The study of excitation transfer between light-harvesting antenna and reaction center in chromatophores from purple bacterium *Rhodospirillum rubrum* by selective picosecond spectroscopy

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1. INTRODUCTION

The primary photophysical processes of photosynthesis are usually subdivided into:

(i) Delivery of excitation from light-harvesting antenna pigments to reaction centers (RCs);

(ii) Conversion of excitation in RC into separated charges.

A portion of excitations (1–10%) is usually lost en route to RCs giving rise to prompt fluorescence of antenna pigments. According to currently accepted ideas the time course of this fluorescence must reflect the kinetics of excitation delivery to RCs and the risetime of RC photooxidation. On this basis, the first picosecond studies of photosynthetic objects were carried out [1–4]. Later it was demonstrated that efficient biexcitonic quenching may be involved in picosecond experiments with powerful laser pulses (see reviews [5,6]), which may obscure considerably the real kinetics of fluorescence decay. Besides, a portion of what was usually considered as fluorescence may just be short-lived delayed emissions [7–9]. The third difficulty is due to the fact that some spectral forms of antenna chlorophylls exhibit fluorescence with a negligible yield and lifetime [10,11]. All these difficulties called for using the absorbance laser methods for monitoring the excitation transfer in antenna.

The difference picosecond laser spectrometer of Vilnius State University [12,13] is adequate now for these studies. This work reports the first picosecond absorption data on:

(i) Transfer and disappearance of excitations from light-harvesting antenna;

(ii) Subsequent appearance of RC oxidation in the native chromatophores, obtained with this spectrometer.

2. MATERIALS AND METHODS

The chromatophores were isolated from the purple non-sulphur photosynthetic bacterium *Rhodospirillum rubrum* as in [14]. For picosecond measurements chromatophore preparations were diluted so that the absorbance of the sample in a 1 mm cuvette at the excitation wavelength (in 890–920 nm range) was $\leq 0.5\ A$/unit.

Picosecond absorption was measured with a difference spectrometer described in [12,13]. This spectrometer allows one to measure photo-induced absorbance changes down to $2 \times 10^{-4}\ A$ unit with the time resolution of $\sim 10^{-11}$ s. The wavelengths of excitation and probing beams are tuned continuously and independently in the 400–1500 nm spectral range. Excitation pulse energy can be varied from 10^{11}–10^{18} photons . cm^{-2} . pulse^{-1}.

3. RESULTS AND DISCUSSION

The P870 photooxidation in *Rh. rubrum* chromatophores with picosecond laser pulses was monitored by the P800 band shift, characteristic of the primary charge separation in reaction centers. The corresponding kinetic of absorbance changes at 810 nm (in P800 band) induced by 900 nm laser pulses is shown in fig.1, curve 1). It reflects:

(i) The appearance and subsequent disappearance

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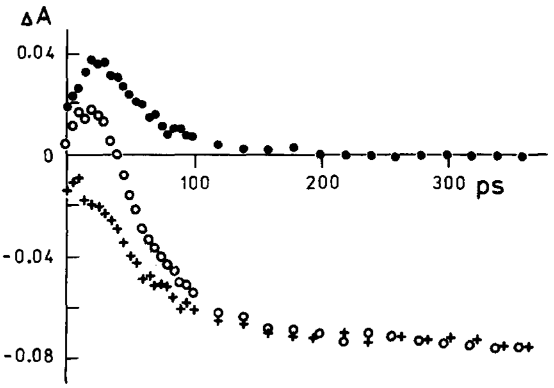

Fig.1. Kinetics of absorbance changes of *Rh. rubrum* chromatophores induced by 900 nm picosecond exciting pulses: curve 1 (○) recorded at 810 nm; curve 2 (●) obtained near 800 nm in the isobestic point for P800 band shift of RCs; curve 3 (+) calculated by the point-by-point subtraction of curve 2 from curve 1. Energy of excitation was $\sim 5 \times 10^{13}$ photons.cm^{-2}.pulse^{-1}, which corresponds to oxidation of $\sim 1/3$ RCs in chromatophores (see fig.3, curve 3)

of excitations in antenna pigments, which manifest themselves as an absorption increase in this spectral range;

(ii) The shift of P800 band due to the primary separation of charges in RCs, which in turn results in some bleaching.

To separate the superimposed signals, the kinetics were recorded at 802 nm in the isobestic point of P800 band shift (curve 2). The kinetic at 802 nm exhibits a very fast absorption increase ($< < 10^{-11}$ s), and this increase relaxes in the time interval commensurable with the duration of laser pulse used.

