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An Iron-Containing Superoxide Dismutase from the Chemolithotrophic *Thiobacillus denitrificans* "RT" Strain

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Abstract. Superoxide dismutase has been purified to homogeneity from aerobically grown *Thiobacillus denitrificans* strain "RT". It has a molecular weight of 43,000, is composed of two identical subunits which are not covalently bound, and contains 1.35 atom of iron per molecule. Absorption spectra and amino acid analysis are similar to those of other Fe-superoxide dismutases from bacteria. Aerobically and anaerobically grown cells contain the same Fe-enzyme with similar levels of activity. Manometric sulfite oxidation measurements suggest for the enzyme a protective function of sulfite against the autooxidation initiated by superoxide free radicals.

Key words: *Thiobacillus denitrificans* — Superoxide dismutase — Sulfite oxidation.

The superoxide free radical (O_2^-) is a common metabolic intermediate in a variety of biological oxidations, and it seems reasonable to expect that it is actually generated intracellularly by organisms exposed to oxygen (Fridovich, 1973). Superoxide dismutase increases about 10^4 fold the spontaneous dismutation rate of the radical ($O_2^- + O_2^- \xrightarrow{2H^+} H_2O_2 + O_2$) (Rabani and Nielsen, 1969). The enzyme was found to be present in all aerobic or facultatively anaerobic microorganisms (McCord et al., 1971) as well as in some anaerobes (Hatchikian and Henry, 1977; Tally et al., 1977; Kanematsu and Asada, 1978), and is assumed to play an important role in the protecting of cells against the deleterious action of superoxide radical (Fridovich, 1975). The involvment of superoxide in the autooxidation of sulfite was also investigated by McCord and Fridovich (1969a) who found that superoxide dismutase could inhibit the chain reaction. Since sulfite is an intermediate in the oxidation of inorganic sulfur compounds by thiobacilli (Suzuki, 1974; Schedel et al., 1975) it was of interest to characterize superoxide dismutase from the facultative anaerobe *Thiobacillus denitrificans*. The present paper reports on the purification and the properties of the Fe-containing superoxide dismutase present in aerobically and anaerobically grown *Thiobacillus denitrificans*, the function of protection of sulfite against autooxidation is discussed.

Non-Standard Abbreviations. DMSO = dimethyl sulfoxide; SDS = sodium dodecyl sulfate; SOD = superoxide dismutase

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Materials and Methods

Organism and Growth Conditions. Thiobacillus denitrificans strain "RT" (DSM 807, ATCC 29685) was grown using thiosulfate as the electron donor as previously reported (Baldensperger and Garcia, 1975). Aerobic cells were grown using ammonium as nitrogen source, and anaerobic growth was performed with nitrate as electron acceptor and sole nitrogen source.

Assay of Superoxide Dismutase. The activity of superoxide dismutase was measured by the cytochrome reduction assay of McCord and Fridovich (1969b) and enzymatic unit was defined as the amount of enzyme required to inhibit the cytochrome reduction by 50%. Changes in absorbance were followed with a Beckman model 25 spectrophotometer. During the course of purification, column eluates were assayed for superoxide dismutase activity by the procedure described by Elstner and Heupel (1976) which is based upon the inhibition of nitrite formation from hydroxylammonium chloride. Nitrite was determined colorimetrically with a M4QIII Zeiss spectrophotometer.

Assay of Sulfite Oxidase. Enzymic and chemical oxidation of sulfite were measured manometrically using a model V 166 Warburg apparatus. Superoxide radicals were produced in the vessel either by means of a potential difference of 1.5 volt impressed accross sealed in platinium electrodes, or by reaction between sulfite (5mM) and dimethyl sulfoxide (0.25 mM). This concentration in dimethyl sulf-

