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Efficiency of excitation trapping by reaction centres of complex B890 from *Chromatium minutissimum*

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The formation and decay of excited states of light-harvesting bacteriochlorophyll (BChl) molecules and primary charge separation in reaction centres (RCs) of pigment-protein complex B890 of the purple bacterium *Chromatium minutissimum* were studied as a function of excitation wavelength by means of picosecond absorbance difference spectroscopy. Selective excitation in the RC absorption band (at 800 nm) induced rapid bleaching of the absorption band of the RC BChl dimer ($\tau \sim 1$ ps) without absorption changes due to excited light-harvesting BChl. It is concluded that excitation energy transfer from the BChl dimer of the RC to light-harvesting BChl molecules is considerably slower than RC charge separation. Therefore, energy transfer from the light-harvesting antenna to the RC in bacterial photosynthesis may be described as a 'migration-limited model'.

Energy transfer; Excitation trapping; Picosecond spectroscopy; Bacterial photosynthesis; Complex B890; (*Chromatium minutissimum*)

1. INTRODUCTION

The primary physical processes of photosynthesis may be divided into two groups: (i) energy transfer via light-harvesting bacteriochlorophyll (BChl) molecules to the reaction centre (RC) and (ii) charge separation in the RC. The two theoretical models in the literature describe the interaction between light-harvesting BChl and RCs during light energy transformation in photosynthesis. According to the first model, RCs display about 100% efficiency of excitation trapping (the 'migration-limited model'); while according to the other, the excitation trapping ability of RC is low ('the trap-limited model') (reviews [1–4]). At present, it is generally accepted [1–4] that excitation trapping by RCs is a limiting stage of bacterial photosynthesis primary processes. However, experimental evidence on these two models is rather sparse [5,6].

Laser picosecond absorption difference spectroscopy with selective excitation and probing enables us to determine through direct measurements which of these two models is implemented in bacterial photosynthesis. This is the subject of the present paper.

2. MATERIALS AND METHODS

The pigment-protein complex B890 was isolated from the purple photosynthetic bacterium *Chromatium minutissimum* according to a modified method of Moskalenko and Erokhin [7]. Samples were suspended in 0.05 M Tris-HCl buffer (pH 8.0). The reduced state of RC of complex B890 was obtained by addition of sodium ascorbate. The sample of complex B890 in solution consists of separated particles, each containing a single RC and about 30 light-harvesting BChl molecules. The absorption spectrum of complex B890 is shown in fig.1. All measurements were carried out at room temperature.

Picosecond absorbance difference measurements were performed with a laser spectrometer described in [8,9]. The apparatus allows one to measure photoinduced absorption changes down to $\sim 10^{-4}$ *A* units. The wavelengths of excitation

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Abbreviations: BChl, bacteriochlorophyll; RC, reaction centre

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and probe pulses are tuned continuously and independently over the spectral range 400–1500 nm. Excitation energy can be varied over the range 10^{11}–10^{17} photons/cm in a pulse.

Kinetic curves were simulated by assuming (i) a Gaussian form for picosecond excitation and probe pulses (FWHM = 18 ps), (ii) the absence of non-linear processes and (iii) monoexponential kinetics of energy transfer from the antenna to the RC or within RC pigments with 100% efficiency. The 0 ps mark on the time scale corresponds to coincident excitation and probe pulses.

3. RESULTS AND DISCUSSION

Previously we [10] and others [11,12] have shown that excitation transfer to the BChl dimer (P870) via RC pigments occurs in the subpicosecond time domain. However, in cell samples as well as in chromatophores of purple bacteria, the excitation energy transfer from antenna molecules to active ('open') RCs and charge separation in RC occur with a time constant $\tau \simeq 60$ ps at room temperature [13,14]. The about 100-fold difference between the time constants of excitation delivery to the BChl dimer of RC via these two excitation transfer pathways is one of the premises of our experiments. Indeed, if the migration-limited model is valid, then selective excitation of RC pigments in chromatophores and cells will result in (i) the absence of signals due to excitation of antenna molecules and (ii) significantly faster kinetics of bleaching of the RC dimer band because of dimer excitation and subsequent charge separation than after selective excitation of antenna molecules. However, if the trap-limited model is valid then the amplitudes of antenna signals and kinetics of RC dimer bleaching will be of the same order for both types of excitation.

