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Determination of the Quantum Yields of the Primary Photosynthesis Events and the Photosynthetic Unit Types in Purple Bacteria

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Summary

1. Photoinduced changes in absorbancy and fluorescence yields were studied in *Chromatium minutissium* and *Ectothiorhodospira Shaposhnikovii* suspensions. These parameters were examined as functions of light intensity in the region of 750–950 nm under aerobic and anaerobic conditions.

2. The fluorescence was shown to consist of two spectrally indistinguishable emissions: "photosynthetic" emission with a yield correlating with the state of reaction centres (P_{890}) and "background" emission with constant yield.

3. The "photosynthetic" fluorescence dependence on the portion of nonactive P_{890} was consistent with the multicentral (statistic) model of photosynthetic unit organization.

4. In anaerobic conditions the amplitude of the photoinduced absorbancy increase around 910 nm was proportional to the portion of nonactive P_{890}.

5. A precise method for determinating the quantum yield of the primary electron donation was proposed. For *Chr. minutissimum* and *E. shaposhnikovii* under aerobic conditions these yields were found to be equal to $0{\cdot}91 \pm 0{\cdot}02$ and $0{\cdot}93 \pm 0{\cdot}02$ respectively.

Introduction

Vredenberg and Duysens[1] have shown for *Rhodospirillum rubrum* that the inverse value of fluorescence yield (φ_f) depends linearly on the portion of photooxidized P_{865}. Similar results were obtained with green photosynthetic bacterium *Chloropseudomonas ethilica*[2] and purple bacteria *Chromatium D.* and *Rh. spheroides.*[3] Clayton[3] and Borisov[4] have shown these results to be consistent with multicentral type of photosynthetic units (PSU). But according to the data of Clayton[3] experimental evidence favours this model only under aerobic conditions. Instead of P_{890} photo-bleaching an absorbancy increases were observed around 850 and 890 nm under anaerobic conditions for some purple bacteria.[5–6] These types of photoinduced absorbancy changes (Δ o.d.) disappeared if the redox potential of the medium was increased.[7]

Vredenberg and Amesz associated these Δ o.d. with the shifts of light harvesting bacteriochlorophyll bands B850 and B890. They believe conformational changes in protein-pigment complex (not associated with primary photosynthetic events) to be responsible for these shifts. On the contrary, Clayton[7] suggested that for *Rh. spheroides*

the absorbancy increase around 870 nm is due to bacteriochlorophyll photoreduction. Cusanovich *et al.*[8] suggested that these Δ o.d. manifest the functionating of the reaction centers P_{905} different from P_{890}.

This paper deals with some aspects of above-mentioned questions and also with precise determinations of quantum yields of the primary electron donation in photosynthesis.

Methods

Three-to-five-day-old cultures of *Chromatium minutissimum* and *Ectothiorhodospira shaposhnikovii* cells grown in modified Larsen's medium[9] were used in the experiments. The cells were grown anaerobically at 30°C under an illuminance of 10^3 lux. Both aerobic and anaerobic cell suspensions were used in the experiments. Cell suspensions

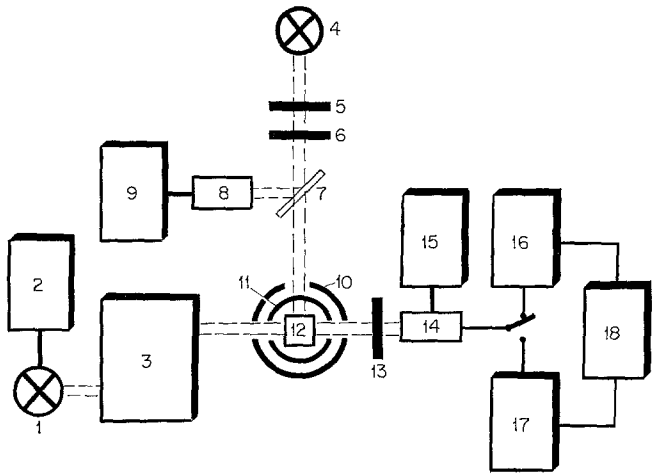

Figure 1. Block diagram of complex optical instrument (see text for descriptions).

were aerated just before experiments by blowing air through the medium for 15 min. Anaerobious was achieved by incubation of cell suspensions under vaseline oil for 1 h.

