Biochimica et Biophysica Acta, 357 (1974) 365–369

BBA 46782

NEW TYPE OF DELAYED LIGHT EMISSION FROM PHOTOSYNTHETIC BACTERIA

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(Received April 8th, 1974)

SUMMARY

A new type of short-wavelength delayed light emission from the photosynthetic bacteria *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* was found. The emission and action spectra suggest that magnesium protoporphyrin IX or a similar compound is the emitter. Anaerobic conditions were necessary to detect this long-lived emission. Some inhibitors of the primary processes of photosynthesis affected the intensity and the decay rate of the delayed light emission.

INTRODUCTION

Arnold and Thompson [1] reported in 1956 that *Rhodospirillum rubrum* emitted light long after the cessation of pre-illumination. The wavelength of the emitted light was in the same wavelength region as that of the fluorescence of bacteriochlorophyll in vivo. Some investigators have studied delayed light emission of the same type in purple photosynthetic bacteria [2–6]. In the present paper we report another component of delayed light emission from purple bacteria with a half-time of 20–40 ms, from a pigment which is different from bacteriochlorophyll.

EXPERIMENTAL AND DISCUSSION

Rhodospirillum rubrum and *Rhodopseudomonas spheroides* were cultured semi-anaerobically under incandescent light at 25–30 °C for 4 or 5 days in a medium containing 0.2 % casamino acids and 0.3 % yeast extract. The pH of the medium was adjusted to 7.3–7.5 with NaOH. After harvesting by centrifugation, the cells were washed with and resuspended in 60 mM phosphate buffer (pH 7.5) containing 0.25 M sucrose.

Delayed light emission was measured with the phosphoroscope described earlier [7]. Actinic light was provided by a high-pressure mercury lamp or a halogen tungsten lamp. The intensity of the actinic beam at the sample position was measured by a calibrated thermopile. The actinic light was chopped by a rotating sector.

Abbreviation: CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

Usually, the actinic beam was passed through two optical filters; Toshiba V-V 42 and Corning 9782. This combination of filters passes light of approx. 370–500 nm. An EMI 9558C photomultiplier (tri-alkali type; spectral sensitivity, S20) covered with a Wratten 22 filter, which passes wavelengths longer than 560 nm, was used to detect the emission from the sample. The photomultiplier was covered during the illumination period by the rotating sector used for chopping the actinic beam to be protected from the strong actinic light and fluorescence. The signal during the measuring period was amplified and recorded on a storage oscilloscope.

When the sample was under aerobic conditions the delayed light emission was not detectable. Therefore, the cell suspension was kept anaerobic by adding glucose oxidase (Sigma Chemical Co.) and glucose at concentrations of 0.3 mg/ml and 70 mM, respectively, or by replacing air by argon gas in a Thunberg-type cuvette. When the cells were densely suspended the delayed light emission could be observed after several minutes without these special procedures because anaerobiosis was achieved by respiration. The emission disappeared when air was re-introduced into the bacterial suspension.

A typical oscilloscopic display of the time course of delayed light emission from the anaerobic cells of *R. rubrum* is shown in Fig. 1. The decay of the emission followed approximately first-order kinetics (see Fig. 5) with a half-time of 20–40 ms.

For the measurement of the emission spectrum light emitted from the sample was passed through a monochromator. Correction was made for the sensitivity of the combination of photomultiplier, filter, and monochromator.