The spectra of absorbance changes (fig.2) suggest that the first component of curve 1 (fig.1) (transient absorption increase) should be attributed to the excitation of antenna molecules. Consequently, the remaining portion of the absorbance changes at 810 nm (long-lived bleaching) may be attributed to the shift of P800 band induced by charge separation in RCs. This conclusion was confirmed by similar kinetic experiments with either a portion or all RCs preoxidized by continuous light or chemical substances – a proportional decrease or complete elimination of the long-lived bleaching was observed. Contrary to this, the kinetics of antenna bacteriochlorophylls (curve 2 of first component of curve 1, fig.1) were apparently the same when chromatophore RCs were in the reduced or oxidized states. But the peak height of curve 2 or of first component of curve 1 rose as did the increase of excitation pulse energy which also sped up its relaxation.

Some kinetics were recorded at 795 nm. They also contained both signals, but at this wavelength the first component (the short-lived absorption increase due to antenna excitation) turn into some long-lived intermediate level of increased absorption due to P800 band instead of long-lived bleaching at 810 nm. This long-lived level was also shown to be proportional to the portion of active RCs and vanished if they were preoxidized.

To separate the net kinetics of RC oxidation (curve 3, fig.1) (via P800 band shift) we subtracted point-by-point curve 2 (the signal of photoexcited antenna molecules) from curve 1 (the signal of photoexcited antenna molecules plus the signal of P800 band shift). In additional experiments the antenna signal was demonstrated to be the same within some 5–10% in 800–810 nm spectral range (see fig.2).

For moderate picosecond excitation ($< 10^{15}$ photons . cm^{-2} . pulse^{-1}), the rates of RC photooxidation in chromatophores (i.e., excitation transfer plus RC oxidation itself) and that of decay

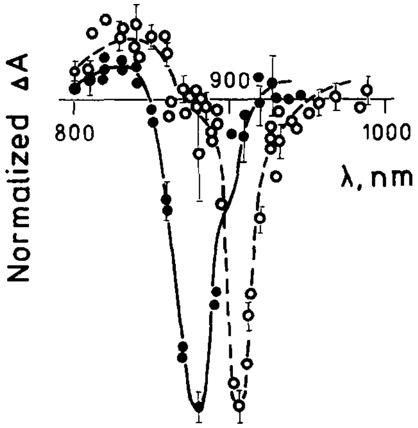

Fig.2. Difference spectra of absorbance changes of *Rh. rubrum* chromatophores, induced by 920 nm excitation pulses with high (●) and low (○) energies (10^{17} and 10^{15} photons.cm^{-2}.pulse^{-1}, respectively). The probing pulse coincided in time with the exciting pulse in both cases.

of photoexcited molecules of antenna bacteriochlorophyll were the same within experimental error and equal to (in monoexponential approximation):

$$\tau_{\text{RC oxidation}} = \tau_{\text{excitation decay}} = 60 \pm 15 \text{ ps}$$

For high pulse energies ($> 10^{17}$ photons$\cdot$cm$^{-2}\cdot$pulse^{-1}), the risetime $\tau_{\text{RC oxidation}}$ had the same value, but the decay time for singlet excitations in antenna bacteriochlorophylls decreased to the level under our time resolution:

$$\tau_{\text{RC oxidation}} = 60 \pm 15 \text{ ps}$$

$$\tau_{\text{excitation decay}} < 20 \text{ ps}$$

Fig.2 shows the spectra of absorbance changes induced by picosecond laser monopulses in chromatophores of *Rh. rubrum*. One can see a drastic difference in the position of bleaching peaks brought about by week and strong pulses. We suggest [15] that the antenna system of *Rh. rubrum* contains a small fraction (~ 5%) of bacteriochlorophyll with a long wavelength absorption band maximum at ~ 905 nm (spectral form B905. The remaining bulk of pigment comprises the well-known B880 form. The B905 minor fraction acts as a trap for singlet excitations in the B880. This trapping appears to occur via inductive resonance in a time interval considerably shorter than the duration of our laser pulse, i.e., ≤ 25 ps. That is why only B905 form exhibits bleaching in the case of weak excitation pulses. Fig.3 shows the light dependences for the amplitudes of B905 and B880 bleaching, as well as for RC photooxidation (again via the P800 spectral shift). One can see that RC photooxidation correlates linearly with the amplitude of B905 bleaching. The bleaching of B880 starts only after saturation of the B905 band bleaching and RC photooxidation. This seems to mean that each B905 complex of molecules, on having received one excitation cannot have others induced in B880 by a strong excitation pulse. Besides the excitation transfer from B880 to B905 molecules there are two quenching processes of $S^1_{B880} + S^1_{B880}$ and $S^1_{B880} + S^1_{B905}$ types [15], where S^1 is the first singlet excited state. But at intensities of ~ 10^{16} photons.cm^{-2}.pulse^{-1} all these channels of B880 deactivation become overloaded. Therefore the concentration of excitations in B880 increases drastically which develops itself into the profound bleaching of 880 nm band.