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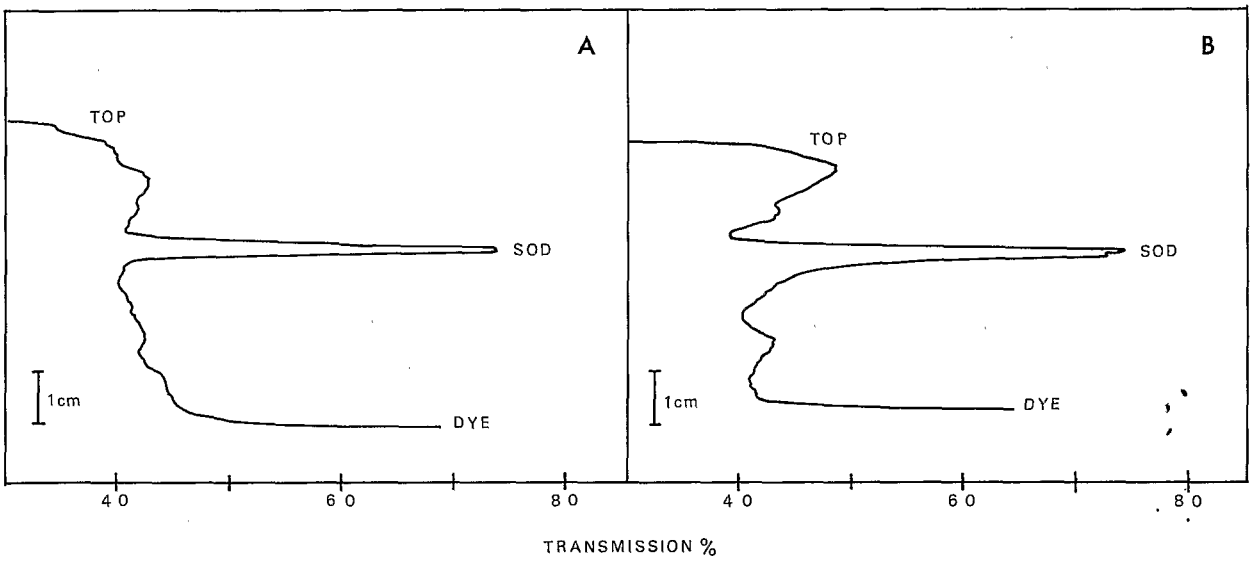

Fig. 1 A and B. Densitometric scans of polyacrylamide gel electrophoregrams stained for SOD activity. Cell free extracts were applied to 7% gels. **A** 0.170 mg protein from anaerobically grown cells ("NO_3" extract). **B** 0.135 mg protein from aerobically grown cells ("O_2" extract)

oxide (DMSO) was found by Puget and Michelson (1974) to have no effect on the superoxide dismutase activity of the Fe enzyme from *Photobacterium leiognathi.*

Determination of Protein. Protein was determined according to Lowry et al. (1951) using bovine serum albumin as the reference.

Absorption Spectra. Visible and ultraviolet absorption spectra were recorded with a Cary model 14 spectrophotometer.

Determination of Molecular Weight. The molecular weight of the purified enzyme was estimated by gel filtration according to Whitaker (1963). Sephadex G-75 column was used with the following standards: bovine serum albumin, 68,000; ovalbumin, 43,000; pepsin, 35,000; soybean trypsin inhibitor, 20,100.

Polyacrylamide Gel Electrophoresis. Analytical gel electrophoresis was carried out with 7% gels (pH 9.5) using the method of Davis (1964). 50 µg of protein were loaded on each gel and protein bands were stained with Coomassie blue. The activity bands were made visible by the photochemical assay of Beauchamp and Fridovich (1971).

The subunit molecular weight was determined by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis according to Weber and Osborn (1969). Samples were incubated at 37°C for 2 h in 0.010 M phosphate buffer pH 7, 1% in SDS, in the presence or absence of 1% β-mercaptoethanol. Electrophoresis was carried out 3 h with a constant current of 10 mA per gel. The following standards were used: bovine serum albumine, 68,000; ovalbumine, 43,000; chymotrypsinogen A, 25,000; soybean trypsin inhibitor, 20,100 and cytochrome C, 12,500.

Metal Analysis. Iron was determined after hydrolysis of the enzyme 10 min at 80°C in 1% HCl as ferrous o-phenanthrolinate according to Massey (1957). Manganese, zinc and copper were assayed by atomic spectrophotometry using a Perkin Elmer 370 atomic absorption spectrophotometer.

