SULFITE OXIDASE OF A FACULTATIVE AUTOTROPH, THIOBACILLUS NOVELLUS*

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Peck (1960) and Peck and Fisher (1962) proposed a mechanism for the oxidation of thiosulfate by thiobacilli, in which thiosulfate is first split to sulfide and sulfite by thiosulfate reductase, and sulfite is then further oxidized to sulfate by APS reductase and ADP-sulfurylase.

During an investigation of T. novellus, which oxidizes both thiosulfate and sulfite to sulfate, an enzyme which oxidized sulfite to sulfate was isolated and purified 34-fold. Enzyme activity was not increased by the addition of AMP as occurred in T. thioparus (Peck, 1961) or by hypoxanthine as in T. denitrificans (Milhaud et al. 1958). The enzyme more closely resembled the sulfite oxidase isolated, and partially purified, from mammalian liver (MacLeod et al. 1961).

MATERIALS AND METHODS

T. novellus, kindly supplied by Dr. R. L. Starkey, was converted to autotrophy by repeated transfer into a mineral salt medium with decreasing concentrations of glucose and increasing concentrations of thiosulfate. After four transfers, the organism metabolized thiosulfate as its sole source of energy, and carbon dioxide as its sole carbon source.

Autotrophic T. novellus was grown on Starkey's medium No. 3 (Starkey, 1935) with frequent neutralization by Na_2CO_3.

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Cells were harvested in a Sharples centrifuge, washed twice in 0.02 M potassium phosphate (pH 7.0), suspended in 0.04 M potassium phosphate (pH 7.0), and sonicated for 15 minutes in a 10 KC Raytheon, under an atmosphere of nitrogen. Unbroken cells and cell debris were removed by centrifugation.

The crude extracts obtained were then centrifuged at 144,000 x g for one hour. The supernatant liquid, after dilution with an equal volume of 0.2 M Tris-HCl (pH 8.0), was treated with crystalline $(NH_4)_2SO_4$. The fraction which precipitated between 45-100% $(NH_4)_2SO_4$ was dissolved in a volume of 0.2 M Tris-HCl (pH 8.0) equal to the original volume of the crude extract, and dialyzed overnight at 5° C against 0.002 M Tris-HCl (pH 8.0). Ten ml dialyzed extract was chromatographed on a column of DEAE-cellulose (1 x 12 cm) in 0.002 M potassium phosphate (pH 7.0). The enzyme was eluted with 0.02 M potassium phosphate (pH 7.0).

The enzyme activity was assessed by the method of Peck (1961) which is based on the spectrophotometric measurement of the reduction of $K_3Fe(CN)_6$. The purified enzyme solution was concentrated in a dialysis bag placed in crystalline sucrose. The concentrated enzyme was dialyzed against 0.002 M Tris-HCl (pH 8.0) for several hours, and made up to the original volume with the same buffer. Protein was determined according to the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

Table I shows the results of enzyme purification. The enzyme showed no loss of activity with repeated freezing and thawing over several months, but the activity was completely destroyed by heating at 60° C for 1 min.

Table II shows the results obtained when the purified enzyme was assayed in the presence of AMP and substrate. It can be seen that AMP did not stimulate the enzyme activity, and thiosulfate could

TABLE I. Purification of sulfite oxidase

	Total protein (mg)	Specific activity* (μmoles $Fe(CN)_6^{\equiv}$ red/mg protein/hr)
Crude extract	12.3	79
144,000 x g supernatant	11.0	84
45-100% $(NH_4)_2SO_4$	8.2	100
Purified enzyme	0.19	2710

*The reaction mixture contained in μmoles: Tris-HCl (pH 8.0), 5.0; $K_3Fe(CN)_6$, 1.5; Na_2SO_3 in 5 mM EDTA, 5.0; and enzyme. Final volume 1.5 ml.

not replace sulfite as substrate. With crude extracts, thiosulfate reduced ferricyanide though much more slowly than sulfite did.

TABLE II. Activity of sulfite oxidase

System	μmoles $Fe(CN)_6^{\equiv}$ reduced/mg protein/hr
No AMP	2710
Complete	2030
No enzyme	0
No $Fe(CN)_6^{\equiv}$	0
No $SO_3^{=}$	0
No $SO_3^{=}$ plus $S_2O_3^{=}$	0

Complete reaction mixture contained in μmoles: Tris-HCl (pH 8.0), 5.0; $K_3Fe(CN)_6$, 1.5; Na_2SO_3 in 5 mM EDTA, 5.0; AMP, 10; purified enzyme, 19.0 μg; and where indicated $Na_2S_2O_3$ 5.0 μmoles. Final volume 1.5 ml.

Since AMP did not stimulate the reduction of ferricyanide by sulfite either with the crude or purified enzyme, this enzyme could not be APS-reductase (Peck, 1961). Also, while the latter enzyme did not readily couple to naturally occurring electron acceptors, sulfite oxidase readily reduced mammalian cytochrome c, and a native c-type cytochrome.

A comparison with an enzyme found by Milhaud et al. (1958) in whole cells of T. denitrificans showed that neither crude extracts, nor whole cells, of T. novellus consumed significantly greater quantities of oxygen measured manometrically in the presence of hypoxanthine than in its absence.

MacLeod et al. (1961) isolated and purified a sulfite oxidase from mammalian liver. The bacterial enzyme possessed several of the characteristics of this enzyme, but unlike it, was not associated with cytochrome b_5, did not couple directly to oxygen or reduce methylene blue anaerobically in the presence of sulfite.

Sulfite oxidase of T. novellus was inhibited by increasing concentrations of Tris-HCl, Tris-acetate, NaCl, KCl and potassium phosphate, all of which gave similar results. The phosphate inhibition of enzyme is illustrated in Table III.

TABLE III. Phosphate inhibition of sulfite oxidase

Potassium phosphate added	% inhibition* at pH 6.0	% inhibition* at pH 7.0	% inhibition* at pH 8.0
1.67×10^{-3} M	35	13	9
3.3×10^{-3} M	46	13	9
1.67×10^{-2} M	88	74	50

*Activity in absence of potassium phosphate taken as 100%.

Of interest also is the observation that crude extracts of T. novellus oxidized thiosulfate completely to sulfate without Norit A treatment or dialysis, unlike the system found by London and Rittenberg, (1964) in a strain of T. thioparus which required these treatments for complete conversion of thiosulfate to sulfate.

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