The intensity of the emission at 10 ms after the cessation of illumination was

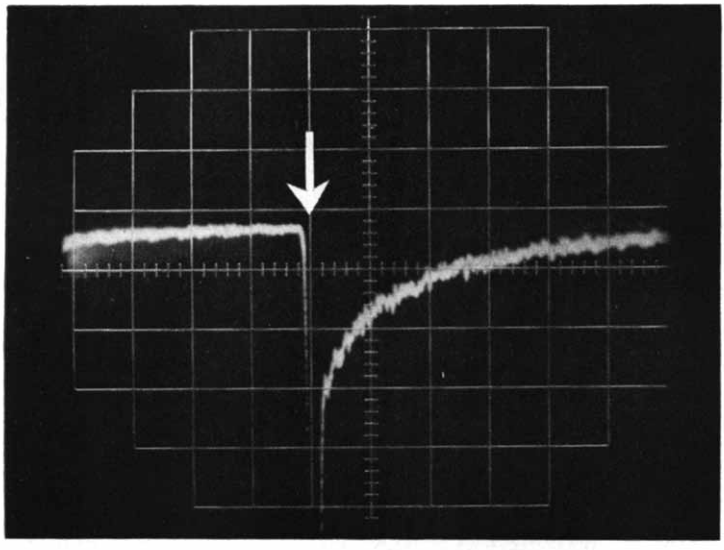
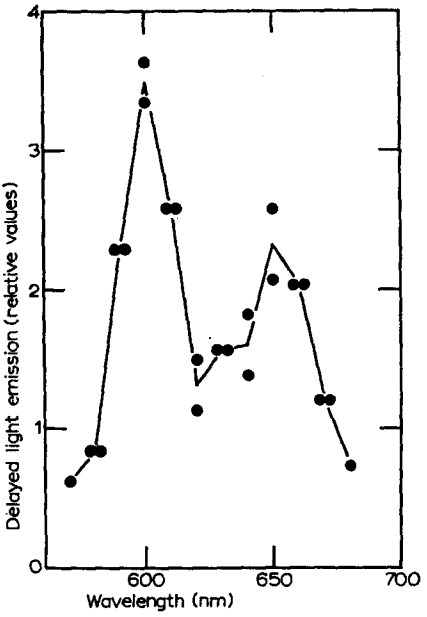


Fig. 1. Time course of the delayed light emission from *R. rubrum*. Cells were suspended in 60 mM phosphate buffer (pH 7.5) containing 0.25 M sucrose. The bacteriochlorophyll concentration of the sample was 5.6 μM. Air was replaced by argon gas. The sample was illuminated intermittently by blue light at an intensity of about $1.2 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. The arrow indicates the time of cessation of illumination. The downward deflection corresponds to the increase of intensity of emitted light. Oscilloscope settings: 20 ms/div.; 20 mV/div.



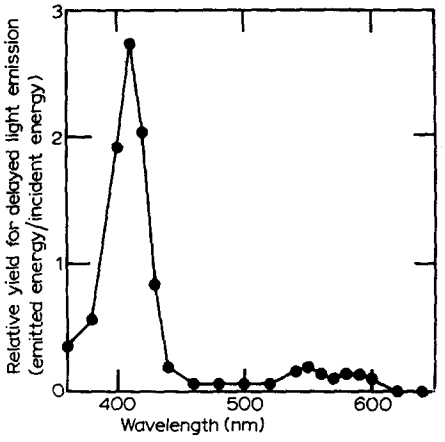


Fig. 2. Spectrum of delayed light emission from *R. rubrum*. Cells were suspended in 60 mM phosphate buffer (pH 7.5) containing 0.25 M sucrose. The bacteriochlorophyll concentration of the sample was 0.34 μM. Glucose oxidase and glucose were added at concentrations of 0.3 mg/ml and 70 mM, respectively. The sample was illuminated by blue light at an intensity of $1.0 \cdot 10^5$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. The slit width of the monochromator corresponded to 19.2 nm. Correction was made for the sensitivity of the combination of photomultiplier, monochromator and filter.

Fig. 3. Action spectrum for the delayed light emission from *R. rubrum*. Cells were suspended in 60 mM phosphate buffer (pH 7.5) containing 0.25 M sucrose. The bacteriochlorophyll concentration of the sample was 1.9 μM. Air was replaced by argon gas. Emitted light with wavelengths longer than 570 nm was detected. The slit width of the monochromator for excitation corresponded to 19.2 nm.

plotted against the wavelength to construct the emission spectrum (Fig. 2). At the bacteriochlorophyll concentration used (0.34 μM), the effect of reabsorption of the emitted light was not significant. The spectrum showed two peaks at about 600 and 650 nm. The emission from bacteriochlorophyll could not be detected, probably due to the very low sensitivity of the photomultiplier in the near-infrared region.

Fig. 3 shows the action spectrum for the delayed light emission. A monochromatic actinic beam was obtained by passing the light from a halogen tungsten lamp through a monochromator and an appropriate cut-off filter. The intensity of the actinic beam was maintained between 100 and 600 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1} using Toshiba neutral density filters. The magnitude of the observed signal (emission longer than 570 nm) was linear with the intensity of the actinic beam at all wavelengths tested. The size of the signal divided by the intensity of the actinic light was plotted against the wavelength of the actinic light. The action spectrum showed a main peak at about 410 nm and two smaller peaks at 550 and 590 nm.

