MINOR COMPONENT B-905 OF LIGHT-HARVESTING ANTENNA IN *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES AND THE MECHANISM OF SINGLET–SINGLET ANNIHILATION AS STUDIED BY DIFFERENCE SELECTIVE PICOSECOND SPECTROSCOPY

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1. Introduction

Primary photophysical events in the photosynthetic apparatus include: (i) transfer of photo-induced excitation along the light-harvesting chlorophyll-like antenna towards the reaction centers (RCs); (ii) conversion of this excitation energy into the energy of separated charges in the RCs [1,2].

Laser studies on excitation transfer along antenna pigments have been performed only with the fluorescence picosecond spectrometers because chromatophores, thylakoids, chloroplasts, and intact cells are characterized by a relatively high fluorescence yield [3–5]. Attempts at employment of absorption-picosecond spectroscopy for this purpose have not yielded any significant results due to difficulties in reliable registration of very small amplitudes of absorbancy changes ($\sim 10^{-2}$–10^{-3}) in those intact preparations.

The progress in picosecond laser spectroscopy in Vilnius State University made it possible to construct the first picosecond instrument for working in the mode of a difference absorption spectrometer with the sensitivity up to 2×10^{-4} in absorbance and time resolution close to 10 ps. Below we report the data on the dynamics of excitation transfer in antenna pigments of *Rh. rubrum* chromatophores obtained by picosecond measurements of the absorption changes occurring due to the conversion of BChl molecules into a singlet excited state.

A minor fraction of pericentral BChl was found, which appears to focus excitations in the nearest proximity of RC. A new insight into the mechanism of non-linear processes (single–singlet annihilation) in the light-harvesting antenna was also advanced.

2. Materials and methods

Chromatophores were isolated from purple non-sulphur photosynthetic bacterium *Rhodospirillum rubrum* according to [6]. In picosecond experiments, chromatophore concentrations were usually chosen in such a way that the absorbance of the sample in a 1 mm cell was $\leqslant 0.5$ unit for the excitation light beam and $\leqslant 1.0$ unit for the probing beam.

Picosecond absorption measurements were carried out with a spectrometer described in [7,8]. This spectrometer allowed one to measure photoinduced absorption changes up to 2×10^{-4} units with the time resolution of $\sim 10^{-11}$ s. The wavelengths of excitation and probing beams are tuned continuously and independently in a 400–1500 nm spectral range. Excitation pulse energy can be varied from 10^{11}–10^{18} photons . cm^{-2} . $pulse^{-1}$.

3. Results

Fig.1. shows the difference spectra of absorption changes in the picosecond time range in *Rh. rubrum* chromatophores induced by exciting picosecond pulses. At high intensity of exciting 920 and 532 nm pulses ($\sim 4 \times 10^{17}$ $h\nu/cm^2$ in a pulse), the difference spectrum exhibited a bleaching band with the max-

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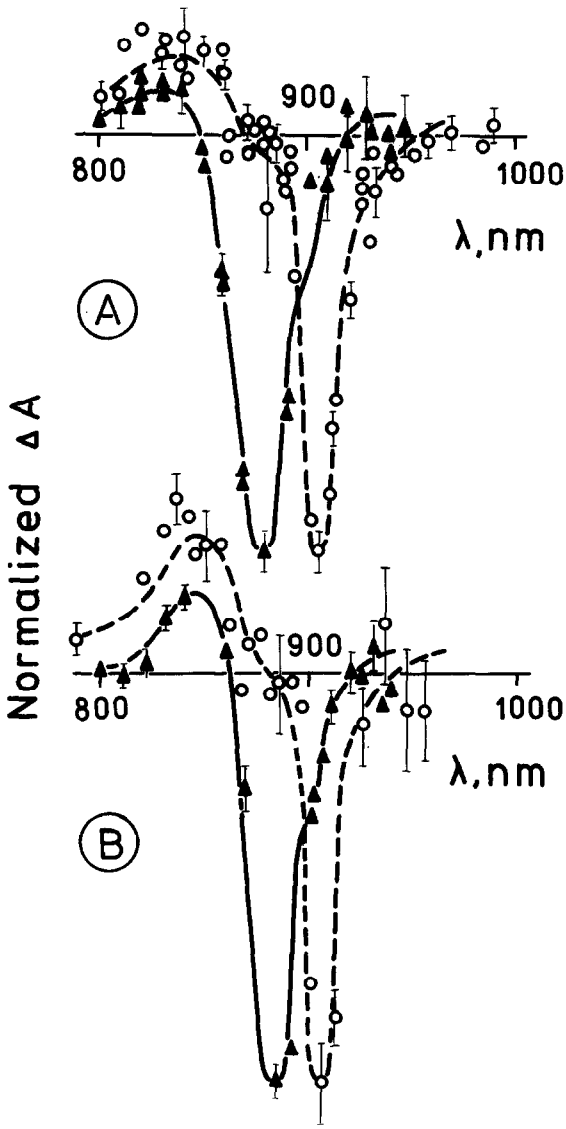