Photo-induced changes in absorbancy and relative fluorescence yield were examined with a complex optical instrument shown in Fig. 1. This single-beam type instrument allows one to measure in the region of 400–1000 nm the following parameters: (a) transmittance spectra; (b) photoinduced Δ o.d. with a sensitivity up to $3 \cdot 10^{-4}$ optical density; (c) relative fluorescence yields; (d) relative delayed emission yields in the time region from $5 \cdot 10^{-4}$ sec to 10^{-1} sec.

The light beam from tungsten lamp (1) fitted by stabilized power supply (2) is filtered by a diffraction-type monochromator (3). Then monochromatic light is directed onto the measuring cuvette (12) through holes in rotating modulator (11). Exciting light from powerful (500 W) tungsten lamp (4) is filtered with optical (5) and interference (6) filters. In our experiments these filters transmit light around 597 nm with a bandpass of 8 nm. The minor part of exciting light is reflected by a glass plate (7) onto the calibrated photocell (8). The photocurrent is amplified by photometer (9). The measuring and

exciting beams never fall on the sample simultaneously thus preventing the scattered exciting light from getting on the cathode of the photomultiplier (14), fitted from stabilized power supply (15). In our experiments cesiumoxide type of photomultiplier was used. Photocurrent changes associated with light-induced Δ o.d. were amplified by amplifier (16) and registrated with a pen-recorder (18). Prompt and delayed emissions were excited by lamp (4).

The modulating cylinder (11) was dismounted when fluorescence yields were measured. In this type of experiment an optical filter was put in front of the photomultiplier cathode in order to protect it from scattered exciting light. The rotating modulator frequency could be changed from 10^3 to 10^4 r.p.m. thus permitting the decay kinetics examinations of delayed emissions. Respective photocurrents were amplified by a special amplifier (17) supplied with synchronous detector and recorded with (18).

Results and Discussion

The photoinduced Δ o.d. spectra for *Ectothiorhodospira Shaposhnikovii* and *Chromatium minutissimum* are shown in Fig. 2A and 2B. Under aerobic conditions (curves 1) the spectrum for *E. Shaposhnikovii* shows negative maxima centered at 812^-, 850^-, 892^- nm and a positive maximum—at 792^+ nm (+ and − hereafter denote the sign of respective Δ o.d. bands); similar peaks for *Chr. minutissimum* are—810^-, 845^-, 894^- and 792^+. Such spectra were observed and discussed in a great number of papers. It is currently accepted that long-wave negative peaks are associated with photoinduced (or chemoinduced) P_{890} bleaching. Two short-wave peaks (positive and negative) manifest a slight shift in the absorption band of the P_{800} bacteriochlorophyll fraction, which seems to be closely associated with P_{890}. The Δ o.d. spectra under anaerobic conditions (curves 2 in Fig. 2A and 2B) are greatly different from those discussed above. The peaks in the photoinduced Δ o.d. spectra are located at 848^-, 775^+, 860^+, 912^+ nm for *E. Shaposhnikovii* and at 810^-, 844^-, 783^+, 866^+, 906^+ nm for *Chr. minutissimum*. It is evident from energy considerations that fluorescence compete with P_{890} for the pool of photoinduced electronic excited states. This idea was confirmed qualitatively in a number of papers. In this work the φ_f was observed to increase (Fig. 3) as the P_{890} become photobleached thus decreasing the efficiency of primary electron donation to the photosynthetic electron transport chain. For *E. Shaposhnikovii* and *Chr. minutissimum* these increases were usually 3–4 fold. The φ_f dependences on the portion of oxidized P_{890} were derived from Fig. 3. In Fig. 4A and 4B the data are shown for *E. Shaposhnikovii* and *Chr. minutissimum* in aerobiosis. According to Vredenberg and Duysens,[1] Sybesma and Vredenberg,[2] Clayton,[3] and Borisov *et al.*[10] the primary electron donating process in the photosynthesis of purple bacteria is mediated by singlet type electronic excited states. It can be easily shown that regardless of the PSU type φ_f must increase $1 + \varphi_e/\varphi_\Sigma$ times when primary electron donation is blocked. There φ_e and φ_Σ are quantum yields of primary electron donation and overall energy losses in the state when active photosynthesis is involved. According to this formula $\varphi_e = 0{\cdot}75$ and $0{\cdot}7$ for *E. Shaposhnikovii* and *Chr. minutissimum*. But the model proposed first by Vredenberg and Duysens[1] predicts very definite (hyperbolic) dependence of the φ_Σ value (and also the φ_f value if we assume that $\varphi_f/\varphi_\Sigma =$ const) on a portion of photooxidized $P_{890}(P^+/P + P^+)$. The theoretic curves are shown in Fig. 4A and 4B (solid curves 1). They are substantially aside from experimental

