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Observations on the changes in ultraviolet absorbance caused by succinate and light in *Rhodospirillum rubrum*

CLAYTON[1] found that illumination of *Chromatium*, *Rhodopseudomonas spheroides*, and *Rhodospirillum rubrum* chromatophores caused changes in their ultraviolet absorbance. Between 240 and 295 mμ, the absorbance changes were similar to those which resulted from the reduction of ubiquinone (UQ). These findings appeared to implicate UQ as a participant in bacterial photosynthesis. Using an extraction method, SUGIMURA AND OKABE[2] demonstrated that succinate reduced the endogenous UQ of *R. rubrum* chromatophores in the dark.

RUDNEY[3] found that ubiquinone with 2 isoprenoid units in the side chain (UQ-2) stimulated photophosphorylation in chromatophores of *R. rubrum* if the cells had been grown in the presence of diphenylamine. RUDNEY[4] later concluded that the effect of UQ-2 was merely to poise the redox potential, and that it did not necessarily reflect a role of UQ in photophosphorylation.

Recently, REDFEARN[5] used an extraction method to study the effect of illumination on UQ in *R. rubrum* cells and chromatophores. Light did not cause a detectable reduction, even in cells which had been treated with phenylmercuric acetate. In fact, illumination of chromatophores caused an appreciable oxidation of UQ. REDFEARN's report prompted the submission of the following observations.

R. rubrum was grown and chromatophores prepared and stored as described elsewhere[6]. Suspensions of chromatophores (50–100 μM BChl) were suspended in 0.1 M glycylglycine buffer (pH 7.4). Whole cells were centrifuged on the fourth day of culture, resuspended in fresh growth medium and used immediately. Continuous actinic light from a tungsten lamp was filtered through several Wratten 88A filters and 1 cm of water. Absorbance changes were measured with a CHANCE double-beam spectrophotometer[7]. The cuvette had a 0.5-cm optical path.

Fig. 1 shows absorbance changes which light causes in whole cells. Trace B monitors cytochrome *c*; trace C, an unidentified pigment which has been the subject of much discussion (see, *e.g.*, ref. 6). At low actinic intensity, absorbance changes due to the latter are inconspicuous, as are those in the ultraviolet (trace A). At high intensity, more cytochrome and the 440-mμ pigment are involved, and the absorbance at 275 mμ increases with respect to 290 mμ. This would be in the direction of UQ oxidation as REDFEARN's[5] data suggest. The kinetics of the ultraviolet absorbance change show a lag, which may match a small overshoot at 440 mμ. Fig. 3A shows the spectrum of the ultraviolet absorbance changes. The maximum is near 275 mμ.

The suspension of cells which was examined in Fig. 1 was treated with phenylmercuric acetate, whereafter illumination caused the absorbance changes of Fig. 2. At first, cytochrome oxidation at low intensity was not greatly affected, but a higher intensity did not cause additional oxidation of cytochrome (trace B). Later, illumination no longer caused cytochrome oxidation (trace C). The character of the absorbance changes at 440 and 275 mμ was affected immediately after the addition of phenylmercuric acetate, and then remained the same for at least 60 min. The 440-mμ absorbance change became more pronounced, particularly at low intensity

Abbreviations: UQ, ubiquinone; UQ-2, ubiquinone with 2 isoprenoid units in the side chain.

(trace D). The ultraviolet absorbance changes altered in direction and kinetics (trace A).

In chromatophores, illumination caused ultraviolet absorbance changes similar

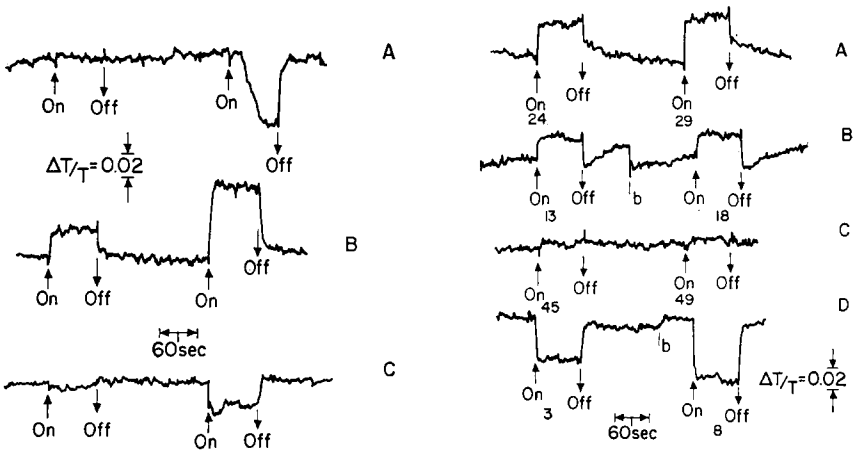

Fig. 1. Absorbance changes induced by light in *R. rubrum* whole cells. The left-hand responses resulted from illumination with approx. 0.56 $nE \cdot cm^{-2} \cdot sec^{-1}$; the right-hand responses, from illumination with 2.7 $nE \cdot cm^{-2} \cdot sec^{-1}$. (These were incident intensities, measured with a silicon solar cell.) A, 290–275 mμ; B, 440–425 mμ; C, 500–440 mμ. An upward deflection of the trace indicates an absorbance increase at the first wavelength with respect to the second. Arrows indicate initiation and termination of illumination.

