SHORT COMMUNICATIONS

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Photosynthetic electron transport induced by flashing light in the purple photosynthetic bacterium *Rhodospirillum rubrum*

In previous publications[1,2] evidence was presented for two separate electron transport chains driven by two distinct light reactions in the purple photosynthetic bacterium *Rhodospirillum rubrum*. One light reaction is the oxidation of a reaction center consisting of specialized bacteriochlorophyll molecules, P890, and a subsequent oxidation of a cytochrome C428. The other light reaction induces a cyclic electron transport in which another cytochrome, cytochrome c_2, participates. The reaction center for this reaction consists of P890 molecules, which in the reduced form, interact with another form of bacteriochlorophyll, P800 (ref. 3). The cessation of this interaction upon oxidation of the P890 molecules in the reaction center causes a blue shift of the 805-nm absorption band. The evidence resulted from experiments in which light-induced absorbance changes and action spectra of such changes were measured with continuous actinic light. Since in such experiments the observed light-induced absorbance changes include contributions of more than one pigmented reactant, the interpretation of the time courses becomes difficult and is not always unique. If the sample is submitted to a series of flashes separated by dark times which are in the order of the photosynthetic time constant, the importance of this complication can be diminished substantially[5,6].

The operation of a set up for such experiments[5] is illustrated in Fig. 1. The sample is illuminated by a series of actinic flashes and by two alternating measuring

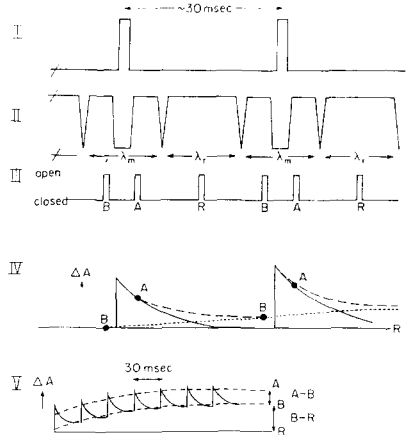

Fig. 1. Illustration of the operation of an apparatus for the measurement of repetitive flash-induced absorbance changes. Explanation of symbols is in the text.

beams, one at a wavelength λ_m at which the absorbance change is to be measured and the other at some "inert" reference wavelength λ_r. A rotating wheel selects the flashes (Trace I, Fig. 1). The same wheel screens the photomultiplier when a flash is on. A vibrating mirror which operates synchronously with the wheel alternates the measuring light from the measuring wavelength, λ_m, to the reference wavelength, λ_r, and *vice versa*. The signal seen by the photomultiplier thus can be represented schematically by Trace II, Fig. 1. The photomultiplier is connected to a gating circuit, the operation of which is illustrated in Trace III, Fig. 1. Signals transmitted through the gating circuit are samples of the absorption a short time before each flash (B), the absorption a short time after each flash (A), and the absorption at an "inert" reference wavelength (R). The B signal is subtracted from the A signal and the R signal is subtracted from the B signal. The A—B and B—R difference signals are amplified separately and displayed on two recorders. Reaction components which cycle faster than the dark time between the two flashes (in this set up about 30 msec), but slower than the time between the flash and the A signal (about 1.5 msec), will show up in the A—B measurement, while reaction components which cycle slower than the dark time between the flashes will show up in the B—R measurement (Traces IV and V, Fig. 1).

This communication reports a few measurements made with *R. rubrum* with the described technique. The bacteria were grown as described previously[1]. The culture bottles were rotated in front of an incandescent light source, thus ensuring homogeneous illumination of the cells in the cultures during growth. Samples of 1-, 2-, and 3-day-old cultures, suspended in the original culture medium, were used. The temperature in most of the measurements was 30°, equal to the temperature at which the organisms were grown.

Fig. 2 shows the A—B and B—R signals as a function of the measuring wavelength in the Soret region induced by white flashes of moderate intensity. The A—B signal showed the reduced-*minus*-oxidized spectrum of apparently only cytochrome c_2 (refs. 1 and 2), demonstrating a turnover time of this cytochrome less than 30 msec. The B—R signal had the reduced-*minus*-oxidized spectrum of cytochrome C428 (refs. 1 and 2) showing that this cytochrome accumulated in the oxidized form in

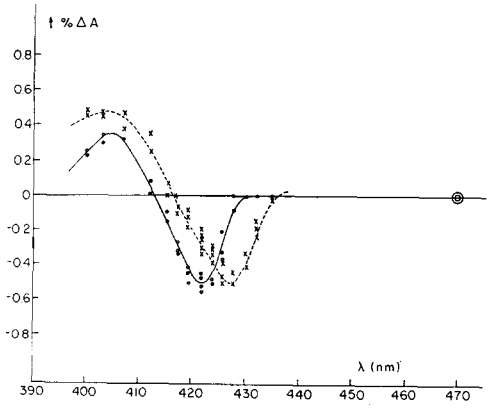

Fig. 2. Spectra of repetitive flash-induced absorbance changes in *R. rubrum*. ●—●, A—B measurement; ×---×, B—R measurement; λ_r = 470 nm. Explanation of symbols is in the text.

the flashing light. At higher intensities of the flashes, the spectrum of the originally negative A—B signal became positive with a broad maximum at about 435 nm.

