost time-independent anisotropy ($r(0) = r(\infty) = 0.21$, see table 1). At shorter wavelengths (880–900 nm), B875 and B896 were excited simultaneously and some depolarization occurred in the transfer step B875 ⟶ B896; this is shown by a small initial decay of $r(t)$, with a time constant of about 20 ps, from a value $r(0) > 0.2$ to a steady value $r(\infty) = 0.17$ (fig.2A). At even shorter wavelengths (< 880 nm), where B875 was excited almost exclusively, $r(t)$ was low and time independent (fig.2B). These findings suggest that little depolarization occurs within B896 but that energy transfer within B875 and between B875 and B896 results in efficient depolarization.

The time constant for the decay of the B896 component (650 ± 50 ps) observed here agrees with the fluorescence lifetime (640 ± 30 ps) of isolated B875 at 77 K [14] and establishes BChl 896 as the terminal emitter in these prepartions. In *R. sphaeroides* chromatophores at 77 K, τ = 190 ± 10 ps was observed for the slow quenching of BChl 896* by closed reaction center phototraps [10]. Otherwise, the kinetic and absorbance changes were similar to those reported here, but the decay pattern was complicated by the presence of additional B800-850 pigments [15] in the intact system. It now appears that in chromatophores, the ΔA spectrum in the 860–880 nm region [10] has contributions that arise from a special pigment that mediates energy transfer from B850 to B875 [15].

Based on the finding that fast energy transfer to B896 is observed even in isolated and purified B875

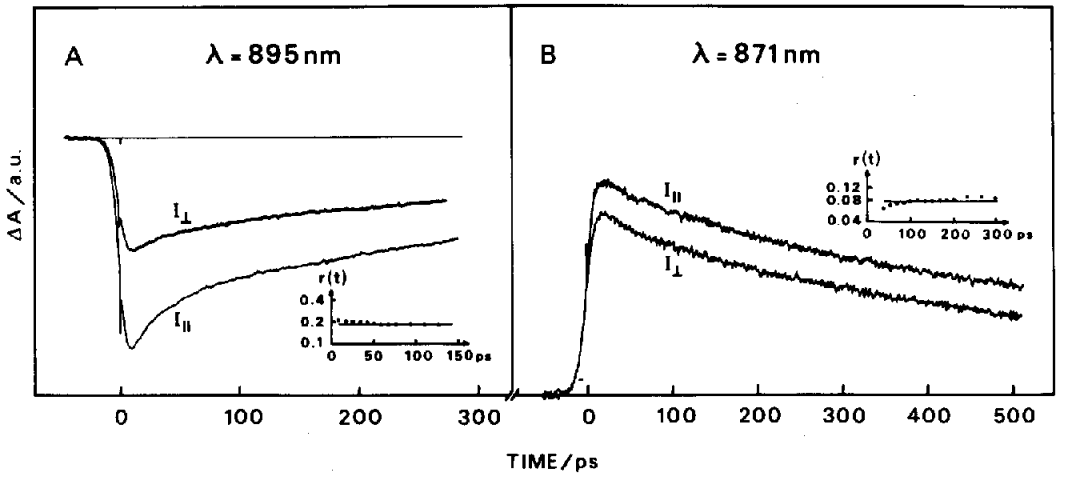

Fig.2. Decay of induced anisotropy, $r(t)$, of B875 at 77 K. (A) λ = 895 nm; (B) 871 nm.

preparations, it appears that B896 is coupled tightly to B875 and is possibly intrinsic to this complex. Estimates of oligomerization states from mobility in lithium dodecyl sulfate-polyacrylamide gel electrophoresis indicate that the B875 minimum unit migrates as a trimer containing 6 BChl molecules [12]. From singlet-singlet annihilation measurements on excised B875 gel bands at 295 K, 8 connected BChl molecules were calculated [16]. Since ratios of 6–7 in amplitude for the respective deconvoluted BChl 875 and 896 absorption bands have been estimated [7], it is possible that one of these BChls is in a unique 'B896 environment'. This would provide a simple explanation for the lack of depolarization of BChl 896*, even at room temperature [17]. Alternatively, B896 could arise from interactions between two BChl molecules on different B875 complexes with essentially parallel transition moments. Some support for involvement of interacting BChl molecules on different complexes in the origin of the B896 redshift is provided from a broadening observed on the red side of the near-IR absorption band in B875 preparations of higher aggregation states in gel electrophoresis (not shown).

In intact membranes, B896 may also arise from intercomplex associations, if it is assumed that this component is not intrinsic to the minimum B875 unit. At low temperature the excitations are rapidly localized on B896 and efficient energy transfer occurs among different BChl 896 molecules in the core antenna [18]. In order to account for the long-lived high absorption anisotropy of B896* and the high fluorescence polarization of the terminal emitter [7], their transition dipoles would have to be approximately parallel. It was proposed that at 4 K, two to four reaction centers are interconnected by B896 [18]. Based on the stoichiometry of the core antenna (~20 BChl 875 and 4 BChl 896 per reaction center), this would imply that a high degree of order exists in which the reaction center and the B875-896 complex serve as a building block. Thus, the function of B896 may be to concentrate the excitation energy in the vicinity of the reaction center and thereby increase the efficiency of the final energy transfer step to the special pair in the reaction center. Possibly this mechanism is common to all photosynthetic organisms.

Table 1

Summary of isotropic lifetimes and limiting values of anisotropy ($r(0)$ and $r(\infty)$) of B875 at 77 K

Wavelength (nm)	τ_f (ps)	τ_s (ps)	$r(0)$	$r(\infty)$
905	20 (weak)	650 ± 20	0.21	0.21
900	20 ± 5	607 ± 15	0.21	0.18
895	18 ± 3	620 ± 30	0.22	0.18
890	20 ± 5	625 ± 20	0.23	0.16
886	13 ± 1	–	–	–
880	12 ± 3	650 ± 25	0.09	0.08
870	very weak	680 ± 30	0.08	0.08
850	weak	650 ± 25	–	–

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