Fig.1.(A) Spectra of photo-induced absorption changes in *Rh. rubrum* chromatophores at high (▲——▲) and low (○——○) intensities of excitation 920 nm light pulses (4×10^{17} and $\leqslant 10^{15}$ photons/cm^2, respectively). The probing pulse coincides in time with the arrival of the excitation 920 nm pulse (duration of both pulses at half-height ~25 ps). Measurements were done in a 1 mm cell at an absorbance of the chromatophore sample of 2.5 units with the maximum of the absorption spectrum at 880 nm and of 0.4 units at 920 nm. Intensity of the probing pulse: $\leqslant 10^{12}$ photons/cm^2. The spectra are normalized to unity in the maxima at 880 and 905 nm, respectively. (B) Same spectra as in fig.1(A), but with 532 nm excitation light pulses. The spectra measured at high (▲——▲) and low (○——○) intensities of the excitation pulses. Absorbance of chromatophores in a 1 mm cell at 532 nm was 0.35 units and at 880 nm ~0.7 units.

imum at 880 nm and the width at half-height ~25–30 nm. The maximum of this band coincides with that of the absorption spectrum of *Rh. rubrum* chromatophores. Contrary to that, at low intensity of the exciting pulses ($\leqslant 10^{16}$ $h\nu$/cm^2), we observed the bleaching of the band with the maximum located at 905 ± 5 nm and the half-width 15–20 nm at the long wavelength end of the chromatophore absorption spectrum. On both sides of this bleaching band increase in absorption was recorded. At the short wavelength end this increase in both cases had the form of a broad band with an indistinct maximum at 840 ± 10 nm. Its magnitude was 10–30% of that of the bleaching band depending on the excitation energy. At the long wavelength side, wide diffuse absorption appeared with the amplitude of ~5% of that of the bleaching band.

The dependences between the amplitudes of the 880 and 905 nm bleaching bands and the excitation energy are given in fig.2. Curve 1 shows the process of bleaching for the 880 nm band. It appears only at exciting pulse energies $\geqslant 10^{16}$ $h\nu$/cm^2, and then grows linearly up to 10^{17} $h\nu$/cm^2. Curves 2 and 3 in the range 10^{11}–10^{16} $h\nu$/cm^2 show the bleaching of the 905 nm band. Its amplitude grows monotonously and reaches the plateau at intensities within 10^{15}–10^{16} $h\nu$/cm^2. Above 10^{16} $h\nu$/cm^2 the spectral changes in the 880 nm band are superimposed onto this band. In the 920 nm region, the differential spectrum contains the zero point changes for high intensity exciting pulses. Probing at this point showed that this plateau starts at ~10^{15} $h\nu$/cm^2 and reaches at least 5×10^{17} $h\nu$/cm^2.

The insertion to fig.2 represents light dependence for 880 and 905 nm bands when exciting 532 nm pulses were used. They do not differ much from the corresponding curves obtained upon excitation of chromatophores at the long wavelength end of the absorption spectrum.

The kinetics of disappearance of the photo-induced changes in the 905 nm band are mono-exponential with the time constant of 60 ± 15 ps and do not depend or depend weakly, on the intensity of exciting pulses. The kinetics of regeneration of optical changes in the 880 nm band consist (at high intensity) of 2 components. The lifetime of the long-lived component within the experimental error coincides with that for the band with maximum at 905 nm. The lifetime of the short-lived component decreases from ~35 ps (10^{16} $h\nu$/cm^2) to $\leqslant$20 ps (4×10^{17} $h\nu$/cm^2). The amplitude of the short-lived component rises as excitation increases.

