PHOTOCHEMICAL SULFATE REDUCTION BY RHODOSPIRILLUM RUBRUM[1]

M. L. Ibanez[2] and E. S. Lindstrom

Department of Bacteriology,
The Pennsylvania State University, University Park, Pennsylvania

Received October 15, 1959

The chromatophore of Rhodospirillum rubrum bears two photochemical activities that may have a functional relation. Attempts have been made to relate photooxidation to photosynthetic phosphorylation (Ibanez and Lindstrom, 1959). As these isolated processes are aerobic and anaerobic, respectively, a search was made for a physiologically plausible substitute for oxygen in the photooxidase assay. Sulfate was found to substitute for oxygen in the photooxidase assay much as will fumarate (Vernon, 1959). Data are presented that indicate a photochemical sulfate reduction is mediated by the chromatophores from R. rubrum.

R. rubrum S-1 was grown anaerobically in the light in a lactate-yeast extract medium. Extracts were prepared by grinding with alumina, and chromatophores were purified by centrifugations at 14,000 x G for 10 minutes and 25,000 x G for 30 minutes, and were washed and collected by centrifugations at 100,000 x G for 30 minutes in 0.1 M phosphate buffer pH 7.5. Anaerobic photooxidation was assayed in Thunberg tubes by spectrophotometrically measuring the light-dependent oxidation of reduced 2,6-dichlorophenol indophenol (DPIP) (Vernon and Kamen, 1953).

Data in Table I indicate that photooxidation of reduced DPIP will take place anaerobically in the presence of sulfate. The activity in the presence of sulfate is routinely twice that obtained in air. Addition of

---

[1]Authorized for publication as paper No. 2401 in the journal series of the Pennsylvania Agricultural Experiment Station. Support of the National Science Foundation (G 4343) is also acknowledged.

[2]National Science Foundation Cooperative Fellow.

exogenous diphosphopyridine nucleotide (DPN) or adenosine triphosphate (ATP) depresses both aerobic and anaerobic photooxidase activity as measured by DPIP oxidation.

Table I

Anaerobic Photooxidation in the Presence of Sulfate

Assay conditions	Photooxidase Activity
Aerobic	
Without $SO_4^=$	0.42
+ 5 μM $SO_4^=$	0.42
+10 μM DPN	0.14
+10 μM ATP	0.0
Anaerobic	
Without $SO_4^=$	0.0
+ 5 μM $SO_4^=$	0.87
+ 5 μM $SO_4^=$ + 10 μM DPN	0.34
+ 5 μM $SO_4^=$ + 10 μM ATP	0.0

All values are μM of DPIP oxidized/mg protein/hour. Curvette or Thunberg cuvette contents: chromatophores, 10 mg protein; sulfate, $\pm$ 5 μM; phosphate buffer (pH 7.5), 100 μM; reduced DPIP, 0.5 μM; DPN, $\pm$ 10 μM; ATP, $\pm$ 10 μM; and H_2O to final volume of 3.0 ml. Anaerobic assay cuvettes were flushed 5 X with purified N_2. Aerobic assay was in air. Assay period was 3 minutes in light at 30°C.

Data in Table II show that the photochemical reducing power apparently reduces sulfate to sulfite. The photooxidase assay was carried out as detailed in Table I except that $Na_2S^{35}O_4$ was added as the only exogenous sulfate source. At the end of the incubation period of 15 minutes, 100 μM of $BaCl_2$ were added to the rinsed Thunberg sidearm and the reaction mixture was acidified. After standing overnight, the contents of the sidearm were carefully evaporated and the radioactivity of the volatile sulfur compounds ($BaSO_3$) determined. These data suggest that a photochemical reduction of sulfate took place with sulfite as a possible intermediate.

Aliquots were also banded on paper chromatograms which were developed with a butyric-ammonium solvent to isolate and detect labeled nucleotides. Only inorganic sulfur was detected, but the total radioactivity measured on the dried chromatograms was much less with aliquots from tubes exposed to the light. This provided additional evidence for a photochemical reduction of sulfate by the chromatophore.

Table II

Photochemical Production of Volatile Sulfur from Sulfate

Assay Conditions	Radioactivity from Volatile S	Radioactivity from Residual $SO_4^=$
Light	51 cpm/ml	557 cpm/ml
Dark	13	3,209
No enzyme	0	3,367

All values are counts/minute/ml from S^{35}. Contents of Thunberg tubes: chromatophores, 10 mg protein; $S^{35}O_4$, 3,300 counts/minute/ml of reaction mixture; phosphate buffer (pH 7.5), 100 μM; reduced DPIP, 0.5 μM; H_2O to 3.0 ml. Flushed 5 X with N_2 and incubated 15 minutes in the light at 30°C.

The physiological function of this chromatophoral sulfate reduction is still unknown. That no product other than volatile sulfur has been detected could be evidence that this is another non-specific manifestation of the light-generated reducing power. Indeed, this reaction could be merely the transfer of electrons from reduced DPIP to sulfate mediated by chlorophyll. We consider this unlikely as nitrate would not substitute for sulfate. The extract used here was essentially free of contamination with soluble enzymes. These enzymes may be essential for the further metabolism of the photochemically reduced sulfate.

The anaerobic system of Vernon, utilizing fumarate, was heat sensitive (no photooxidation was measured after heating the extract) due to the denaturing of the chromatophoral succinic dehydrogenase (Woody and Lindstrom, 1954). Photooxidase was remarkably heat resistant with oxygen as the acceptor (Vernon and Kamen, 1953), and it retained this heat

resistance if sulfate was substituted for oxygen. This suggested that the photooxidase and sulfate reduction were closely related.

The inhibition by ATP and DPN of photooxidation may be due to competition for the photochemically produced oxidants and reductants by the indigenous photosynthetic phosphorylation. Or the photochemically reduced DPN may yield electrons to the photooxidized DPIP resulting only in an apparent inhibition.

In summary, sulfate was shown to substitute for oxygen and to make possible an anaerobic photooxidation of reduced 2,6-dichlorophenol indophenol. ATP and DPN inhibited both the aerobic and anaerobic photooxidations. The available evidence demonstrated that the bacterial chromatophore is capable of a photochemical sulfate reduction.

References

1. Ibanez, M. L. and Lindstrom, E. S., Bact. Proc. 55 (1959).

2. Vernon, L. P., J. Biol. Chem. 234, 1883 (1959).

3. Vernon, L. P. and Kamen, M. D., Arch. Biochem. Biophys. 44, 298 (1953).

4. Woody, B. R. and Lindstrom, E. S., J. Bacteriol. 69, 353 (1954).