the active area of the enzyme containing the essential thiol groups. The case studied here was found to be different, since glutamate within the limits of concentrations 10^{-3} to 10^{-2} *M* had no protective effect. Thus, while it is possible that also in the present case phosphate and thiol reagents compete for an active site of the enzyme surface, this site does not appear to be identical with the one which binds the substrate.

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Is oxidized bacteriochlorophyll an intermediate in bacterial photosynthesis?

DUYSENS *et al.*[1] have suggested that intracellular bacteriochlorophyll in an excited electronic state may react to form a reductant and an oxidized form of bacteriochlorophyll analogous to the product formed[2,3] when a methanol solution of bacteriochlorophyll is treated with $FeCl_3$. Intracellular oxidation of bacteriochlorophyll was inferred on the basis of absorption increases at 432 mμ and 790 mμ and an absorption decrease near 890 mμ observed upon illumination of *Rhodospirillum rubrum* suspended in aerated water[1,4]. CHANCE AND SMITH[5] also observed the appearance of the absorption band at 432 mμ upon irradiation of aerobic suspensions of *Rsp. rubrum* in the presence of phenylmercuric acetate. Similar absorption changes have also been observed in *Chromatium*[1,6]. The present study shows that in *Chromatium* the absorption change at 432 mμ is *not* correlated with the absorption changes in the near infrared.

Chromatium, strain D, was grown in a liquid inorganic medium[7] containing sulfide, thiosulfate, and bicarbonate as substrates. Cultures were illuminated in a light cabinet. The bacteria were concentrated by centrifugation and resuspended in aerated supernatant liquid.

An earlier version of the spectrophotometer has been described previously[8]. A detailed description of the new split-beam arrangement is in preparation[9]. The monochromatic measuring beam from the monochromator is split into two parts, one of which passes through a sample cuvette and the other of which passes through an optical-density wedge. Both parts of the beam are focused onto a photomultiplier. The light from a super-high-pressure mercury lamp is filtered and focused on the sample cuvette to provide actinic irradiation in the region 500 to 600 mμ. By means of two concentric rotating discs with appropriate openings the intervals during which the actinic light beam irradiates the sample cuvette and the intervals during which the two measuring beams strike the photomultiplier are separated in a fixed time sequence as shown in Fig. 1. An actinic light flash lasting 2 msec is given every

60 msec, and the intensity of the measuring beam passing through the cuvette is measured for 7 msec after each flash (interval *a*) and for 7 msec before the next flash (interval *b*). The intensity of the beam passing through the optical wedge (*w*) is measured during the dark period. The difference between the intensities *a* and *b* is averaged over several flash-dark cycles and registered on one recorder as signal *ab*,

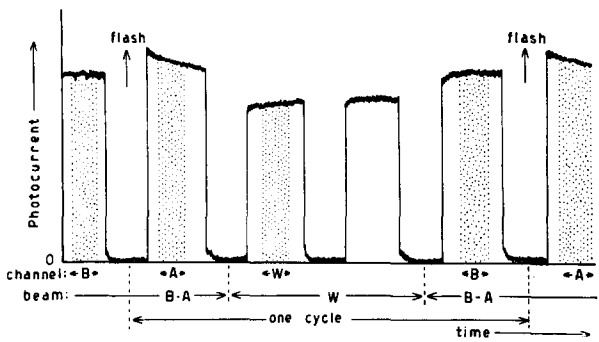

Fig. 1. Time sequence of actinic light flashes and observation intervals during one cycle of spectrophotometer operation. The variation in the photocurrent of the photomultiplier is plotted.

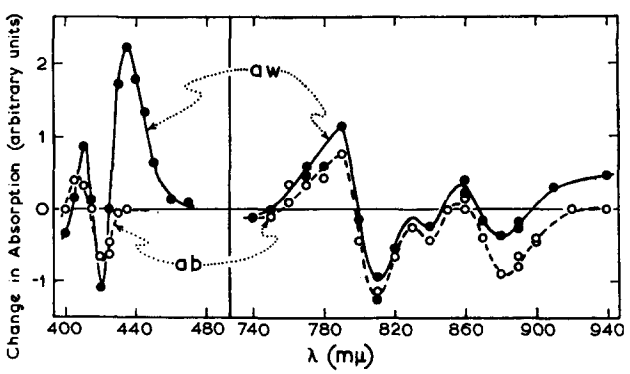

Fig. 2. Spectra of absorption changes caused by 20 sec irradiation of an aerobic *Chromatium* suspension in the presence of 0.1 m*M* phenylmercuric acetate. The scale for the reversible change *ab* has been enlarged by a factor 2.3 with respect to the scale of the steady-state change *aw*.

while the difference between *a* and *w* is similarly registered on a second recorder as signal *aw*. The change in recorder deflection upon turning on the actinic light is the measure of the light-induced absorption change. Signal *ab* is proportional to absorption changes which occur during one light flash and then decay in the dark interval between flashes. Signal *aw*, using the optical-density wedge as a standard, measures the cumulative change in the steady state due to all the light flashes given. Signal *ab* is therefore a measure of changes which are rapid and reversible between flashes, whereas signal *aw* measures both rapid and *slow* changes independently of the decay time.

The spectra of the reversible (*ab*) and steady-state (*aw*) absorption changes induced by irradiation of *Chromatium* under aerobic conditions in the presence of 0.1 m*M* phenylmercuric acetate are shown in Fig. 2. The intensity of the actinic light

was high enough to give 70 to 80 % of the maximum steady-state effect at 436 mμ and yet was low enough to avoid high light-intensity effects in other spectral regions. The spectrum *aw* in the blue shows a sharp trough at about 420 mμ and a broader peak at about 436 mμ. Previous studies[6] have shown that this trough and peak are the net result of a decrease of absorption at 422 mμ due to oxidation of cytochrome and an increase of absorption at 432 mμ caused by some other reaction. The spectrum *ab* shows only the trough at 422 mμ. There is no indication of an absorption increase in the region of 436 mμ in this spectrum. In fact, one sample showed a small absorption *decrease* at 435 mμ for the reversible change *ab*. Thus the large peak at 436 mμ in the spectrum of the steady-state change *aw* reflects a change which is *not* reversible between flashes.

In the region 600 to 740 mμ no significant changes in absorption were observed. In the near infrared region, where the bacteriochlorophylls *B 800*, *B 850*, and *B 890* have their main absorption bands, the spectra of the steady-state and reversible changes are essentially the same (except for a scale factor) within the precision of the measurements. Both spectra have peaks at 790 mμ, major troughs at 810 and 884 mμ, and a minor trough at 840 mμ (*cf.* DUYSENS *et al.*[1]). The reaction(s) responsible for these changes in spectrum are therefore rapid and largely reversible between flashes. The flash intensity necessary for half the maximum steady-state change at either 810 or 890 mμ is at least 4.6 times the intensity necessary for half the maximum change at 436 mμ.

The absence of correlation between the absorption change at 436 mμ and the changes in the near infrared invalidates the hypothesis that these changes are caused by one reaction, specifically the oxidation of bacteriochlorophyll. Although the infrared shifts undoubtedly reflect changes in the bacteriochlorophylls, the substance which is responsible for the appearance of an absorption band at 432 mμ in both *Chromatium* and *Rhodospirillum rubrum* cannot be oxidized bacteriochlorophyll.

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