ABSENCE OF DELAYED LIGHT EMISSION IN THE MILLISECOND TIME RANGE FROM A MUTANT OF RHODOPSEUDOMONAS SPHERIOIDES WHICH LACKS FUNCTIONING PHOTOSYNTHETIC REACTION CENTERS

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All photosynthetic organisms emit a long-lived dim glow from the first excited singlet state of their functionally active chlorophyll (Strehler and Arnold, 1951; Bertsch, 1962). Although the physical mechanism of this delayed light emission is unclear, there is ample indirect evidence that the delayed light is closely associated with the mechanism of photosynthesis: (1) both processes have the same action spectrum (Strehler and Arnold, 1951; Arnold and Thompson, 1956; Goedheer, 1962); (2) both processes show a complex interaction between two pigment systems (Bertsch, 1962); (3) both processes have an absorption cross section equal to 300-400 chlorophyll molecules (Arnold, 1955), implying the existence of photosynthetic units; (4) both processes are heat-inactivated at the same temperature; (5) poisons of photosynthetic energy conversion invariably have a drastic effect on the kinetics of delayed light emission (Bertsch et al., 1963).

We now report the first piece of direct evidence that the machinery for photosynthetic energy conversion is responsible for the delayed light emission: in photosynthetic bacteria the emission of delayed light depends on the presence of functional photosynthetic reaction centers.

We have compared the intensity of delayed light emission from wild-type Rhodopseudomonas spherioides to the delayed light emission from a mutant which was isolated by Sistrom et al. (1963) and which is believed to lack photosynthetic reaction centers. The mutant cannot grow photosynthetically even though it has

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a normal complement of photosynthetic pigments. When grown aerobically in the dark, the mutant (PM-8) and the parent (wild-type, strain Ga) have similar absorption spectra, but the bacteriochlorophyll of the mutant serves no discernible photochemical function. The following evidence indicates that the mutant lacks functional photosynthetic reaction centers. The mutant shows none of the reversible light-induced absorbancy changes which occur in the wild-type (Sistrom and Clayton). That is, the mutant shows no photooxidation of photosynthetic cytochromes, no photoreduction of ubiquinone, no shift in carotenoid absorption bands, and no photooxidation of P870 (which is associated with the bacterial photosynthetic reaction center). The mutant does not show the light-induced electron spin resonance signal which is present in the wild type (Sistrom and Clayton). The mutant shows no light-induced transients in fluorescence (Sistrom and Clayton). Chromatophores extracted from the mutant are unable to catalyze either the photooxidation of reduced cytochrome c, or the photooxidation of reduced phenazine methosulfate coupled with reduction of ubiquinone (Clayton and Sistrom, Clayton et al.). Thus the bacteriochlorophyll of the mutant is photosynthetically inert.

The yield of bacteriochlorophyll fluorescence in the mutant is greater than that in the wild-type (Sistrom and Clayton). In contrast, as we shall report here, delayed light is emitted by the wild-type, but not by the mutant.

EXPERIMENTAL

The intensity of delayed light emission, averaged from 1 to 5.5 msec after the center of an exciting flash, was measured with a modification of the Becquerel phosphoroscope. This instrument was constructed by W. F. Bertsch in collaboration with William Arnold and J. B. Davidson at the Oak Ridge National Laboratory. It consisted of a pair of spinning discs mounted on a common shaft, between which the cell suspension was held stationary in a 2-ml cellulose nitrate test tube. In order to provide adequate light-baffling we mounted the discs so that they would spin in narrow slots within an aluminum housing. The cells were illuminated through two oppositely spaced holes in the front disc, so that when the discs were spinning the cells received a flash of exciting light every 6.6 msec. The cells were viewed by a photomultiplier placed behind the rear disc. This rear disc was cut into the shape of a paddle wheel, the blades of which were aligned with the illuminating holes in the front disc. The rear disc thus allowed the cells to be viewed by the photomultiplier for a period (1-5.5 msec) between each exciting flash, but for the

duration of each flash the blades of this disc protected the light-measuring equipment from the bright exciting light.

