THE PHOTOGENERATION OF SUPEROXIDE BY ISOLATED PHOTOREACTION CENTER FROM *Rhodospirillum rubrum**

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SUMMARY.

The photochemical activity of isolated photoreaction center is accompanied by the formation of superoxide radical anions. This is shown by the photoinduction of the aerobic chain oxidation of sulfite or of adrenalin and by the reduction of ferricytochrome c. These phenomena can be inhibited by superoxide dismutase. Since O-phenanthroline does not affect the formation of superoxide, the photoreduction of oxygen is supposed to take place on or before the primary electron acceptor.

The substance of this work is based on the observation that preparations of photoreaction center show a photoinduced oxygen uptake. This oxygen uptake is inhibited by bovine erythrocyte superoxide dismutase. In view of the fact superoxide ions $O_2^{\cdot-}$ have been shown to be photogenerated in isolated chloroplast preparations, presumably through photoreaction 1 (1,2,3), the phenomenon appeared to be of sufficiently general occurrence to be worthy of attention.

We report evidence which indicates that, in isolated photoreaction center, molecular oxygen may react close to or at the site of the primary electron acceptor to form $O_2^{\cdot-}$.

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MATERIALS AND METHODS.

Wild type *Rhodospirillum rubrum* (ATCC, no 11170) was grown as described previously (4). Photoreaction center was obtained from isolated chromatophores (5) of this organism. The preparation procedure was essentially that of Noël *et al.* (4) except that the preparation was further purified by chromatography on DEAE-cellulose according to a procedure analogous to that of Okamura *et al.* (6).

Horse heart cytochrome c (type VI) was purchased from Sigma Chemical Co. It was found to be 5 % in the reduced form.

Bovine erythrocyte superoxide dismutase (3000 units per mg) was obtained from Truett Laboratories, Dallas, Texas.

Oxygen concentration was measured with a Yellow Springs Instruments (Model 53) Oxygen Monitor. The sample was illuminated by a 150 W Trungsten-Halogen lamp (Sylvania A/216). The light was filtered with a Schott RG8 (690 nm cutoff) glass filter.

Spectrophotometric measurements were performed with a Cary 14 R recording spectrophotometer equipped with a cross-illumination attachment described elsewhere (4).

Adrenochrome formation was followed with this instrument by setting the analyzing beam at 480 nm. An ε_{480} of 4.02 $mM^{-1} \cdot cm^{-1}$ was used (7).

RESULTS

Photoreaction center preparations show a slow oxygen uptake in the dark. The rate was found to vary somewhat with the age of the preparations. Autoxidation is a likely explanation for this observation.

Near-infrared light stimulates the rate of oxygen uptake and this stimulation is inhibited by superoxide dismutase but not by catalase (table 1). This indicates that excitation of the porphyrin pigments leads to the formation of $O_2^{\cdot-}$.

Superoxide radicals are known to initiate the chain oxidation of sulfite into sulfate (8) and of adrenalin into adrenochrome (9). Both chain reactions are propagated by $O_2^{\cdot-}$ which is formed at the expense of molecular oxygen.

As shown in table 1, sulfite or adrenalin greatly increase the rate of oxygen consumption of photoreaction center illuminated by near-infrared light. This effect is counteracted by superoxide dismutase but not by O-phenanthroline.

The formation of adrenochrome from adrenalin was also monitored spectroscopically at 480 nm, the absorption maximum of adrenochrome.

TABLE I

Light-induced oxygen uptake (nanomoles/min)

Reaction mixture in 10 mM Tris-Cl (pH 8.0)	No additions	Superoxide dismutase (50 µg/ml)
Photoreaction center (2 µM)	4.4	2.5
+ catalase (25 µg/ml)	4.4	-
+ O-phenanthroline (10 mM)	5.5	-
+ sulfite (10 mM)	48.0	5.0
+ adrenalin (0.1 mM)	20.0	9.0

Light intensity : 10^6 ergs·cm^{-2}·sec^{-1} through a 3 mm thick Schott RG 8 filter.

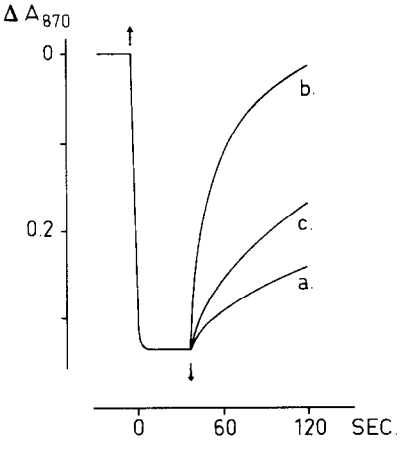


Figure 1 :

Light-induced adrenochrome formation measured at 480 nm. Actinic light (10^5 ergs· cm^{-2}sec^{-1}) filtered through a 870 nm Baird Atomic interference filter (15 nm half-band width) a - 1 µM photoreaction center and 0.5 mM adrenalin in 50 mM carbonate-bicarbonate (pH 10.0). b - As in a except adrenalin omitted.

