BBA 43216

Light-induced reactions of P890 and P800 in the purple photosynthetic bacterium *Rhodospirillum rubrum*

In most photosynthetic bacteria at least two (probably *c*-type) cytochromes are oxidized by light[1–5]. Recently published action spectra for the light-induced oxidation of the cytochromes in *Chromatium*[6] and *Rhodospirillum rubrum*[5] suggested that these cytochromes do not share the same reaction center. Thus in *R. rubrum*[5], the substrate coupled cytochrome C428 seems to be oxidized by light mainly absorbed in the major absorption band at 883 nm, while the cytochrome in the cyclic reaction, cytochrome c_2, is oxidized by light absorbed in both, the major absorption band peaking at 883 nm and the minor absorption band peaking at about 805 nm.

Convincing evidence exists[7–10] that the efficient light-induced decrease in absorbance at about 883 nm in *R. rubrum* (and similar light-induced changes in the near infrared absorbance in other photosynthetic bacteria) is due to the oxidation of a constituent, P890, of a reaction center, a site at which excitation energy is converted to chemical energy. CLAYTON[11] has shown that the blue shift of the minor absorption band at 805 nm, which always seemed to occur together with the light-induced decrease in absorbance at 883 nm (refs. 7–9 and 12) is due to a reaction of a different entity, P800. K. SAUER (personal communication) has demonstrated that this blue shift could be a spectral change, due to the oxidation of P890, of P800 molecules (presumably bacteriochlorophyll) which have formed complexes with P890.

The present communication describes experiments with *R. rubrum*, in which light-induced absorbance changes measured under different conditions, in both the near infrared spectral region and the blue spectral region which are indicative for the cytochrome reactions, were compared.

Cultures of *R. rubrum* were grown as described previously[5]. After a series of daily transfers, aliquots of such 1-day-old cultures were centrifuged and resuspended in a substrate-free medium. The measurements were carried out at approx. 3°. The instrument used was a split-beam absorption difference spectrophotometer[5], which features two actinic beams from d.c. fed, 1-kW filament lamps.

As has been shown previously[5] in substrate depleted cells at 3° temperature, actinic light of low intensity caused a relatively rapid oxidation followed by a slower reduction in the dark of only cytochrome C428. The light-*minus*-dark difference spectrum measured under these conditions is shown in Fig. 1a (●—●). In the near infrared spectral region the same low intensity actinic light induced mono-phasic absorbance changes which were slow and often somewhat retarded. The light-off reaction always was rapid. The light-*minus*-dark difference spectrum of these absorbance changes given in Fig. 1b (●—●) showed a substantial bleaching centered at 883 nm and only small changes in the 780–830-nm spectral region. If the cell suspension was illuminated with background light of just enough intensity to almost saturate the oxidation of C428, additional actinic light oxidized only cytochrome c_2 as can be seen from the light-*minus*-dark difference spectrum given in Fig. 1a (×---×). The light-*minus*-dark difference spectrum of the absorbance changes in the near infrared spectral region, given in Fig. 1b (×---×) showed both a bleaching centered at 883 nm and a substantial blue shift of a band at about 805 nm.

Phenyl mercuric acetate in a low concentration (37.5 μM) inhibited the light-

induced reaction of C428 but did not affect the light-induced reaction of cytochrome c_2 as is shown in Fig. 2 (top tracings). Under such conditions one would expect that the P890 molecules which are connected with C428 are oxidized rapidly in the light

