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4. PHOTOSYSTEM I

ULTRAFAST PROCESSES IN BACTERIAL ANTENNAS STUDIED BY NONLINEAR POLARIZATION SPECTROSCOPY (FREQUENCY DOMAIN)

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1. I NTRODUCTION

The main absorption maxima of bacteriochlorophyll a (Bchla) in the light harvesting antennas (LH) of photosynthesizing bacteria are distinctly red-shifted as compared to the S_0-->S_1 transition of Bchla in solution. In *Rhodobacter sphaeroides* there are three basically different elementary cell-like arrangements of Bchla in the protein matrices, wich built the peripheral parts of LH with absorption maxima at 800 and 850 nm and the core LH (875 nm). Besides this global differentiation there are indications of a substructure (inhomogeneity) for each of these absorption bands, which seem to play in important role in the ultrafast energy transfer through the antennas to the reaction center. To characterize this substructure, up to now absorption and emission spectroscopy in the time domain (with ps/fs pulses) at room and low temperatures is used as well as spectral hole burning at low temperatures (cp. [1, 2, 3]). Recently we have shown [4] that the nonlinear polarization spectroscopy in the frequency domain (NLPF) [5], which was not applied in the field of photosynthesis before, is an additional informative method. It gives insight into band broadening and spectral heterogeneity as well as energy and phase relaxation in the ps/fs region.

In the following we report on NLPF measurements in the B850 region of chromatophores from *Rb. sphaeroides* and discuss the results in comparison with those obtained recently at the isolated B800-850 complex [4].

2. MATERIALS and METHOD

Chromatophores of *Rhodobacter sphaeroides 2.4.1.* (ATCC 17023) were prepared by repeated passage of bacteria through French pressure cell (20 000 Psi), centrifugation of raw cell fragments (1h at 15 000 ·g) and ultracentrifugation of supernatant (1h at 240 000 · g). Chromatophores were washed 3 times with tris-buffer (20 mM tris/HCL pH 8).

Isolated B800-850 antennas were prepared as described elsewhere [4]. For the NLPF measurements samples with $OD_{850} \cong 2$ were used in 0.1 cm sample cells.

The principle of the NLPF method [5], the experimental set-up and the theoretical approach are described in detail elsewhere [4, 6]. In short, nonlinear interaction between a linearly polarized pump beam (frequency ω_1) and an also linearly polarized, but 45° twisted probe beam (ω_2) in the sample creates a new polarization component of the probe beam, which can be detected behind crossed polarizers. We register this NLPF signal under stationary conditions (15 ns-pulses) as a function of the frequency difference between the tuned ω_1 and the fixed ω_2. Two dye lasers ($\delta\lambda \leq 0.2$ cm^{-1}) are used, which are simultaneously pumped by an excimer laser.

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In case of a homogeneously broadened band with band maximum at ω_0 the NLPF signal shows (for $\omega_2 = \omega_0$) two maxima: one at resonance between pump and probe frequency ($\omega_1 = \omega_2$), the second one at resonance between pump frequency and ω_0. Increasing inhomogeneity (increasing spectral width of distribution of the center wavelengths of the homogeneous subbands) diminishes the height of the second maximum; in case of extremely inhomogeneous broadening it is completely absent. The energy and phase relaxation times (T_1, T_2) are obtained by fitting the NLPF signals with the appropriate lineshape functions [7].
T_1 determines mainly the half-width of the signal around $\omega_1 = \omega_2$ whereas T_2 is reflected in the wings of the signal.

3. RESULTS and DISCUSSION

NLPF signals of the chromatophores from *Rb. sphaeroides* were measured at room temperature at several frequencies in the B850 region. A typical result is given in Fig. 1a: With probe frequency ω_2 at 11 634 cm^{-1} (859.5 nm), the NLPF signal for a large ω_1 - scanning range is shown. For comparison, in Fig. 1b the corresponding NLPF signal with the isolated B800-850 complex is shown, registered unter identical conditions.