At this wavelength, photoreaction center alone undergoes a light-induced absorbance increase which rapidly levels off (figure 1). In the presence of adrenalin, the same preparation also shows a slower kinetic component

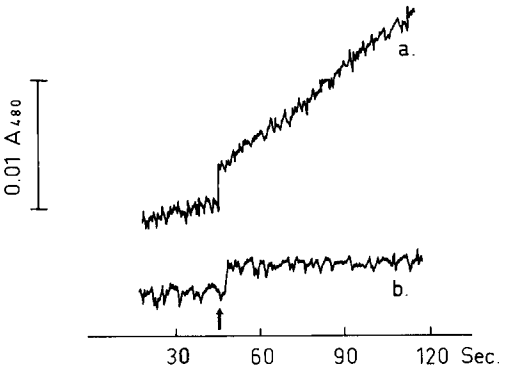


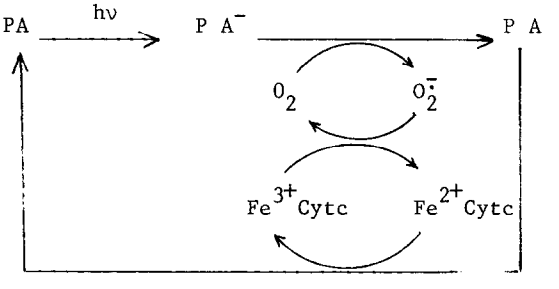
Figure 2 :

Inhibition of the light-induced formation of adrenochrome by increasing concentrations of superoxide dismutase. Conditions as under figure 1.

attributable to the formation of adrenochrome. The initial rates of this reaction were obtained from recordings such as shown in figure 1.

Its inhibition by superoxide dismutase exhibits a characteristic concentration dependency: 90 % inhibition is observed with 6 µg/ml of enzyme (figure 2).

We have also used cytochrome c as a probe for O_2^- : ferricytochrome c is easily reduced to its ferro form by superoxide anions (10). However, ferrocytochrome c is also known to be an excellent reductant for P_{870} (11, 5). A system composed of isolated photoreaction center, ferricytochrome c and superoxide anions is therefore expected to undergo a cyclic electron flow such as the following :



As a consequence, the reduction of P_{870} after the actinic light is turned off should be accelerated by added ferricytochrome c if

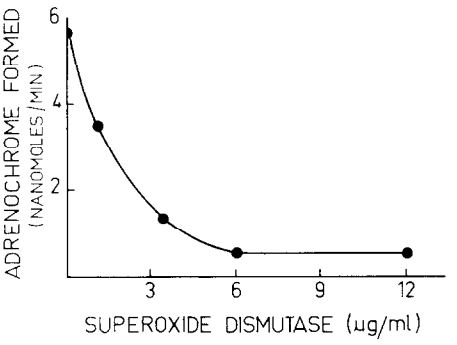


Figure 3 :

Light-induced bleaching and dark recovery of P_{870}. a - 2 µM photoreaction center in 10 mM Tris-Cl (pH 8.0), b - as in a but with 150 µM ferricytochrome c, c - as in b but with 50 µg/ml superoxide dismutase.

$O_2^{\cdot-}$ is present.

Figure 3 shows the bleaching of P_{870} in the light and its recoloration in the dark. The rate of this dark recovery is several times faster in the presence of added ferricytochrome c. This effect is counteracted by superoxide dismutase. These observations are consistent with the existence of a cycle such as predicted above. They also provide further evidence for the photogeneration of $O_2^{\cdot-}$ by photoreaction center preparations. Orthophenanthroline does not inhibit this reaction.

DISCUSSION

The photochemical activity of photoreaction center isolated from *Rhodospirillum rubrum* is attended by the formation of superoxide anions. This has been shown by the initiation of the chain oxidations of sulfite and of adrenalin; both reactions were inhibited by superoxide dismutase. This interpretation is also supported by the fact that added ferricytochrome c increases the recovery rate of P_{870} in the dark and by the inhibition of this effect by superoxide dismutase.

O-phenanthroline, a well known inhibitor of electron transfer between the primary and secondary acceptors (12,13), has no apparent effect

on the photogeneration of superoxide anions. This would indicate that the site of univalent oxygen reduction is at or before the primary electron acceptor.

The real significance of this phenomenon cannot be assessed at present. In particular, its quantum requirement has yet to be determined. However, since it appears to be of fairly general occurrence in photosynthetic organisms (1,2,3), it may be a reflection of the primary mechanism of charge separation.

REFERENCES.

1. Asada, K. and Kiso, K. (1972) Eur. J. Biochem., 33, 253-257.
2. Asada, K., Kiso, K., and Yoshikawa, K. (1973) J. Biol. Chem., 249, 2175-2181.
3. Bolton, J.R. and Harbour (1975) Biochim. Biophys. Res. Comm., 64, 803-807.
4. Noël, H., van der Rest, M. and Gingras, G. (1972) Biochim. Biophys. Acta, 275, 219-230.
5. van der Rest, M. and Gingras, G. (1974) J. Biol. Chem. 249, 6446-6453.
6. Okamura, M.Y., Steiner, L.A. and Feher, G. (1974) Biochemistry, 13, 1394-1402.
7. Green, S., Mazur, A., and Shorr, E. (1956) J. Biol. Chem., 220, 237-255.
8. Fridovich, I. and Handler, P.J. (1960) J. Biol. Chem., 235, 1835-1838.
9. Fridovich, I. and Misra, H.P. (1972) J. Biol. Chem., 247, 3170-3175.
10. Mc Cord, J.M. and Fridovich, I. (1969) J. Biol. Chem., 244, 6049-6055.
11. Ke, B., Chaney, J.H. and Reed, D.W. (1970) Biochim. Biophys. Acta, 216, 373-383.
12. Parson, W.W. and Case, G.D. (1970) Biochim. Biophys. Acta 205, 232-245.
13. Clayton, R.K., Fleming, H. and Szuts, E.Z. (1972) Biophys. J. 12, 46-63.