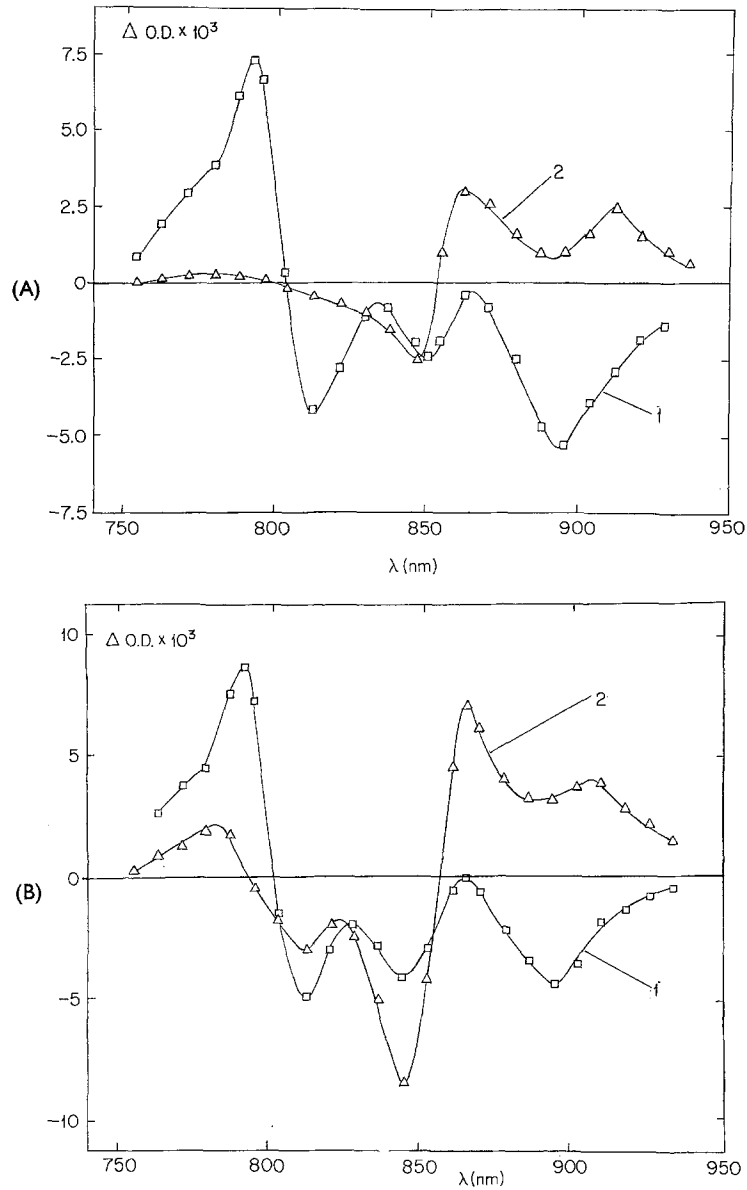

Figure 2. Differential absorbancy spectra (light-dark type). A. For *E. Shaposhnikovii*; B. For *Chr. minutissimum* 1. aerobiosis; 2. anaerobiosis. Exciting light: $\lambda = 597$ nm, $\Delta\lambda = 8$ nm, Exciting light intensity = $6{,}2 \cdot 10^3$ erg/cm^2.sec. Optical densities at 895 nm, 0·5 and 0·6 for *E. Shaposhnikovii* and *Chr. minutissimum* respectively.

points. But we have noticed that experimental data fit the above mentioned model if the zero point on the ordinate axis is shifted up. We have made an assumption that fluorescence consists of two spectrally indistinguishable emissions: the "background" one with a constant yield and the "photosynthetic" one with φ_f depending on the redox state of P_{890}.