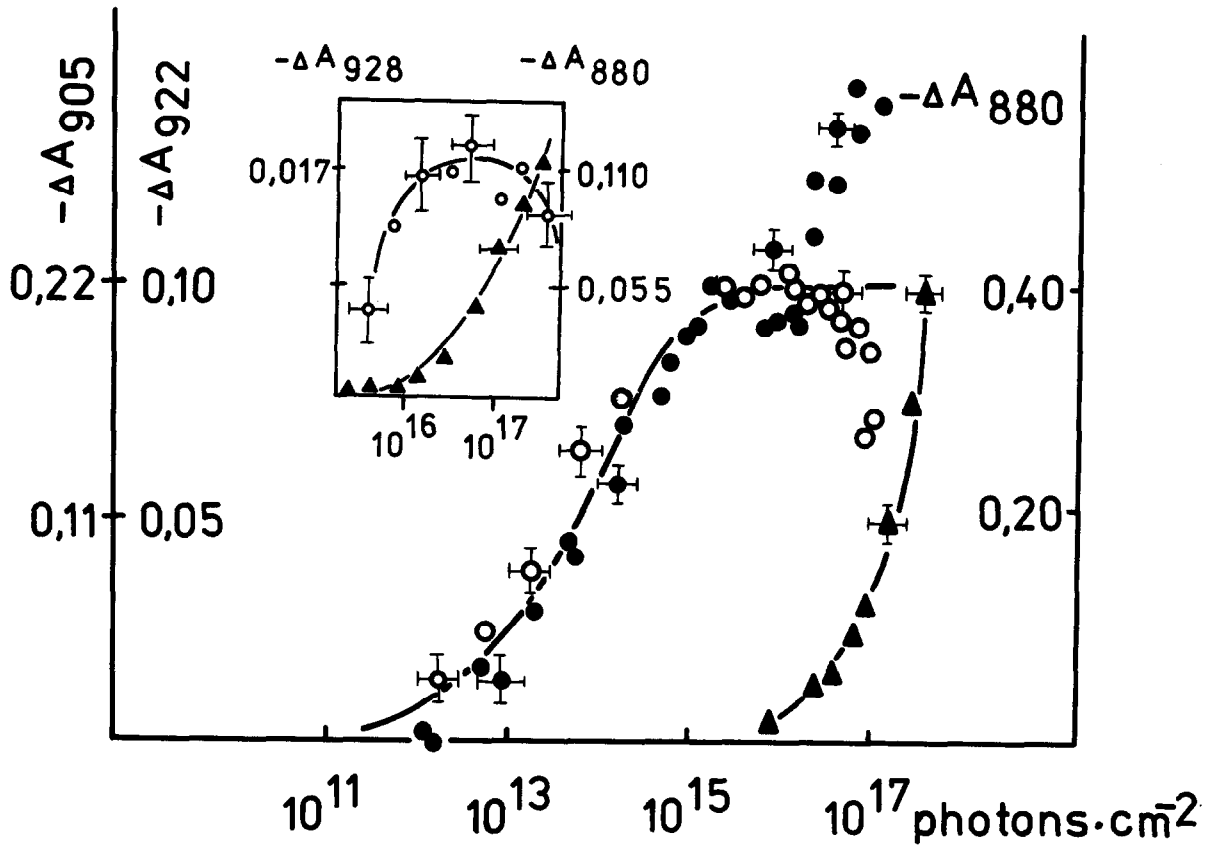

Fig.2. Dependence between photo-induced changes in absorption of *Rh. rubrum* chromatophores and the intensities of excitation light pulses. Probing and excitation light pulses coincide (zero time delay). Light curves are normalized to unity; for each curve its own ordinate scale is given: curve 1 (▲——▲) was obtained at λ_{ex} = 905 nm, λ_{pr} = 880 nm; curve 2 (●——●) at λ_{ex} = 905 nm, λ_{pr} = 905 nm; curve 3 (○——○) at λ_{ex} = 890 nm and λ_{pr} = 922 nm. Absorbance of chromatophore preparation in a 1 mm cell was ~1 units at 890 nm. In the insertion, dependence between photo-induced changes in chromatophore absorption and the intensity of excitation of 532 nm light pulses are given. Absorbance of the preparation in 1 mm cell in the course of these measurements was 0.9 units at 532 nm.