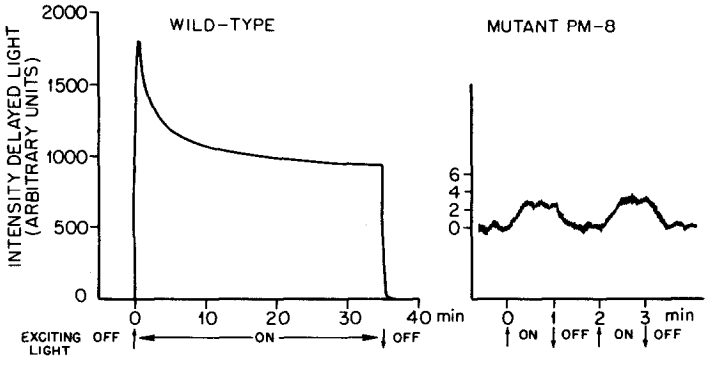
The exciting light consisted of wavelengths between 7200 and 11,000 Å. To obtain this range of wavelengths the emission of a 1500-watt tungsten bulb was filtered through 5 cm of water and a Corning glass filter CS7-69. The filaments of the tungsten lamp were focused on the cells to give an image of about 1 cm^2. With this exciting light, essentially all of the emitted light was in the wavelength region of bacteriochlorophyll fluorescence.

The photomultiplier was an RCA 7102, cooled with liquid nitrogen, and operated at either 1260 or 1420 volts. The output of the photomultiplier was smoothed by an electrical filter (10 sec time constant), amplified by a vibrating reed electrometer, and plotted on a Brown recorder.

Cultures of R. spherioides, wild-type strain Ga and the nonphotosynthetic mutant strain PM-8, were grown in the dark under limited aeration as described elsewhere (Sistrom and Clayton).

RESULTS

The figure shows the intensity of delayed light emitted by R. spherioides,



RHODOPSEUDOMONAS SPHERIOIDES

Intensity of delayed light, averaged from 1 to 5.5 msec after excitation, emitted by Rhodopseudomonas spherioides wild-type strain Ga and non-photosynthetic mutant strain PM-8. The same arbitrary units of light emission are used in both graphs. The small signal from strain PM-8 appears to be unrelated to photosynthesis. The two cell suspensions, each containing about 5 X 10^9 cells per ml, were adjusted to the same bacteriochlorophyll concentration, about 2 μg/ml.

wild-type and nonphotosynthetic mutant PM-8. The two cell suspensions were adjusted to the same cell density and had equal concentrations of bacteriochlorophyll. The arbitrary units of intensity of emission in the figure are the same for both cell types; the graph for the mutant represents a much more sensitive measurement than that for the wild-type. It can be seen that the delayed light signal with wild-type cells was 1800 units at the peak of an initial transient and about 900 units in the steady state, whereas the signal with the mutant was only 3 units.

After being heated to 90° C for 4 min, both types of cells gave a delayed light signal of 6 units. With distilled water in place of a cell suspension we observed a signal of about 30 units, attributable to a light leak through the phosphoroscope. With cells in the sample holder the effect of such a leak would be smaller because of absorption and scattering of light by the cells. These results indicate that the 3-unit signal observed with mutant cells (Fig., b) was unrelated to photosynthesis and could be ascribed in part to a light leak.

CONCLUSION

We have measured the delayed light emitted by two strains of photosynthetic bacteria: a nonphotosynthetic mutant of Rhodopseudomonas spherioides and the photosynthetically competent parent (wild-type) strain. The mutant possesses a normal complement of bacteriochlorophyll but lacks functional photosynthetic reaction centers. Delayed light emission, averaged from 1 to 5.5 msec from the center of a flash of exciting light, was found to be more than 300 times as intense in the wild-type (during steady-state photosynthesis) as in the mutant. This result shows that functional reaction centers must be present in order for delayed light to be emitted from these photosynthetic bacteria.

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