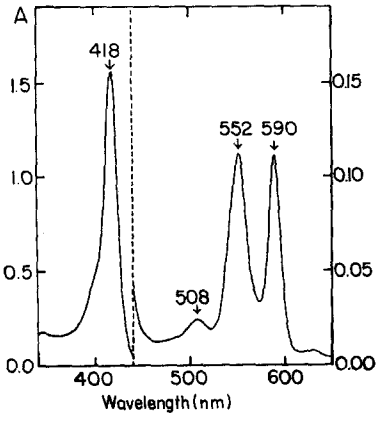
A similar delayed light emission was observed in chromatophore preparations. Cells of *R. rubrum* were broken in a French pressure cell at 200 kg $\cdot$ cm^{-2} and centrifuged at 10 000 $\times g$ for 20 min. The supernatant was centrifuged at 80 000 $\times g$

for 1 h and the precipitate obtained was resuspended in the same buffer. Anaerobic conditions were also necessary to detect the long-lived emission. The lifetime of the emission from chromatophores was similar to that from intact cells.

Cells of *R. spheroides* also emitted delayed light of the same type. Its emission and action spectra were the same as those of *R. rubrum*.

The action spectrum in Fig. 3 resembles the absorption spectrum of magnesium protoporphyrin IX [8]. This suggests that the delayed light emission of the type reported here is emitted from magnesium protoporphyrin IX or a similar compound. Magnesium protoporphyrin IX is known to be a precursor in the biosynthesis of bacteriochlorophyll [9, 10]. Jones [9] found that magnesium protoporphyrin monomethyl ester was secreted into the culture medium of *R. spheroides*. Iron deficiency in the culture medium increased the amount of magnesium protoporphyrin monomethyl ester secreted [9]. Although we did not check the iron deficiency in our culture medium, magnesium protoporphyrin IX or a similar compound was always found in the acetone–methanol (7 : 2, v/v) extracts of *R. rubrum* and *R. spheroides* cells. Magnesium protoporphyrin IX in the extracts was transferred into ethyl ether by partition. The concentrated ether solution was spotted onto a silica gel plate and was developed with a *n*-hexane–benzene–acetone–methanol–water (130 : 70 : 50 : 20 : 2, by vol.) mixture. The fluorescent purple spot was extracted with ethanol from silica gel. The isolated substance had the same absorption spectrum as that of magnesium protoporphyrin IX [8] (Fig. 4). The fluorescence spectrum of the isolated substance was the same as that of the delayed light emission from bacterial cells (not shown here). Different amounts of magnesium protoporphyrin were found in cells depending on the bacterial species, and length and conditions of culture.

The delayed light emission of the type reported here was affected by certain



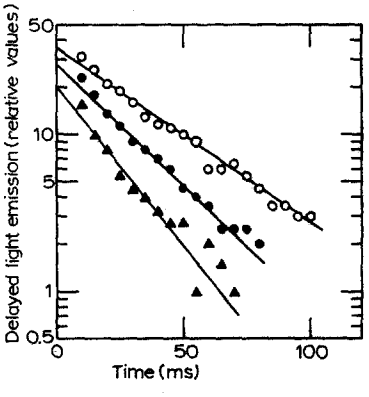


Fig. 4. Absorption spectrum of the pigment isolated from *R. spheroides* (ethanol solution). The absorbance scale is 10 times magnified on the right side.

Fig. 5. Effect of CCCP on the time course of delayed light emission from *R. rubrum* chromatophores. Chromatophores were suspended in 60 mM phosphate buffer (pH 7.5) containing 0.25 M sucrose. Glucose oxidase and glucose were added at concentrations of 0.3 mg/ml and 70 mM, respectively. Air was replaced by argon gas. CCCP was added as ethanol solution. The sample was illuminated by blue light at an intensity of about $1.2 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. ○, control; ●, 2 μM CCCP; ▲, 10 μM CCCP.

inhibitors of photosynthesis such as carbonylcyanide *m*-chlorophenylhydrazone (CCCP), *o*-phenanthroline and 2,4-dinitrophenol. The effect of CCCP on the time course of the delayed light emission from the magnesium protoporphyrin-like pigment in chromatophores of *R. rubrum* is shown in Fig. 5. CCCP decreased the intensity of delayed light emission and accelerated the decay. Dinitrophenol and *o*-phenanthroline had effects similar to that of CCCP. It is possible that this short-wavelength-delayed light emission is closely associated with the primary processes of photosynthesis or is reflecting the physical or chemical states of the energy-transducing membranes of photosynthesis. Further research is in progress.

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