Preliminary Notes

PN 1274

Absence of light-induced absorbancy changes in a mutant of *Rhodopseudomonas spheroides* unable to grow photosynthetically

CLAYTON[1,2] has shown that illumination of bacterial chromatophore fractions causes changes in light absorption at wavelengths corresponding to the absorption bands of bacteriochlorophyll. These changes have been interpreted as being due to an alteration in the absorption spectrum of a special bacteriochlorophyll when it is oxidised. CLAYTON has suggested that the oxidation of this special bacteriochlorophyll is the primary photochemical reaction in bacterial photosynthesis.

We have recently isolated several mutants of *Rhodopseudomonas spheroides* which are unable to grow photosynthetically, although they appear to have the normal complement of photopigments when grown semi-aerobically. We report here some preliminary experiments with one of these mutants, strain PM-8. This strain contains normal bacteriochlorophyll but does not show the light-induced changes in absorption described by CLAYTON.

The mutants were isolated by the following procedure. The parental strain was *R. spheroides* Ga. The only carotenoid pigments formed by this strain in large amounts are neurosporene and hydroxyneurosporene (chloroxanthin); only traces of more unsaturated carotenoid pigments are found. This circumstance considerably simplifies analyses of the pigments in photosynthetic-deficient mutants. A suspension of strain Ga was irradiated with ultraviolet light and the survivors plated on a solid medium. After 2–3-days incubation two replica plates were prepared; one was incubated aerobically, the other anaerobically in the light. Colonies which appeared to be normally pigmented on the aerobic plate but did not occur on the anaerobic plate were isolated and purified.

One of these mutants, PM-8, has been examined. The growth rates of PM-8 and the parental strain are the same when the cultures are aerated with air or with 5% or 1% oxygen; both form approximately equal amounts of photopigments in the lower oxygen tensions. If a culture of PM-8 grown in 1% O_2 is transferred to anaerobic conditions and illuminated it does not grow; under the same conditions strain Ga grows almost immediately.

Chromatographic analysis of the pigments indicates that strain PM-8 has a normal complement of carotenoid pigments. In addition to large amounts of neurosporene and hydroxyneurosporene, a trace of an orange pigment, which may be dihydroxylycopene was detected. The bacteriochlorophyll of strain PM-8 purified by chromatography on powdered sugar has a spectrum which is identical to that of bacteriochlorophyll prepared from strain Ga. The spectra in ether are identical to that obtained by HOLT AND JACOBS[3] (see Table I). Furthermore, the spectrum of strain PM-8 *in vivo* is similar to that of strain Ga, with absorption bands at 800, 850 and 875 mμ. The inability of strain PM-8 to grow photosynthetically is not therefore explicable in terms of any gross alterations in its photopigments.

TABLE I

RELATIVE ABSORBANCIES AT ABSORPTION MAXIMA OF BACTERIOCHLOROPHYLL PREPARED FROM *R. spheroides* STRAIN Ga AND STRAIN PM-8 AND OF CRYSTALLIZED BACTERIOCHLOROPHYLL

The absorbancies are given relative to that at 770 mμ. The figures in Column A were calculated from the data of HOLT AND JACOBS[3]. The figures in Columns B and C were calculated from the spectra of the major chromatographic fraction of bacteriochlorophyll from strain Ga (Column B) and from strain PM-8 (Column C).

Wavelength (mμ)	*Relative absorbancies in ether*		
	A	*B*	*C*
770	100	100	100
574	21.6	21.6	21.8
392	50.2	52.5	53.0
357	76	79.9	78.5

TABLE II

LIGHT-INDUCED ABSORBANCY CHANGES IN CHROMATOPHORE FRACTIONS PREPARED FROM *R. spheroides*, STRAINS Ga AND PM-8

Cell extracts were prepared by sonic oscillation; the crude chromatophore fraction was isolated by centrifugation at 100000 × *g* for 1 h; the pellet was resuspended in water and recentrifuged. It was finally resuspended in water; the absorbancy at 590 mμ was adjusted to 1.6. Both cultures were grown in 1 % O_2.

Strain	*Absorbancy changes at different wavelengths*			
	785 mμ	*815 mμ*	*900 mμ*	*1250 mμ*
Ga	+0.015	−0.032	−0.014	+0.006
PM-8	0.00	0.00	0.00	0.00

Although we could demonstrate light-induced absorbancy changes in chromatophore fraction of extracts of strain Ga, such changes were totally lacking in similar fractions of strain PM-8 (Table II). These experiments were conducted with a Zeiss spectrophotometer; the exciting light was filtered through a blue-green filter (Corning No. 4-97) and two thickness of red-cellophane placed in front of the photocell served as a blocking filter.

These results show clearly that the light-induced absorbancy changes reported by CLAYTON are not the result of a change in the spectrum of the bulk of the bacteriochlorophyll. Furthermore, they strongly support the notion that the oxidation of the special bacteriochlorophyll is an obligatory step in bacterial photosynthesis.

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