Amino Acid Analysis. The purified enzyme was dialyzed against water and hydrolyzed under vacuum in 6N HCl at 110° for 24 h. The residues were analyzed in a LKB model 3201 amino acid analyzer. Tryptophan content was determined by the photometrical method of Edelhoch (1967).

Results

Superoxide Dismutase in Crude Extracts from Thiobacillus denitrificans

16 g wet weight of cells were harvested from a 20 l carboy of anaerobic medium "DB" and cell-free extract ("NO_3" extract) was prepared as previously described (Baldensperger and Garcia, 1975). Aerobic growth was conducted in a 20 l fermentor on aerobic medium "A" maintained at pH 6.8 by titrating sterile 10% (w/v) Na_2CO_3. Cells were harvested with similar cell yield and the extract from aerobically grown cells ("O_2" extract) was prepared as for anaerobic cultures. 5 µl (containing about 150 µg of protein) of both extracts were subjected to polycrylamide gel electrophoresis and to staining of superoxide dismutase activity. A sole activity band (RF = 0.40) was observed in both extracts, and corresponded to the activity stained in gels from the purified iron superoxide dismutase described below. Densitometric scans of stained gels from extracts of anaerobically and aerobically grown cells are shown in Fig. 1. Specific activities of "NO_3" extract and "O_2" extract were respectively 7.2 and 7.5 unit/mg protein. Superoxide dismutase was purified from aerobically grown cells since "O_2" extract exhibited a slightly higher specific activity than "NO_3" extract.

Sulfite Oxidation in Relation with Superoxide Dismutase Activity

For the measurement of the AMP-independent sulfite oxidase activity, a crude extract was prepared from

aerobically grown cells by centrifugation at 10,000 g for 30 min of washed bacteria, suspended in pH 7.5 0.050 M Tris-HCl buffer (15 g wet wt./30 ml buffer). Sulfite oxidation was assayed manometrically in the presence or absence of KCN 0.001 M. Results are presented in Table 1.

With 1.3 mg of protein from crude extract, 36% inhibition of oxygen uptake was noticed in the presence of 0.001 M KCN. The inhibition rate increased to 75% with 13 mg of proteins, while in the presence of 26 mg of the crude extract proteins it was noticed that oxygen uptake decreased also in the absence of KCN as compared to the uptake with 13 mg.

An attempt was made to produce superoxide radical in the Warburg vessel, either by means of sealed in platinium electrodes according to Fridovich and Handler (1961) or by dimethyl sulfoxide (DMSO) reacting with sulfite (McCord and Fridovich, 1969a). Concentrations in EDTA, sulfite, and DMSO were adjusted as described by the authors to obtain initiation of the chain reaction. In this condition, superoxide dismutase present in crude extract was found to inhibit autooxidation of sulfite, as shown in Tables 2 and 3. Complete inhibition of sulfite oxidation initiated by 1.5 V between the platinium electrodes was obtained by 18 mg of crude extract proteins. In the DMSO sulfite system, a 98% inhibition was measured.

Table 1. Manometric measurement of sulfite oxidation by crude extract from *Thiobacillus denitrificans*

mg protein of crude extract	0	1.3	2.6	13	26
µl O_2 utilized (min^{-1}) minus KCN	5.8	4.7	5.0	18.3	14.3
µl O_2 utilized (min^{-1}) + KCN	5.8	3.0	1.5	4.5	3.0
Inhibition (%)	0	36	70	75	79

Final concentration in Warburg vessels: sulfite, 15 mM; EDTA, 0.015 mM; KCN, 1 mM; phosphate buffer pH 7.6, 70 mM; crude extract, as indicated. The sodium sulfite (freshly prepared) was added from the side arm as solution in EDTA at zero time. Gas phase air. Temperature 37° C

Table 2. Inhibition of electrolytic initiation of sulfite oxidation by crude extract from *Thiobacillus denitrificans*

mg protein of cude extract	0	18
µl O_2 utilized (min^{-1}) 0 Volt	5	14.5
µl O_2 utilized (min^{-1}) 1.5 Volt	8	14.5