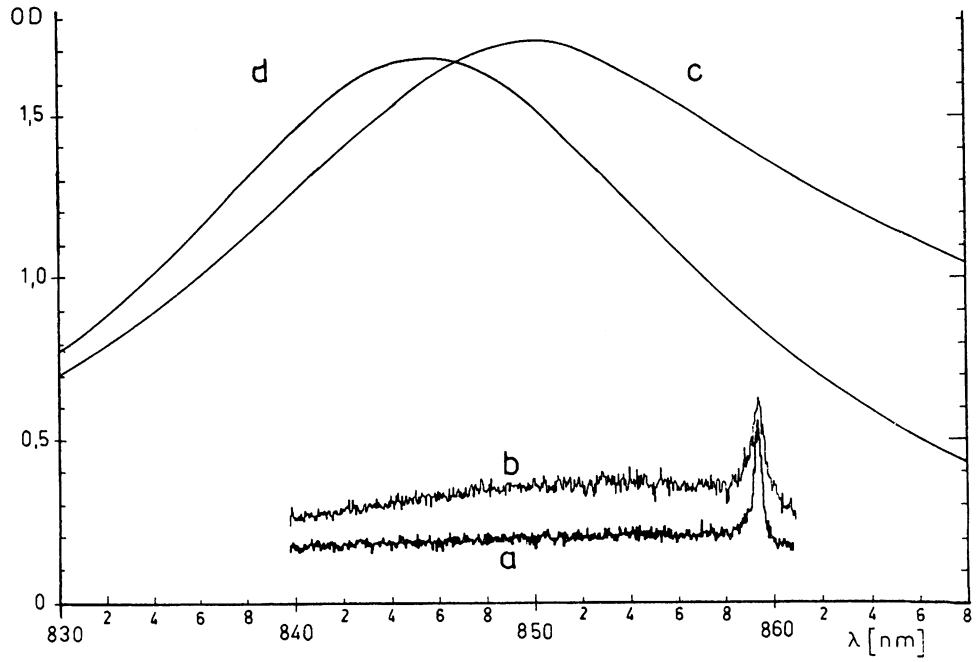

Figure 1 . NLPF signals of chromatophores from *Rb. sphaeroides* (a) and of B800-850 complex from *Rb. sphaeroides*/LDAO (b), both probed at 859.5 nm. Corresponding absorption spectra: chromatophores (c), B800-850 complex (d).

Also shown are the relevant parts of the absorption spectra of both samples. Obviously, the NLPF signal from the chromatophores represents the case of extremely inhomogeneous broadening.

From the NLPF shape the values T_2=40fs and a corresponding homogeneous with of 260 cm^{-1} were obtained. There is little change of these values when changing ω_2 in the B850 region of the chromatophores. This is different from the result with the B800-850 complex, where we have found variations in the homogeneous widths between 30 and 210 cm^{-1} in the also inhomogeneously broadened B850 band, dependent on ω_2 [4]. Whereas hints to heterogeneity of B850 at room temperature came also from time resolved energy transfer [1, 2] and fluorescence quenching measurements [7], Reddy et al [3] have found a homogeneous behaviour with a line width of 220 cm^{-1} for B850 of chromatophores at 4.2 K.

To compare results at these different temperatures, one has to take into consideration, that

i) there is a change in the fwhm of the B850 absorption band of the chromatophores from 280 cm^{-1} (4.2 K) [3] to 550 cm^{-1} (293 K)

ii) there is a change in the number of Bchla 850 per domain from about 30 (4.2 K) to at least several hundreds (293 K) [2].

Measuring NLPF signals with highest spectral resultion around $\omega_1 = \omega_2$ gives information on T_1. At ω_2 = 11 634 cm^{-1} we obtained $T_1 \cong$ 8 ps, which indicates a contribution of exciton annihilation.

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