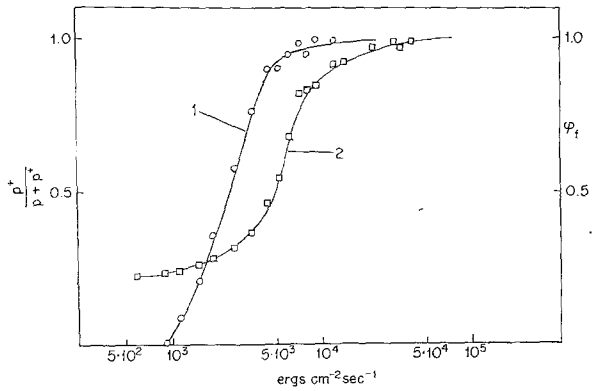

Figure 3. Fluorescence yield of BChl and P_{890} photobleaching as functions of exciting light intensities for *E. Shaposhnikovii* under aerobic conditions. 1. Normalized P_{890} (peak at 892 nm) photobleaching. 2. Relative fluorescence yield. Exciting light: $\lambda = 597$ nm, $\Delta\lambda = 8$ nm.

We tried to determine the background level (dashed curves in Fig. 4A and 4B) so as to obtain the best coincidence of experimental and theoretic data. It is seen in Fig. 4A and 4B that experimental points for *E. Shaposhnikovii* lay between the theoretical curves corresponding to the φ_e values 0·91 and 0·95 and for *Chr. minutissimum*—between the curves corresponding to the φ_e values 0·89 and 0·92. Notice how much deviate the curves corresponding to φ_e values differing from one another only by 3–4%. It is evident that such precise φ_e determination cannot be achieved by measuring the rates of P_{890} or cytochromes photooxidation. Such precision is the result of the fact that the theoretic curves depend on the φ_Σ values. Hence, the closer φ_Σ to zero ($\varphi_\Sigma = 1 - \varphi_e$) the more precisely φ_e may be determined.

For determination of the real values of φ_e *in vivo* it was necessary to study the nature of

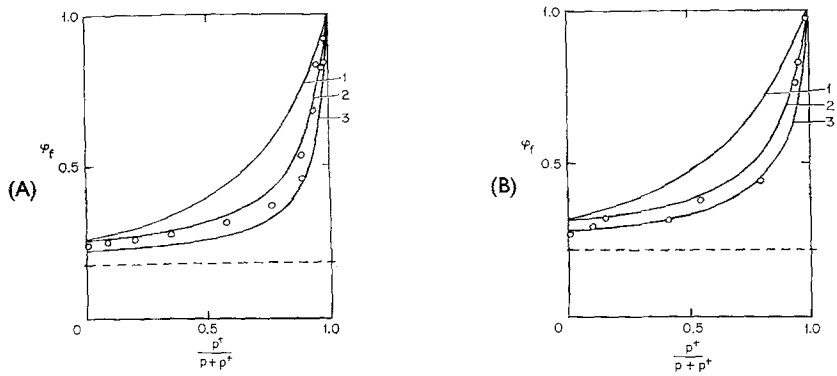

Figure 4. Fluorescence yield dependence on the portion of photooxidized reaction centres in aerobiosis. A. for *E. Shaposhnikovii*. 1. Theoretical curve for 0·75 quantum yield of P_{890} photooxidation related to abscissa axis. 2 and 3. New theoretical curves for 0·91 and 0·95 φ_e values related to background level (dashed curve). B. for *Chr. minutissimum*. 1. Theoretical curve for 0·70 quantum yield of P_{890} photooxidation related to abscissa level. 2 and 3. Theoretical curves for 0·89 and 0·92 φ_e values related to background level (dashed curve).

the background emission. The experiments with phase fluorometer performed in our group[11] revealed it to be of prompt fluorescence type. Combining the data on the fluorescence lifetime and yield dependences on exciting light intensities made it possible to appreciate the portion of electronic excited states migrating to the background bacteriochlorophyll molecule to be $\cong 15\%$ in the case of *E. Shaposhnikovii*.[11] For *Chr. minutissimum* this portion is equal to $\cong 10\%$.