4. Discussion

The above results could be interpreted as follows: The light-harvesting antenna of the chromatophores from purple bacterium *Rh. rubrum* is made up of two components. The predominant component B880 has the bleaching peak at 880 nm (fig.1), which coincides with long wavelength band of *Rh. rubrum* chromatophores absorption spectrum (not shown). When a portion of its molecules converts into the first singlet excited state either due to direct absorption of light (excitation at 920 nm) or due to energy migration from carotinoids or deactivation of its higher excited state (excitation at 532 nm), a characteristic bleaching band appears in the difference spectrum at 880 nm. At low energies of the excitation pulse its lifetime apparently reflects the rate of energy migration from this component.

Besides, there is a minor component of the antenna BChl, B905, whose bleaching peak in the differential spectrum localizes at 905 nm. It can readily accept electronic excitations from B880.

The number of BChl molecules belonging to B905 can be estimated. Upon various treatments and association of pigment molecules their most conservative parameter is $\int\Sigma(\nu)d\nu$ which changes by $\leqslant$20% [9]. Consequently, we may simply compare those integrals for our minor fraction, i.e., the square under the B905 bleaching band upon its saturation at high excitation energies with that for B880 bulk BChl. This

prompts us to estimate the portion of B905 as 3–5 BChl molecules/RC. We believe this fraction to form only one tightly-bound complex containing all the 3–5 molecules.

Detection of the B905 minor component in the light-harvesting antenna of *Rh. rubrum* chromatophores makes it possible to detail and develop the idea about non-linear processes of singlet annihilation observed in the antenna upon laser excitation [10]. The most important fact is the absence of the non-linear dependence between optical changes and the intensity of excitation pulses in the difference spectrum of B905. This proves that all the BChl molecules of B905 form a condensed strongly interacting ensemble apparently a single pigment–protein complex, and their excitation leads to formation of a collective excited state. Contrary to this, in the major component of the light-harvesting antenna, B880, a number of excited states may exist simultaneously; they will interact with excitation in B905 and with each other according to the following schema:

$$S^1_{905} + S^1_{880} \rightarrow S^n_{905} + S^o_{880} \rightarrow S^1_{905} + S^o_{880} + \text{heat} \quad (1)$$

$$S^1_{880} + S^1_{880} \rightarrow S^n_{880} + S^o_{880} \rightarrow S^1_{880} + S^o_{880} + \text{heat} \quad (2)$$

where S^o, S^1 and S^n denote the ground, first and higher singlet states of BChl molecules, respectively. When S^1_{905} and S^1_{880} interact, the pigment–protein complex B905 will almost always remain in the excited state since the energy of S^1_{905} is lower than that of S^1_{880}. It is noteworthy that the singlet–singlet annihilation processes in the intensity range up to 10^{16} $h\nu/cm^2$ follow eq. (1), whereas at excitation intensities $>10^{16}$ $h\nu/cm^2$ it is the processes described by eq. (2) that are predominant.

Some minor antenna components shifted towards the long wavelength region with respect to the absorption band of the RCs have been discussed in investigations on plant photosynthesis (components with fluorescence maxima at 730–740 nm enhancing at nitrogen temperatures [11,12]). It is accepted that photo-induced excited states begin to leak to these forms at low temperatures due to inactivation of RCs. However, our experimental data allow us to make a different conclusion (at least for purple bacteria): singlet excited states transfer rapidly from the main antenna B880 to the minor pericentral form B905, and only from the latter do they enter RC. At first glance, this seems to be senseless since in B905 a portion of electronic energy (~55 meV) is lost, and the excitation energy becomes lower than that of the absorption band of the RC dimer P870. However, we believe that this is no problem since entropy is gained here: the excited state localizes in the vicinity to RC. It increases the possibility of its being entrapped by RC significantly.

The energetic and kinetic characteristics of this entrapment will be reported later.

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