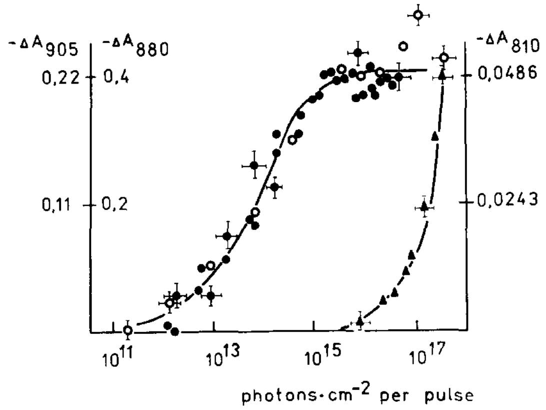

Fig.3. Dependence of absorbance changes of *Rh. rubrum* chromatophores on the energy of 900 nm excitation pulses (light curves): curve 1 (▲) measured at 880 nm; curve 2 (●) measured at 905 nm; probing pulse coincided in time with exciting pulse in both cases; curve 3 (○), corresponding to P800 band shift due to RC oxidation, was recorded at 810 nm with 450 ps time delay for probing pulse relative to exciting pulse.

The above experimental data lead us to the following conclusions:

(1) The longer wavelength position of the B905 band as compared to that of the $(P870)_2$ dimer appears to be due to strong interaction of more than two BChl molecules. This interaction may be not only of dipole–dipole but also of exchange type at a very short distance. It may well be that, say, 3–5 BChl molecules/RC form such a tightly bound complex [15].

(2) These B905 complexes are located in the immediate proximity of RCs. The fact of the identical decay times of the excitation population in B905 and the risetime of RC photooxidation proves that RC traps excitations from B905 which thus acts as an efficient coupling arrangement between the bulk of antenna pigment and RCs. It must be fluorescence of the B905 form that is observed around 910–915 nm with a lifetime of $\tau_{\text{flu}} = 50$ ps [1] approximately equal to the above mentioned decay and risetime.

(3) The energy gap formed between B880 and B905 amounts to 39 meV which means that the downhill and uphill migration rates relate as 5:1. But bearing in mind that in *Rh. rubrum* we have some 50–80 B880 molecules/3–5 B905 molecules, we arrive at the conclusion that the ratio of the quasi steady state concentrations in B880 and B905 should be proportional to 3:1 (in favour of B880) provided these forms can freely exchange their excitations via an inductive resonance mechanism. But this is not the case. The bulk of excitations is localized in B905 (fig.2, weak excitation). To explain this discrepancy, we suggest that the strong electron-exchange interaction in B905 complexes leads to formation in them of singlet excitations mixed with charge transfer complexes (like in associations of dye molecules). This phenomenon eliminates the backward transfer of excitation quanta to the B880 bulk pigment.

The energy scheme of the primary processes is shown in fig.4. The solid arrow represents a very fast ($\leq$ 5 ps) excitation migration from the B880 antenna to the B905 complexes leading to formation of the mixed state

$$\left\{\begin{matrix} B905^* \\ B905^+ B905^- \end{matrix}\right\}$$

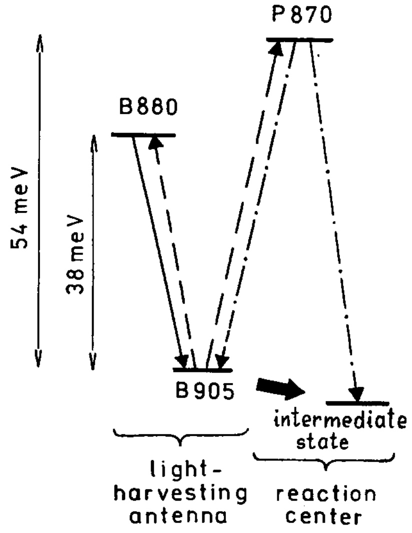

Fig.4. Energy scheme for energy transfer between light-harvesting antenna and reaction center in *Rh. rubrum* chromatophores (details in text).

The dashed arrows represent the excitation migration from B905 back to B880 and to the RC dimer $(P870)_2$ via an inductive resonance mechanism. One can see that the energy gap between B905 and $(P870)_2$ ($\sim$ 55 meV) must reduce this rate constant $\sim$ 10-fold at room temperature and much stronger at low temperatures. But we do not believe that this mechanism plays the key role in the energy transfer from B905 to $(P870)_2$ of RC.

We suggest the 'tunnelling of charge transfer state' from the above-mentioned B905 mixed state (presumably, a charge transfer complex) just to some state of RC with separated charges or with charge transfer complex without formation of $(P870)_2$ singlet excited state. This process is indicated in fig.4 by a thick arrow. In this case the energy transfer would require no activation energy, because the corresponding energy levels are nearly coincident with those of *Rh. rubrum*.

Now we are investigating the temperature dependence of RC photooxidation in chromatophores in order to discriminate between two alternatives in energy transfer from B905 to RCs.

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