Final concentration in Warburg vessels: sulfite, 15 mM; EDTA, 0.23 mM; phosphate buffer pH 7.6, 70 mM; crude extract, as indicated. The sodium sulfite (freshly prepared) was added from the side arm as solution in EDTA at zero time. Gas phase air. Temperature 37° C. Voltage was impressed in the reaction mixture by means of platinium electrodes (0.5 mm diameter, 3 mm length) sealed in the vessel at a distance of 3 mm

Table 3. Inhibition of the DMSO initiated sulfite oxidation by crude extract from *Thiobacillus denitrificans*

mg protein of crude extract	0	22
µl O_2 utilized (min^{-1}) minus DMSO	0.2	8.8
µl O_2 utilized (min^{-1}) + DMSO	9.4	8.9

Final concentration in Warburg vessels: sulfite, 5 mM; EDTA, 0.1 mM; DMSO, 0.25 mM; phosphate buffer pH 7, 100 mM; crude extract, as indicated. The sodium sulfite (freshly prepared) was added from the side arm as solution in EDTA at zero time. Gas phase air. Temperature 37° C

Purification of the Enzyme

All purification procedures were performed at 4° C, and unless otherwise noted phosphate buffer was used at pH 7.8.

a) Preparation of Crude Extract. Cells of *Thiobacillus denitrificans* were harvested by centrifugation during log phase and washed twice in 0.05 M phosphate buffer pH 6.8. The pellet (120 g wet w.) was resuspended in 100 ml 0.05 M phosphate pH 7,8 and homogeneized before passing through a French pressure cell at 20,000 PSI. A few cristals of deoxyribonuclease were added to the preparation which was stirred for 10 min at room temperature before centrifugation 30 min at 30,000 g. The crude extract contained 65.2×10^3 units of superoxide dismutase, its specific activity was 7.5 unit/mg protein.

b) Precipitation at 60° C. The crude extract was agitated in a thermostatic bath at 60° C during 5 min and chilled. The precipitate was discarded after centrifugation for 30 min at 30,000 g. The clear supernatant exhibited a specific activity of 12.9 unit/mg protein.

c) Ammonium Sulfate Fractionation. The preparation was brought to 45% of saturation in $(NH_4)_2SO_4$ by addition of a saturated solution and stirred during 5 min at room temperature. The precipitate was discarded after 30 min at 30,000 g. The supernatant was then brought to 80% $(NH_4)_2SO_4$ saturation by addition of the solid salt, and the mixture was centrifuged for 40 min at 20,000 g. The supernatant was free from superoxide dismutase activity.The precipitate was dissolved in a minimum of 0.01 m phosphate buffer pH 7.8, and dialyzed against 2 l of the same buffer, which was exchanged 3 times every 8 h.

Table 4. Purification of superoxide dismutase from *Thiobacillus denitrificans*

Purification step	Volume (ml)	Total protein (mg)	Total activity (unit) $\times 10^{-3}$	Specific activity (unit/mg protein)	Yield %	Purification fold
Crude extract after centrifugation	150	8,700	65.2	7.5	100	1
Supernatant from heat step	124	4,340	55.9	12.9	86	1.7
50–80% $(NH_4)_2SO_4$	53	1,670	45.3	27.1	69.5	3.6
Sephacryl	140	252	38.4	153	59	20.4
Hydroxylapatite	35	63	26.7	424	41	56.5
DEAE 52	38	12.5	18.1	1,448	36	193
Ultrogel AcA 4/4	29	8.5	16.5	1,940	25	259

d) Sephacryl Gel Filtration. The dialyzed solution (53 ml) was applied to a column of Sephacryl (5 × 84 cm) equilibrated with 0.01 M phosphate buffer pH 7.8. The column was washed with the same buffer at a flow rate of 0.9 ml per min, and the active fractions (140 ml) were concentrated in a 320 ml ultrafiltration cell using a PM-10 membrane filter (Amicon) before dialyzing against 0.002 M phosphate buffer (pH 7.8).