In the far red spectral region, the spectrum of the A—B signal, induced by moderate intensities of white flashes showed a bleaching of an absorption band at 883 nm and a blue shift of an absorption band at about 805 nm (*cf.* ref. 4). The spectrum of the B—R signal showed a predominant bleaching centered at 883 nm and in most cases no or only small changes around 800 nm.

The effect of the wavelength of the actinic flashes was investigated at different intensities for both the bleaching at 883 nm and the 805-nm blue shift, measured as the increase of absorbance of 795 nm. Fig. 3 illustrates the results of such experiments.

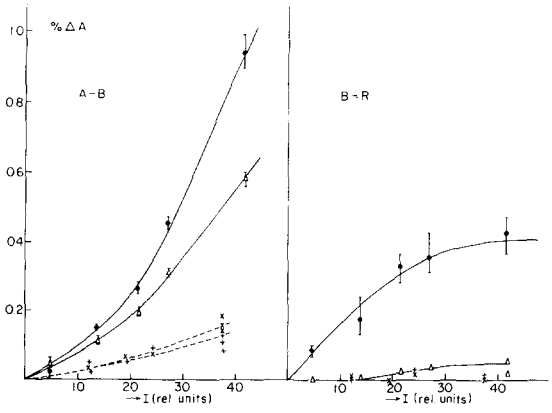

Fig. 3. Repetitive flash-induced absorbance changes in the A—B measurement (left side) and B—R measurement (right side) as a function of the flash intensity in *R. rubrum*. ●—●, decrease in absorbance at 883 nm, induced by 880-nm flashes; △—△, increase in absorbance at 795 nm induced by 880-nm flashes; ×---×, decrease in absorbance at 883 nm induced by 800-nm flashes; +---+, increase in absorbance at 795 nm induced by 800-nm flashes. λ_r = 700 nm. Explanation of symbols is in the text.

The A—B signals (left side) and the B—R signals (right side) are plotted as a function of the incident intensity of the flashes. With 880-nm flashes the B—R signals followed a normal saturation curve for the 883-nm bleaching and very little change in absorbance at 795 nm; the A—B signals showed sigmoid curves for both the 883-nm bleaching and the 795-nm absorbance increase. Flashes at 800 nm gave no signal at all in the B—R measurement, while in the A—B measurement both the 883-nm bleaching and the 795-nm increase of absorbance showed up in a ratio of 0.2–0.3 of the same absorbance changes measured with 880-nm flashes. Saturation is not reached in the A—B measurements for the maximum intensities used. Measured with an Ulbricht integrating sphere, the absorption at 800 nm was 32% of the absorption at 880 nm.

The present results can be considered as a direct demonstration of the occurrence of two light-driven reactions in *R. rubrum*, and thus as an independent confirmation of the conclusions made earlier[1,2]. One reaction causes an accumulation of oxidized P890 and oxidized cytochrome C428 in the flashing light (the B—R measurements). In this reaction the P890 is not aggregated with the bacteriochlorophyll type P800, as demonstrated by the lack of the blue shift around 800 nm. Actinic flashes of

800 nm do not induce the reaction at all. The rate-limiting step in this reaction apparently is the re-reduction of cytochrome C428 by the substrate. The other reaction is a relatively rapid turnover of both P890 and cytochrome c_2. The P890 participating in this reaction is coupled to P800, as demonstrated by the blue shift of the 800-nm absorption band in the A—B measurement. This reaction is induced by flashes at 880 nm as well as at 800 nm. The sigmoid character of the light curve for this reaction indicates a cycle. The results are qualitatively consistent with the action spectra for the oxidation of the cytochromes c_2 and C428 induced by continuous actinic light[1].

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Department of Physiology and Biophysics, University of Illinois, Urbana, Ill. 61801 (U.S.A.) and
CHRISTIAAN SYBESMA

Research Institute for Advanced Studies, Baltimore, Md. 21227 (U.S.A.)
BESSEL KOK

1 C. SYBESMA AND C. F. FOWLER, *Proc. Natl. Acad. Sci. U.S.*, 61 (1968) 1343.
2 C. SYBESMA, *Biochim. Biophys. Acta*, 172 (1969) 177.
3 K. SAUER, E. A. DRATZ AND L. COYNE, *Proc. Natl. Acad. Sci. U.S.*, 61 (1968) 17.
4 R. K. CLAYTON, *Photochem. Photobiol.*, 1 (1962) 201.
5 B. KOK, *Plant Physiol.*, 34 (1959) 184.
6 B. KOK, B. COOPER AND L. YANG, in *Microalgae and Photosynthetic Bacteria*, special issue of *Plant Cell Physiol.*, (1963) 373.

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