The difference spectra of absorption changes of complex B890 are shown in fig.1. The first (closed circles) is determined by excitation of antenna molecules, whereas the other (open circles) is by charge separation in RCs. One observes that excitation of antenna molecules is followed by an absorption increase at 860 nm, and charge separation in RC results in bleaching at this wavelength. The kinetic curves for absorption changes of complex B890 at a wavelength of 860 nm, measured with selective excitation in the antenna absorption band (at 930 nm) and in the RC absorption band (at 800 nm), are shown in fig.2.

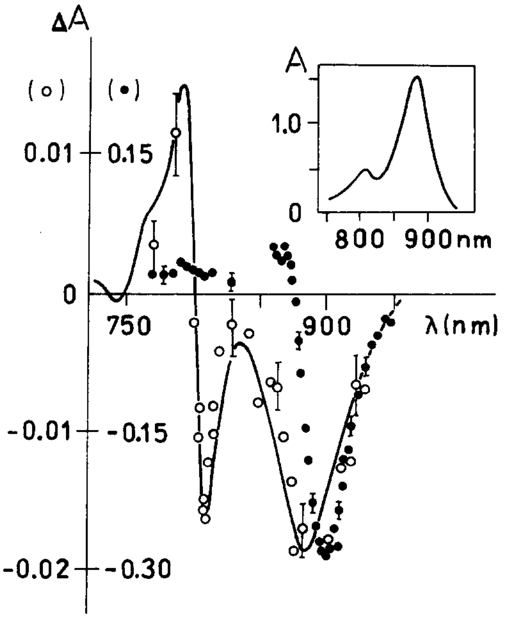

Fig.1. Absorbance difference spectra of complex B890 at 0 ps (closed circles) and 500 ps (open circles) after the excitation flash. The absorption of the sample was about 1.5 at 885 nm in a 1 mm cell (about 0.5 at 840 nm). The intensity of the 840-nm excitation pulses corresponded to absorption of about one light quantum per B890 complex in a pulse. Solid line: absorbance difference spectrum of RC photooxidation ('light-minus-dark') of complex B890, measured in continuous light. Inset: absorption spectrum of complex B890.

The intensity of picosecond excitation pulses was selected so that both curves reached the same final level, i.e. the same quantities of RCs were oxidized in the excited volume of the sample cell. Independent measurements with a calibrated photodiode show the absorption of equal quantities of light quanta at both wavelengths. It should be noted that the intensities used for the excitation pulses were very low – charge separation being registered in about 10% of the total number of RCs in the excited volume of the sample cell. This corresponds to the absorption of about 0.1 light quantum per B890 complex (i.e. per 'domain') in a pulse, since complex B890 is monocentral. Such excitation intensities practically eliminate nonlinear processes of excitation annihilation in the antenna of complex B890.

As seen from fig.2, the signal of excited antenna molecules was significantly lower for excitation in the absorption band of RC monomer BChl (experimental curve shown by closed squares) than for excitation in the antenna band. Moreover, the

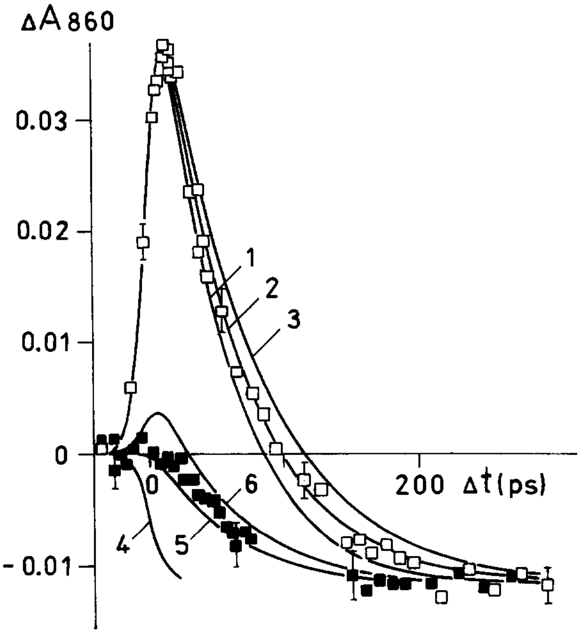

Fig.2. Absorbance change kinetics in complex B890 at 860 nm for excitation in the light-harvesting BChl absorption band at 930 nm (open squares) and in the monomer BChl absorption band of RC at 800 nm (closed squares). The theoretical curves 1–6 (continuous lines) are described in the text. Absorption of the complex B890 was 2.43 at 885 nm in a 1-mm cell (0.15 at 930; 0.75 at 800 nm; 0.95 at 860 nm).