e) Hydroxylapatite Column Chromatography. The dialyzed solution was applied to a column of hydroxylapatite (2.5 × 9 cm) equilibrated with the dialysis buffer and washed with 235 ml of a non linear gradient of phosphate buffer (0.002→0.050 M). Elution of the activity occurred at 0.030 M and was achieved after 45 ml of 0.050 M buffer. The active fractions (35 ml) were dialyzed against 2 l of 0.005 M phosphate buffer pH 7.8 exchanged 3 times every 4 h.

f) DEAE-52 Column Chromatography. The dialyzed solution was applied to a column of DEAE-52 (2.5 × 6 cm) equilibrated with the dialysis buffer and washed with 180 ml of a non linear gradient of phosphate buffer (0.005→0.040 M). The activity was completely eluted in 38 ml of 0.020 M buffer and concentrated by ultrafiltration with a PM-10 membrane filter.

g) Ultrogel AcA4/4 Filtration. The concentrated solution was applied to a column of Ultrogel AcA 4/4 (2.5 × 83 cm) equilibrated with 0.010 M buffer. The elution was performed with the same phosphate buffer at a flow rate of 0.5 ml per min. The superoxide dismutase activity was eluted as a single peak which was congruent with a peak of 280 nm absorbance. The active fractions were dialyzed against 0.002 M phosphate buffer pH 7.8 before concentrating by filtration with a UM-10 membrane filter (Amicon). The purification procedure is summarized in Table 4, and in the final step the enzyme preparation showed a 259-fold purification with a recovery of 25% of the original activity.

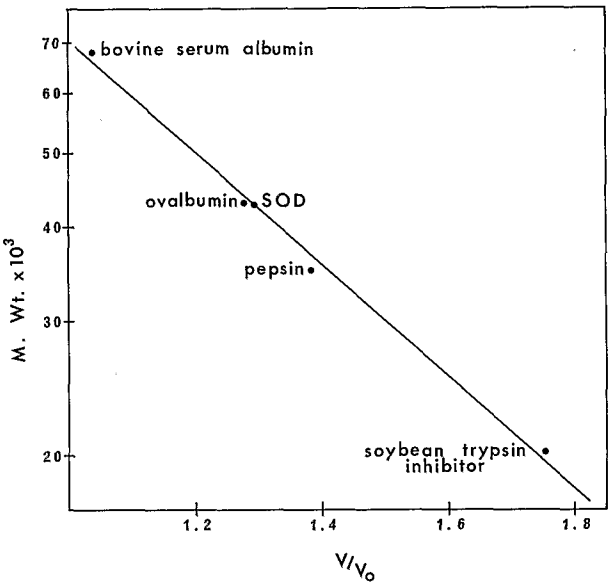

Fig. 2. Determination of the molecular weight of superoxide dismutase from *Thiobacillus denitrificans* (RT strain) by gel filtration. The purified SOD (1.4 mg in 0.2 ml) was subjected to a Sephadex column G-75, the elution volumes of the enzyme and the standards proteins were collected and accurately measured. The bed volume was determined using Dextran blue

Purity of the Enzyme

The superoxide dismutase activity was eluted from Ultrogel filtration as a symetrical peak of 280 nm

