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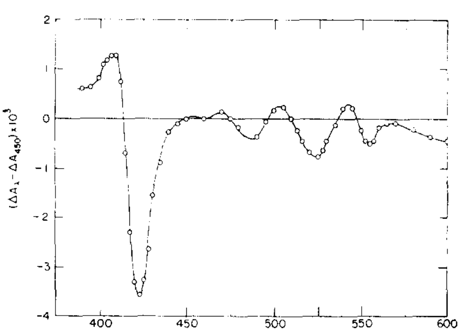
Light-induced absorbance changes of cytochromes and carotenoids in a sulfur bacterium containing bacteriochlorophyll *b*

Thiococcus sp., strain Nidelven, is a photosynthetic sulfur bacterium recently isolated by EIMHJELLEN, STEENSLAND AND TRAETTEBERG[1]. It contains bacteriochlorophyll *b* as does the heterotrophic nonsulfur bacterium *Rhodopseudomonas viridis*[2]. The absorption spectra of both organisms are characterized by an extreme far-red absorption band at 1.03 μ due to bacteriochlorophyll *b*. The carotenoid peaks in Thiococcus[1] are located at 465, 493 and 530 nm in contrast to the peaks at 420, 452 and 482 nm in *R. viridis*[3].

Thiococcus was grown anaerobically at 30° in an incandescent light cabinet on an inorganic medium[4] containing Na_2CO_3 as the carbon source and Na_2S as the reductant. Absorption spectra of whole cell suspensions were recorded by a Shimadzu MPS-50L spectrophotometer. Light-induced absorbance changes were recorded by an Aminco-Chance dual wavelength spectrophotometer. Actinic light (1.01 μ) was provided by a microscope illuminator (American Optical Co., Type 653) and an interference filter (Baird-Atomic, Type B-NIR). Actinic light intensity measured with a radiometer (YSI-Kettering Model 65) was 1.5 $mW \cdot cm^{-2}$. A 3-ml sample was pipetted directly from a culture into a 1-cm cuvette and then covered with paraffin oil for observation. The absorbance at 1.03 μ of the samples used varied between 0.1 and 0.2.

Light-induced absorbance changes were recorded at regular wavelength intervals from 380 to 600 nm. A fixed cycle of light and darkness was repeated at each measuring wavelength (*e.g.*, 30 sec light, 90 sec dark). The light-*minus*-dark steady-state difference spectrum (Fig. 1) shows a peak at 408 nm and troughs at 422 nm and 556 nm, which are characteristic of the light-driven oxidation of *c*-type cytochromes in photosynthetic bacteria[3]. In addition, the troughs at 460, 490 and 524 nm and the peaks at 470, 505 and 543 nm indicate a light-induced red shift of the carotenoid absorption bands similar to the shifts observed previously in *R. spheroides*[5,6] and *Rhodomicrobium vannielii*[7]. Thiococcus is the first organism containing



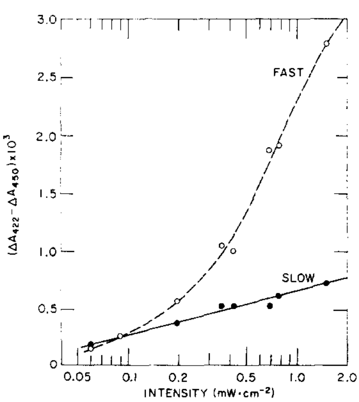


Fig. 1. Light-*minus*-dark difference spectrum (bandwidth = 1.5 nm).

Fig. 2. Magnitude of fast and slow phases of the transition from light to dark as a function of irradiation intensity.

bacteriochlorophyll *b* to exhibit a light-induced red shift of carotenoid bands, since *R. viridis* does not show such a red shift[3].

The kinetics of cytochrome reduction upon cessation of actinic illumination exhibited two distinct phases: a fast phase with a half time less than 2 sec and a slow phase with a half time of 20–30 sec. The fast and slow phases accounted for about 70% and 30%, respectively, of the total absorbance change at 422–425 nm after maximum intensity of actinic illumination. The difference spectrum for the slow phase (dark-*minus*-light) showed a peak at 421 nm and a shoulder at approx. 430 nm, whereas the spectrum of the fast phase showed evidence of only a single component at 422 nm. Light saturation curves (Fig. 2) for the two phases indicate that the cytochrome(s) involved in the slow phase reach the 50% oxidation level at an actinic light intensity (approx. 0.2 $mW \cdot cm^{-2}$) less than half the intensity required for 50% oxidation of the cytochrome(s) responsible for the fast phase (approx. 0.5 $mW \cdot cm^{-2}$).

In analogy to the interpretation of the cytochrome changes in the purple sulfur bacterium *Chromatium*[8], the fast phase is ascribed to the reduction of cytochrome(s) in a cyclic electron transport chain, whereas the slow phase is ascribed to the reduction of cytochrome(s) by S^{2-} and related compounds *via* a noncyclic chain. The close similarity of the light-off kinetics and the light saturation curves for the fast and slow phases in these two sulfur bacteria support a similar interpretation for each organism.

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