Fig. 2. Absorbance changes induced by light in *R. rubrum* whole cells after treatment with phenylmercuric acetate. Same sample as in Fig. 1, following addition of $6.8 \cdot 10^{-4}$ M phenylmercuric acetate. Small numbers below traces indicate time in min after phenylmercuric acetate addition. The instrument baseline was balanced at points marked b. Light intensities and other symbols as in Fig. 1. A, 290–275 mμ; B and C, 440–425 mμ; D, 500–440 mμ.

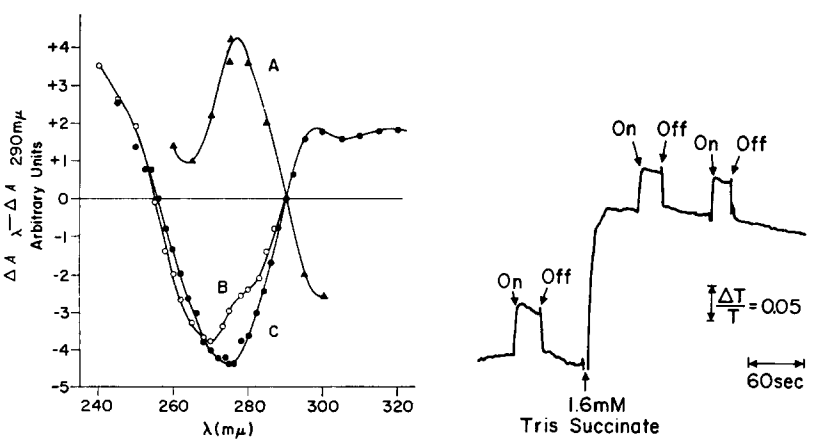

Fig. 3. Difference spectra of absorbance changes in *R. rubrum*. A, whole cells, light *minus* dark; B, chromatophores, light *minus* dark; C, chromatophores, succinate *minus* no additions, in darkness.

Fig. 4. Absorbance changes induced by light and succinate addition in *R. rubrum* chromatophores. 305–275 mμ.

to those which it caused in cells after phenylmercuric acetate treatment (Fig. 4). Succinate also caused a decrease in the absorbance at 275 relative to 290 mμ (Fig. 4), consistent with the reduction of UQ which SUGIMURA AND OKABE[2] described. The absorbance changes caused by succinate and by light were additive (Fig. 4).

Fig. 3 shows spectra of the absorbance changes due to illumination and succinate addition. The latter is maximal at 275 mμ, as expected for UQ reduction. That due to light is different, having a maximum near 270 mμ and a shoulder at 283 mμ. It may be worth noting that rhodoquinone, which occurs in *R. rubrum*, has an absorption maximum at 283 mμ (ref. 8). Both spectra differ from that of oxidized *minus* reduced UQ at wavelengths between 295 and 320 mμ, where both spectra are similar.

There were kinetic differences in the reversals of the absorbance changes due to succinate and light. Those due to light reversed quickly when illumination ceased. Those due to succinate did not recover appreciably, even when malonate was added to block further influx of electrons from succinate. Malonate did block the initial absorbance change if it was added before the succinate. Malonate had no effect on the absorbance changes associated with illumination.

The ultraviolet absorbance changes which light causes seem to be sufficiently complex that one should withhold judgment on whether they are due to UQ. If the absorbance changes caused by succinate and by light are both due to UQ, then they must reflect two different pools which do not communicate rapidly, and which differ in environment enough to have different absorption spectra. Additional work is needed, but the rapid reversal of the changes caused by light, and their small magnitude, will make it difficult to apply extraction methods.

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1 R. K. CLAYTON, *Biochem. Biophys. Res. Commun.*, 9 (1962) 49.
2 T. SUGIMURA AND K. OKABE, *J. Biochem. (Japan)*, 52 (1962) 235.
3 H. RUDNEY, *J. Biol. Chem.*, 236 (1961) PC 39.
4 H. RUDNEY, in A. SAN PIETRO AND H. GEST, *Bacterial Photosynthesis*, Antioch, Yellow Springs, Ohio, 1963, p. 217.
5 E. R. REDFEARN, *Biochim. Biophys. Acta*, 131 (1967) 218.
6 W. W. PARSON, *Biochim. Biophys. Acta*, 131 (1967) 157.
7 B. CHANCE, in R. A. MORTON, *Biochemistry of Quinones*, Academic Press, New York, 1965, p. 460.
8 W. W. PARSON AND H. RUDNEY, *J. Biol. Chem.*, 240 (1965) 1855.

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