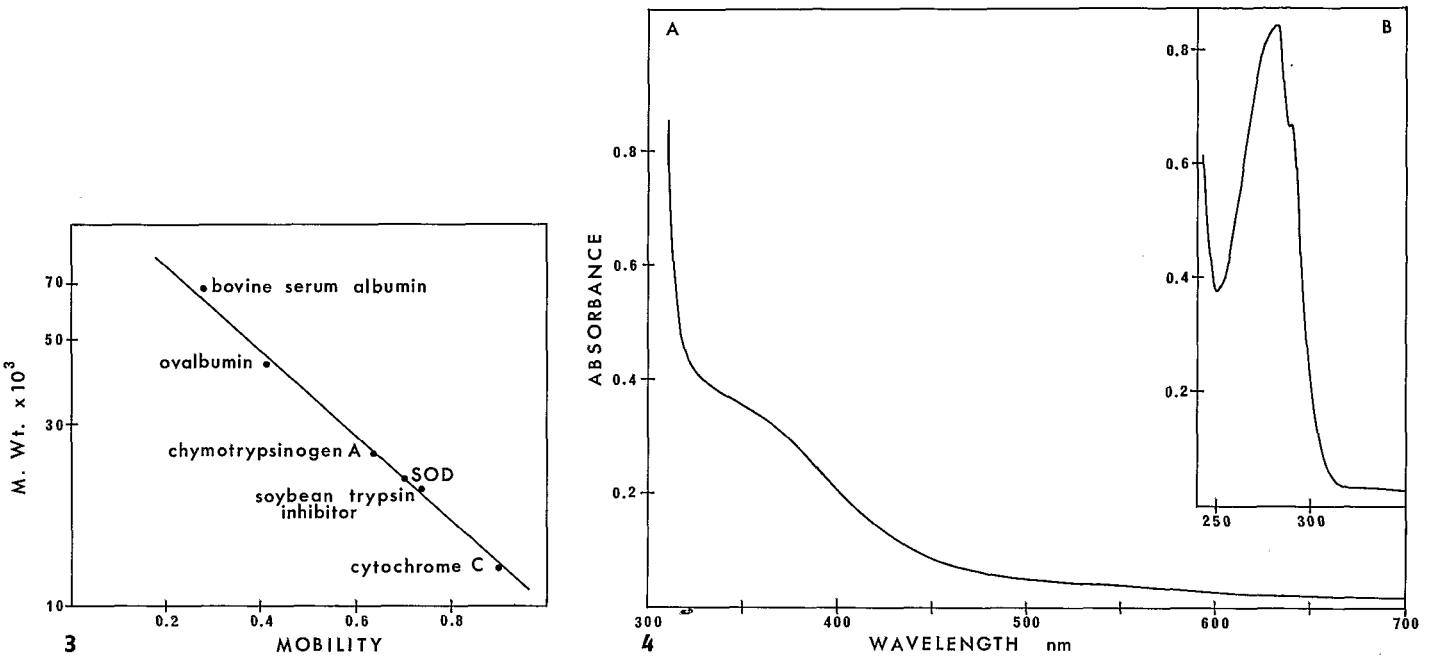

Fig. 3. Determination of the molecular weight of superoxide dismutase subunits from *Thiobacillus denitrificans* by SDS-polyacrylamide gel electrophoresis. 0.8 µg of pure enzyme incubated as described were submitted to SDS-electrophoresis in the presence of 1% β-mercaptoethanol

Fig. 4 A and B. Absorption spectra of superoxide dismutase from *Thiobacillus denitrificans* (1 cm light path). **A** Visible spectrum at 6.83 mg protein/ml in 10 mM potassium phosphate buffer pH 7.6. **B** Ultraviolet spectrum at 0.46 mg protein/ml in the same buffer

absorbance in 13 different fractions which were examined by polyacrylamide gel electrophoresis. In all fractions, the protein band (*RF* 0.40) coincided with the activity zone. It was noticed that in the fractions from the beginning of the elution peak a very weak protein band was also visible (*RF* 0.43) corresponding to a weak activity zone. A similar observation was made by Hatchikian during the course of the purification of superoxide dismutase from *Desulfovibrio desulfuricans* (personal communication). As a single band was reavealed after sodium dodecyl sulfate gel electrophoresis of all fractions it may be assumed that the second activity zone noted in the first fractions collected contained partially dissociated enzyme. Active fractions from Ultrogel were pooled together and concentrated as described, the concentrated enzyme preparation exhibited a single protein band corresponding to the active zone of the crude extract.

Molecular Weight and Subunit Weight

The molecular weight of the purified enzyme was estimated to be 43,000 daltons using a column of Sephadex G-75 (1 × 75 cm) equilibrated with 1 M NaCl in 0.02 M phosphate buffer pH 7.0 (Fig. 2). The subunit molecular weight was estimated by sodium dodecyl sulfate gel electrophoresis, the purified enzyme gave one band both in the presence and in the absence of β-mercaptoethanol. From comparison of its mobility to that of molecular weight standards (Fig. 3) it may be concluded that *Thiobacillus* superoxide dismutase is composed of two subunits of 21,500 daltons that are not joined by bisulfide bridges.