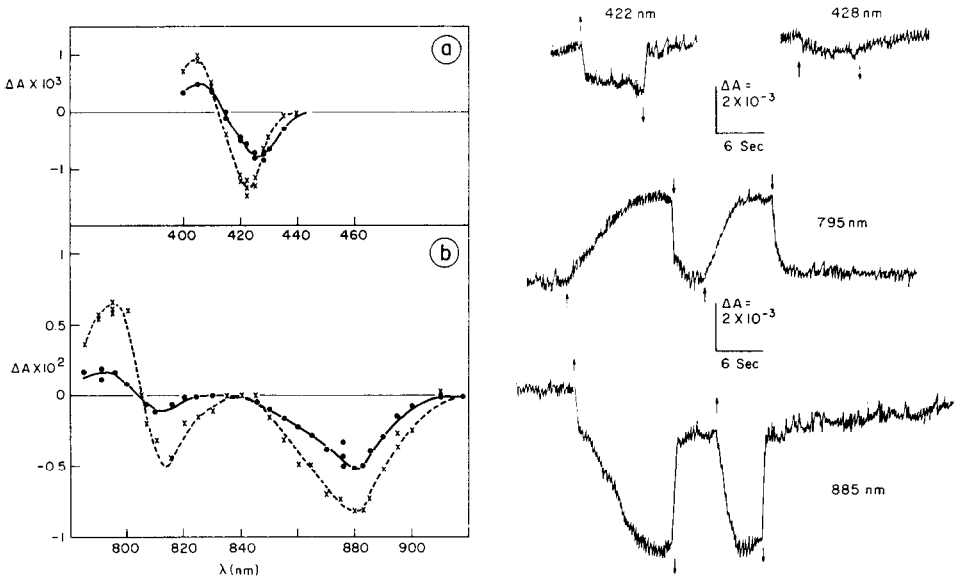

Fig. 1. Light-*minus*-dark difference spectra of absorbance changes in substrate-depleted *R. rubrum*. ●—●, absorbance changes induced by 586-nm actinic light with an intensity of 0.3 nEinstein·cm^{-2}·sec^{-1}; ×---×, absorbance changes induced by 586-nm actinic light with an intensity of 0.6 nEinstein·cm^{-2}·sec^{-1} upon background illumination at 600 nm with an intensity of 0.3 nEinstein·cm^{-2}·sec^{-1}.

Fig. 2. Time courses of light-induced changes at wavelengths indicated in substrate-depleted *R. rubrum* in the presence of 37.5 μM phenyl mercuric acetate. The intensity of the actinic light at 586 nm was 0.6 nEinstein·cm^{-2}·sec^{-1}. The switching on and off of the actinic light is indicated by up and downward pointing arrows, respectively.

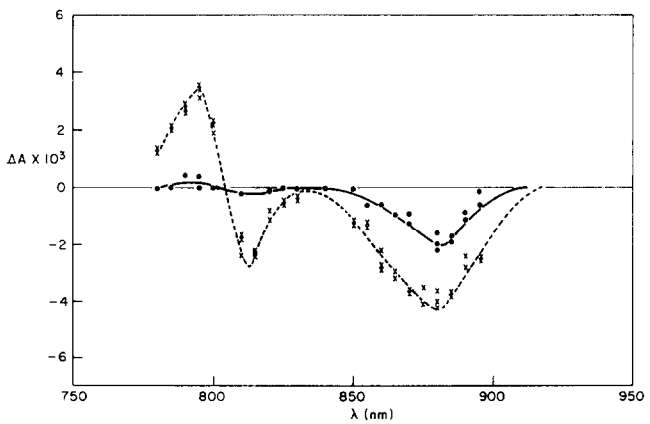

Fig. 3. Light-*minus*-dark difference spectra of absorbance changes in the near infrared spectral region in substrate-depleted *R. rubrum* measured in the presence of 37.5 μM phenyl mercuric acetate. 5 min of complete darkness was allowed after each series of illuminations. ●—●, rapid initial, slowly recovering phase; ×---×, following rapidly recovering phase.

and subsequently reduced very slowly in the dark; the P890 molecules which are connected with cytochrome c_2 should show light-induced oxidation–reduction kinetics similar to the ones observed in unpoisoned cells illuminated with weak background light. The bottom trace of Fig. 2 shows that these kinetics were in fact observed. The light-*minus*-dark difference spectrum of the rapid "light-on" phase, given in Fig. 3 (●—●), showed a bleaching centered at 883 nm but no or very little shift of the 805-nm band. The slower phase of the "light-on" transition, has a light-*minus*-dark difference spectrum (Fig. 3, ×---×) showing a substantial shift of the 805-nm band in addition to a bleaching peaking at 885 nm.

The present experimental results substantiate an interpretation in which the two cytochromes are seen as involved in two different electron transport chains driven by the oxidation of P890 in two different types of reaction center. One type of reaction center contains P890 molecules which are not related to P800. Oxidation of these P890 molecules is coupled through the oxidation of cytochrome C428 to the oxidation of substrate[5]. The other reaction center contains P890 molecules which are closely related to P800 molecules, forming complexes with them. Oxidation of P890 in this type of reaction center, giving rise to a blue shift of the 805-nm band of P800, is coupled to the oxidation of cytochrome c_2 in a cyclic reaction.

Microscopic tests did not reveal any contamination by foreign organisms. Aging, however, can result in profound changes in the pigment ratios. Therefore, since the samples were taken from non-synchronous cultures, the possibility that the data presented above result from measurements with samples in which the cells are in different stages of their development cannot be ruled out completely. However, this possibility must be small, since the majority of the cells can be assumed to be in a similar early stage, due to the series of numerous daily transfers prior to the experiments. Moreover, substantially the same phenomena in respect to the relation of the blue shift of the 805-nm band and the cytochrome reactions were observed in a few experiments with samples from 3-day-old and 5-day-old cultures.

This research was supported by National Science Foundation Grant GB 6732.

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Received September 24th, 1968