THE ROLE OF O^{18} PHOSPHATE IN THIOSULFATE OXIDATION BY *THIOBACILLUS THIOPARUS**

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The thiobacilli convert thiosulfate to sulfate according to the following equation:

$S_2O_3^{=} + 2O_2 + H_2O \longrightarrow 2\ SO_4^{=} + 2H^+$ and they obtain their energy for cell synthesis from this oxidative pathway. Previous experiments (Margulies and Santer, 1958; Vishniac and Santer, 1957) have established that thiosulfate conversion to sulfate by resting cells of *Thiobacillus thioparus* depends on the presence of inorganic phosphate. In the absence of phosphate the oxygen consumption is about 70% of the theoretical amount for complete oxidation and various sulfur compounds can be shown to accumulate in phosphate-free reaction mixtures. In addition it was demonstrated that phosphate controls the rate of thiosulfate oxidation and is acting catalytically. Arsenate can substitute for phosphate.

These data indicated that phosphate might be involved in a substrate level oxidative step in which a sulfur compound (perhaps organically bound) was linked to phosphate. To further test this hypothesis, *T. thioparus* was incubated with thiosulfate and O^{18} labeled inorganic phosphate, prepared according to Cohn (1957). If at some time during the conversion of thiosulfate to sulfate an $S\text{-}O^{18}\text{-}P$ bridge was

* This work was supported in part by funds from grants from the National Institutes of Health, U.S. Public Health Service and the National Science Foundation.

** Lalor Foundation Faculty Research Award.

formed, in which the O^{18} was contributed by the phosphate, and subsequently the $-O^{18}-P$ bond was broken, O^{18} would be found in the sulfate.

Two incubation mixtures were prepared with and without 2,4-dinitrophenol (2,4-DNP); 2,4-DNP stimulates O_2 consumption by _T. thioparus_ with $S_2O_3^{=}$ as substrate (Table I). It was shown by Cohn (1953) and Drysdale and Cohn (1958) that certain levels of 2,4-DNP prevent an exchange reaction by rat liver mitochondria between the oxygen of water and phosphate. Including 2,4-DNP in the reaction mixture would prevent O^{18} incorporation from phosphate into sulfate by way of water. At the end of the incubation period the cells were centrifuged off and the supernatant solution was treated two successive times with a magnesium-ammonium mixture to remove inorganic phosphate; sulfate was subsequently precipitated as the benzidine salt, washed twice with water and dried. The O^{18} content of the sulfate was determined by the method of von E. Doering and Dorfman (1953).

The results demonstrate that O^{18} was incorporated into sulfate; the atom per cent O^{18} in reaction flask 1 was 0.32 and in reaction mixture 2 was 0.34. The phosphate in this experiment was prepared according to Cohn (1957) using water containing 1.5 atom per cent excess O^{18}. Even if there had been a rapid exchange of O^{18} from phosphate to water, which subsequently labeled the sulfate, the O^{18} content of the water would have never reached an amount detectable in the mass spectrometer. In other words in a reaction mixture where there was 1.85 ml of unlabeled water at the start of the experiment ($\sim$100,000 μmoles) and 50 μmoles of phosphate, complete equilibration between the two in the first few minutes of the incubation period would reduce the O^{18} content in the water and the phosphate to an undetectable amount. If the exchange reaction were slow, then initially there would be few or no labeled water molecules to react. From these considerations it is unlikely that the

TABLE I

REACTION MIXTURES TO DETECT O^{18} INCORPORATION FROM PHOSPHATE TO SULFATE

Reaction flask number	1	2
	ml	ml
T. thioparus	1.7	1.7
0.3M O^{18} phosphate, pH7.2	0.1	0.1
$S_2O_3^{=}$, 25 μM	0.1	0.1
2,4-DNP, 4 x 10^{-4}M	---	0.05
KOH, 2.5N (Center well)	0.2	0.2
H_2O	0.05	---

Incubation time 3 hours at 30° C in air.
Cells and reagents prepared in normal H_2O

O^{18} which appears in sulfate got there by way of the oxygens of water.

It appears that the incorporation of the isotope is unaffected by an agent which stimulates O_2 uptake by *T. thioparus*, uncouples electron transport phosphorylation, and limits the exchange reaction between the O in water and phosphate in other systems. Thus the data support the contention that an S-O-P link is formed during the oxidation of thiosulfate to sulfate rather than a mechanism of O^{18} incorporation which would involve water as an intermediary. The incorporation of O^{18} of phosphate into sulfate is impressive since in the absence of 2,4-DNP one might have expected a rapid exchange, catalyzed by the bacteria, between the O of phosphate and the O of water which would tend to dilute the O^{18} content of the phosphate to an undetectable concentration. These incorporation data suggest an additional mechanism for producing high energy phosphate containing compounds in the thiobacilli in addition to those presumably produced as a result of electron transport phosphorylation.

I want to express my thanks to Dr. W. Vishniac for making his laboratory facilities available to me. I am indebted to Dr. K. Okamoto of the Department of Chemistry, Yale University for carrying out the mass analyses.

REFERENCES

1. Cohn, M., J. Biol. Chem., 201, 735 (1953).
2. Cohn, M., in S. P. Colowick and N. O. Kaplan (Editors), Methods in Enzymology, Vol. IV, Academic Press, Inc., New York, 1957, p. 905.
3. Drysdale, G. R., and Cohn, M., J. Biol. Chem., 233, 1574 (1958).
4. Margulies, M., and Santer, M., Bacteriol. Proc., 1958, 121.
5. Vishniac, W., and Santer, M., Bacteriol. Rev., 21, 196 (1957).
6. von E. Doering, W., and Dorfman, E., J. Am. Chem. Soc., 75, 5595 (1953).

Received June 14, 1959