Absorption Spectra

The enzyme exhibited an absorption maximum in the ultraviolet region at 282 nm, with a peak at 290 nm and a shoulder at 258 nm (Fig. 4). Its molar extinction coefficient at 280 was $78.5 \times 10^3\ M^{-1}cm^{-1}$. The visible absorption spectrum exhibited a broad absorption from 320 – 600 nm, with a shoulder around 360 nm and a very small band at 550 nm (Fig. 4). The molar extinction coefficient at the 350 nm shoulder was $2{,}235\ M^{-1}cm^{-1}$.

Metal Analysis

The purified enzyme was dialyzed against 0.010 M Tris-HCl pH 7.5 for 2 days with 4 changes of the buffer and hydrolyzed at 80° C during 10 min in HCl 1%. Iron determination by spectrophotometry using o-phenanthroline indicated the presence of 1.35 atom of iron per molecule on the basis of a molecular weight of

Table 5. Comparative amino acid analysis of iron containing superoxide dismutases

	Thiobacillus denitrificans[a]	*Desulfovibrio desulfuricans*[b]	*Chromatium vinosum*[c]	*Escherichia coli*[d]
Lysine	14	16	8	10
Histidine	9	5	4	6
Arginine	4	4	2	4
Tryptophan	6	6	6	4
Aspartic acid	22	27	24	22
Threonine	12	12	11	13
Serine	10	7	16	10
Glutamic acid	13	18	20	16
Proline	8	8	7	9
Glycine	15	19	14	16
Alanine	20	22	21	26
$^1/_2$ Cysteine		2	1	1
Valine	9	8	14	11
Methionine	3	2	2	0
Isoleucine	5	7	8	8
Leucine	14	15	16	15
Tyrosine	6	8	9	6
Phenylalanine	10	12	8	10

[a] This work
[b] Hatchikian and Henry (1977)
[c] Kanematsu and Asada (1978)
[d] Yost and Fridovich (1973)

43,000. Manganese, copper and zinc were below detection using atomic absorption spectrophotometry.

Amino Acid Analysis

The amino acid analysis of *Thiobacillus denitrificans* superoxide dismutase is summarized in Table 5 and compared to other iron-containing superoxide dismutases isolated from the strict anaerobe sulfate reducer *Desulfovibrio desulfuricans*, the anaerobic purple sulfur bacterium *Chromatium vinosum* and the iron dismutase from *Escherichia coli*. It may be noticed that a great similarity exists between all the iron-containing dismutases, although the *Thiobacillus* enzyme has a higher content of histidine, lysine, tryptophan and methionine and a lower content of glutamic acid.