The P_{890} photobleaching does not take place under anaerobic conditions. But photobleaching (oxidation) is not the only way to make the P_{890} inactive. Photosynthesis saturation may occur with P_{890} in normal (not oxidized) state, if these molecules are unable to give electrons away (e.g., in the case of strongly reduced primary electron acceptor).

Such type of photosynthesis saturation seems to be more physiological and natural as

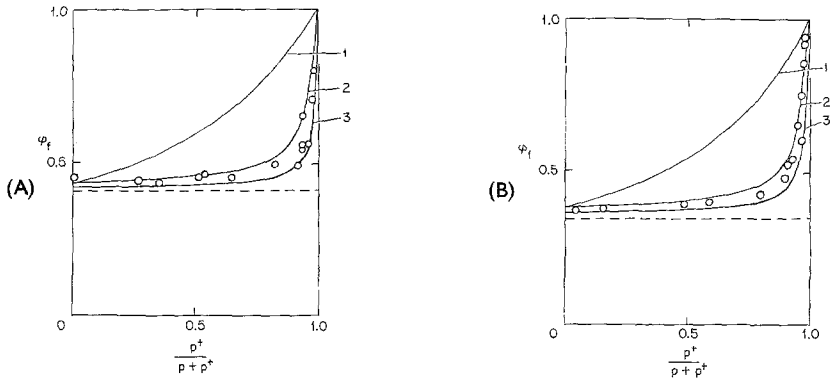

Figure 5. Fluorescence yield dependence on the amplitude of photoinduced Δ o.d. around 910 nm under anaerobic conditions. A. *E. Shaposhnikovii* Δ o.d. at 912 nm. 1. Theoretical curve for 0·58 φ_e value related to abscissa level. 2 and 3. Theoretical curves for 0·968 and 0·988 φ_e values related to background level (dashed curve). B. for *Chr. minutissimum* Δ o.d. at 906 nm. 1. Theoretical curve for 0·63 φ_e value related to abscissa level emission. 2 and 3. Theoretical curves for 0·953 and 0·982 φ_e values related to background level (dashed curve).

compared to aerobiosis. We have also made an attempt to find a correlation between the amplitude of photoinduced Δ o.d. and the portion of nonactive reaction centres under anaerobic conditions. Light dependences (similar to those in Fig. 3) of Δ o.d. and φ_f were studied. Then φ_f dependences on Δ o.d. amplitude were derived as described above. These results are shown in Fig. 5. In these cases after subtracting the background emissions (dashed curves) experimental points also lye on theoretical hyperbolic curves.

This fact leads us to the conclusion that the amplitudes of photoinduced Δ o.d. around 910 nm are proportional to the portion of nonactive P_{890}. The above data do not necessarily indicate that Δ o.d. are associated with P_{890} molecules. These absorption changes may reflect spectral changes in neighbouring BChl molecules, correlating with the nonactive state of P_{890}. The maximal values of φ_e calculated from the data shown in Fig. 5 are a somewhat higher ($\varphi_e \cong 0{\cdot}97$) than those derived from Fig. 4. These φ_e correspond to the φ_Σ values $\cong 3\%$ twice (or even thrice) as small as those calculated for aerobic conditions ($\varphi_\Sigma \cong 7$–9%).

But this fact may be easily explained if we assume that a considerable portion of P_{890}

(50–67%) is oxidized by oxygen in darkness. Thus, under anaerobic conditions maximal φ_e ($0{\cdot}97 \pm 0{\cdot}01$) is realized and $\varphi_e/\varphi_\Sigma \cong 30$–$35$.

Our preliminary data obtained with aerobic suspensions of *Rhodopseudomonas spheroides* also confirmed the ideas about φ_f hyperbolic dependence on the portion of oxidized reaction centres and about high values of $\varphi_e \cong 0{\cdot}90 \pm 0{\cdot}02$.

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