absorption band of the RC BChl dimer bleached significantly more rapidly during excitation in the RC absorption band. Charge separation was registered in 70–80% of the RCs being oxidized at a time when the experimental curve for excitation in the antenna absorption band (open squares) crossed the zero line. Excitation at 800 nm cannot ensure complete excitation selectivity of the RC pigment. About 1/4 of the 800-nm light quanta at the wavelength of 800 nm must be absorbed by antenna molecules (see fig.1). For this reason, it may be suggested that the antenna signal component of the experimental curve (closed squares) results from direct excitation of antenna molecules despite excitation energy transfer from RC pigments to antenna pigments.

The theoretical curves 1–6 are represented by continuous lines. Curves 1–3 are plotted for excitation energy transfer from the antenna to RC for τ = 50, 60 and 70 ps, respectively. The experimental curve (open squares) is approximated well by curve 2 with τ = 60 ps. Curve 4 corresponds to bleaching of the RC dimer absorption band with τ = 1 ps. Curves 5 and 6 are sums of curves 2 (τ = 60 ps) and 4 (τ = 1 ps) with coefficients α and $(1 - \alpha)$ for $\alpha = 0.2$ and 0.3, respectively. The points for the experimental curve (closed squares) correspond approximately to a theoretical curve with $\alpha \simeq 0.25$ (not shown).

It may be concluded that excitation of the RC monomer BChl in the complex B890 results in efficient RC charge separation ($\geqslant$80–90%) and $\tau \sim$ 1 ps. It has been estimated that no more than 10–20% of the excitation is able to migrate from RC to the antenna.

It is usually assumed in theoretical calculations for purple bacteria that the RC BChl dimer is spectrally indistinguishable from antenna molecules, i.e. the energy transfer constants between antenna BChl molecules, from the antenna BChl to the RC, and from the RC to antenna BChl molecules, are the same. If we denote the constant of charge separation as K_e and the constant of energy migration as K_m, the above result may take the form of

$$K_e^{-1} \lll K_m^{-1}, \qquad (1)$$

which corresponds to the migration-limited model.

The value of K_e^{-1}, according to absorbance measurements with femtosecond time resolution, is within 2.5–4 ps [12,15]. The value of K_m^{-1} is not measured directly during the experiments for excitation energy transfer between homogeneous BChl molecules in the light-harvesting antenna. However, it is assumed that $K_m^{-1} \simeq 2/n$ ps, where n is the number of neighbouring molecules to which the energy may be transferred [1,3]. Therefore, it is assumed in the literature that

$$K_e^{-1} \geqslant K_m^{-1} \text{ or more } K_e^{-1} \gg K_m^{-1}$$

(the model of trap limited migration). From our inequality (eqn 1) it follows that (i) there is a rapid, preceding stage with a lifetime of the order of 10–100 fs in RC charge separation if $K_m^{-1} = 2/n$ ps or (ii) excitation migration in the antenna is slow ($K_m^{-1} \geqslant$ 10–20 ps) if K_e^{-1} = 2.5–4 ps. In the latter case antenna BChl molecules ought to be clustered in groups (several molecules in each) in a monolayer around RC.

Some experimental evidence has been obtained for both hypotheses. 'Hole-burning' measurements have provided estimates for the primary photochemical stage in charge separation processes (probably in the RC BChl dimer) of the order of tens and hundreds of femtoseconds [16,17]. On the other hand, the light-harvesting antenna of *Rhodopseudomonas viridis* [18], *Ecothiorhodospira halochloris* [19] and probably the long-wavelength part of the *Chromatium minutissimum* antenna (complex B890) consist of 6 pigment-protein complexes, which surround the RC in a ring-shaped monolayer; each of these complexes contains 4–6 BChls. Energy transfer between BChl molecules within a single complex proceeds with $\tau < 1$ ps and that between complexes with $\tau > 10$ ps [20].

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