Discussion

The contents of superoxide dismutase in aerobically and anaerobically grown *Thiobacillus denitrificans* were identical, and similar to those of other aerobic bacteria (*Pseudomonas ovalis*, 17.2 unit/mg protein, Yamakura, 1975); photosynthetic bacteria (*Chlorobium thiosulfatophilum*, 13 unit/mg protein, Kanematsu and Asada, 1978); or strict anaerobes (*Desulfovibrio desulfuricans*, 3.8 unit/mg protein; Hatchikian, and Henry, 1977). Densitometric scans of gels stained for superoxide dismutase activity showed that both extracts from aerobically and anaerobically grown cells contained only one superoxide dismutase, which was found to be a Fe-SOD. On the contrary, *Escherichia coli* grown under anaerobic conditions contained only Fe-SOD, but exposure to oxygen induced the synthesis of Mn-SOD and New-SOD (Hassan and Fridovich, 1977; Britton and Fridovich, 1977). The presence of the same enzyme at similar levels of specific activity in aerobically and anaerobically grown *Thiobacillus denitrificans* might indicate that the enzyme has a function other than the dismutation of superoxide radical. A similar statement was made by Kanematsu and Asada (1978) concerning the Fe-SOD from *Chromatium vinosum*. The aerobic autooxidation of sulfite has been shown by Fridovich and coworkers to be a free-radical chain reaction, initiated by O_2^- in the presence of minute amounts of EDTA (Fridovich and Handler, 1961; McCord and Fridovich, 1969a), and superoxide dismutase was found to inhibit this reaction. According to Pick et al. (1974), the presence of superoxide dismutase in *Thiobacillus denitrificans* could give the catalytic reaction an advantage of 10^4 over the spontaneous dismutation of superoxide radical, thus limitating the possible interference of the O_2^- initiated autooxidation of sulfite with the enzymic sulfite oxidation by the organism. The present data are consistent with the hypothesis of a protecting function of superoxide dismutase towards sulfite. The difference between the rates of oxygen uptake by the protein-free system and the crude extract inhibited by 0.001 M KCN was found to increase with the amount of protein present in the vessel. According to Aminuddin and Nicholas (1974), the AMP independent sulfite oxidase in the crude extract was inhibited by 0.001 M KCN, but neither the AMP-dependent sulfite oxidase nor the superoxide dismutase activities were affected (Bowen et al., 1966, Asada et al., 1975) by this concentration in cyanide. In such experimental conditions, it might be expected that crude extract would inhibit the spontaneous oxidation of sulfite. When superoxide radicals were produced in the vessel, either at a platinium electrode or by reaction between DMSO and sulfite, complete inhibition of the autooxidation of sulfite was achieved by 18 mg of protein of crude extract from *Thiobacillus denitrificans*. All the data allow us to suggest that superoxide dismutase has an important function in the protection of sulfite, a key intermediate in the oxidation of inorganic sulfur compounds by the organism.

Superoxide dismutase of *Thiobacillus denitrificans* grown under aerobic conditions has been purified to homogeneity as judged from polyacrylamide gel electrophoresis. Some dissociation of the native protein was noticed during the preparative procedure, as

reported earlier by Anastasi et al. (1976) concerning the Fe-enzyme from *Bacillus megaterium*. The properties of the *Thiobacillus* enzyme are very similar to the iron-containing superoxide dismutases so far isolated from other sources, with respect to molecular weight, subunit structure, absorption spectra and amino acid composition.

The ultraviolet spectrum shows a shift of the maximum protein absorbance to 282 nm, and a sharp peak at 290 nm as compared to the previously reported Fe-SOD from other sources. The shoulder at 350 nm observed in the visible absorption spectrum is thought to be due to the iron linked to the protein, the molar extinction coefficient at 350 nm reflecting the iron content of the enzyme.

The *Thiobacillus* enzyme contains 1.35 iron atom per molecule of 43,000 daltons. Intermediate values between 1 iron atom in *Escherichia coli* (Yost and Fridovich, 1973) and 2 iron atoms in *Chromatium vinosum* (Kanematsu and Asada, 1978) have been reported. It is unlikely to attribute the deviation to iron lack during cell growth, as the trace metal solution used for the medium (Baldensperger and Garcia, 1975) gave a high yield in other iron proteins, i.e. cytochromes, as demonstrated by the visible absorption spectrum of the crude extract.

As suggested by Yamakura (1976), it seems more probable that one iron atom is bound strongly to the enzyme molecule, the other being removable through the purification procedure. The differences may also be accounted for by difficulties encountered in the estimation of the iron content (Puget and Michelson, 1974) and/or the protein concentration of the preparations (Slykhouse and Fee, 1976).

Amino acid composition of *Thiobacillus denitrificans* superoxide dismutase shows similarities with that of other iron containing enzymes from bacterial sources, with a high content in histidine, tryptophan and tyrosine. It was suggested by Hatchikian and Henry (1977) that these residues could be the ligands of iron. The *Thiobacillus* enzyme contains methionine which is absent from the *Escherichia coli* Fe-enzyme.

From the present data, we can conclude that aerobically and anaerobically grown *Thiobacillus denitrificans* contains a sole Fe-containing superoxide dismutase protecting the cell against the harmfull action of the radical (Fridovich, 1975). A protecting function of superoxide dismutase towards sulfite is suggested.

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