bacterium *Thiobacillus X*. A fifth fraction (acidic fraction) contained two additional cytochrome components with α bands at 550 and 557 $m\mu$ respectively in the reduced state.

2. All the cytochromes appear to be of the *c* type, are labile to heat and acid and do not combine with carbon monoxide. They are auto-oxidizable to varying degrees.

3. Cytochromes 550 (1) and 557 (1) are basic, and are adsorbed on to Amberlite IRC-50 at pH 7. Their E'_0 values at pH 7 are +0.20 and +0.15 v respectively. Cytochrome 553.5 is acidic but is adsorbed on to Amberlite at pH 5. Its E'_0 value is +0.21 v.

4. The cytochromes of the acidic fraction are not adsorbed on IRC-50. Otherwise their properties are very similar to those of cytochromes 550 (1) and 557 (1) respectively.

5. Addition of thiosulphate to whole cells causes a rapid reduction of cytochrome components with α peaks at 549–558 $m\mu$ and 562 $m\mu$.

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Thiosulphate Oxidation and Cytochromes in *Thiobacillus X*

2. THIOSULPHATE-OXIDIZING ENZYME

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The aerobic sulphur autotroph *Thiobacillus X* oxidizes thiosulphate to sulphate with the intermediate formation of tetrathionate (Trudinger, 1959). In the preceding paper (Trudinger, 1961) the separation from extracts of this bacterium of some cytochrome components and an enzyme oxidizing thiosulphate was reported. In this paper some properties of the enzyme system are described and the role of cytochromes in the oxidation of thiosulphate is discussed.

MATERIALS AND METHODS

Thiosulphate-oxidizing enzyme and cytochromes. The preparation of these is described in the preceding paper (Trudinger, 1961).

Enzymic reaction procedures. All reactions were carried out at 23° unless otherwise stated. Ferrieyanide reduction was followed with a Unicam SP. 600 spectrophotometer as

described by Trudinger (1961). Cytochrome reduction was studied in 5 cm. $\times$ 0.4 cm. tubes which were viewed vertically through a Beck microspectroscope, model 3000, mounted on a microscope stage; the condensing lens was used as a source of illumination. Complete reduction of cytochrome was gauged by comparison with a second sample reduced with $\text{Na}_2\text{S}_2\text{O}_4$.

Gas exchanges were measured by the conventional Warburg manometric technique (Umbreit, Burris & Stauffer, 1949).

Radioactive thiosulphate. $\text{Na}_2(\text{S} \cdot \text{SO}_3)$, labelled with ^{35}S in either the inner ($-\text{SO}_3$) or outer ($-\text{S}$) positions, was obtained from The Radiochemical Centre, Amersham, Bucks. Doubly labelled thiosulphate refers to a mixture of equal activities of inner- and outer-labelled thiosulphate.

Potassium tetrathionate. $\text{Na}_2\text{S}_2\text{O}_8 \cdot 5\text{H}_2\text{O}$ (50 g.) was dissolved in 100 ml. of water and oxidized to completion with iodine. An excess of potassium acetate was added and $\text{K}_2\text{S}_4\text{O}_8$ precipitated by the addition of 4 vol. of absolute ethanol. The material was recrystallized from 0.5N-HCl.

Estimation of thiosulphate and tetrathionate. These were determined by titration with standard iodine in the presence of starch before and after reaction with excess of Na_2SO_3 (Kurtenacher, 1924; Starkey, 1934). Ferricyanide and ferrocyanide interfered with the estimation unless the pH of the mixture was kept above 5 and the titration carried out rapidly. A preliminary analysis was carried out to determine roughly the iodine titre. In a second titration slightly less than the required amount of iodine was pipetted into the mixture and the titration completed as rapidly as possible.

Paper chromatography of thiosulphate and tetrathionate. The rear-phase technique of Pollard, McOmie & Jones (1955) was used with Whatman no. 1 paper. Spots were revealed by spraying the paper with 0.5M- AgNO_3 followed by heating at 100° for 2 min. Radioactive areas were detected by exposing the paper to Kodirex X-ray film.

Paper electrophoresis. An apparatus similar to that described by Wood (1954) was used for the electrophoresis of thiosulphate and polythionates. The material to be analysed was spotted 11 cm. from one end (negative) of a 51 cm. $\times$ 2 cm. Whatman no. 1 paper strip wetted with 0.05M-sodium citrate, pH 5.2. The papers were placed in the electrophoresis apparatus under carbon tetrachloride and a voltage of 1000 v was applied for 1 hr. The use of the higher voltage for shorter times than those used by Wood (430 v for 3 hr.) resulted in sharper bands and greater reproducibility. Average mobilities ($\mu/\text{sec.}/\text{v}/\text{cm.}$) were 3.65 for thiosulphate and 3.10 for tetrathionate. Some variation in ion movement occurred from day to day owing to changes in room temperature.

Determination of radioactivity. Samples containing less than 1 mg. of dry matter were dried on lens tissue mounted in aluminium or glass planchets. After drying, radioactivity was determined with an end-window Geiger tube. No corrections for self-absorption were necessary under these conditions.

Degradation of radioactive tetrathionate with mercuric chloride. Tetrathionate reacts with HgCl_2 according to equation (1) (van der Heijde, 1955).

To 1 ml. of the solution to be analysed was added 0.5 ml. of 40% (v/v) formaldehyde and 0.5 ml. of 5% (w/v) HgCl_2 in 2% (w/v) sodium acetate. After 1 hr. at room temperature the mixture was centrifuged and the precipitate was washed twice with 1 ml. portions of 0.01M- Na_2SO_4 . The combined supernatants were then mixed with 20 mg. of inactive $\text{K}_2\text{S}_4\text{O}_6$ and the above described procedure was repeated. The supernatants, containing the sulphate, were combined and made up to standard volume. The precipitates of sulphur and $\text{HgCl}_2, 2\text{HgS}$ were oxidized with conc. HNO_3 , saturated with bromine and made to standard volume. Portions of each solution were plated on glass planchets and counted.

Degradation of radioactive tetrathionate with cyanide. Tetrathionate reacts with cyanide according to equation (2) (Brodskii & Eremenko, 1955).

The sample to be analysed (1 ml. of approx. 0.01M) was made alkaline to phenolphthalein and mixed with 1 ml. of 0.1M-KCN. After 5 min. at room temperature the products

were separated by chromatography on Dowex-2 $\times$ 8 (acetate). Sulphate was eluted with 2M-ammonium acetate, pH 5, thiosulphate with 5M-ammonium acetate, pH 5, and thiocyanate with 2N- HNO_3 . The identities of sulphate and thiosulphate were checked by electrophoresis on paper and that of thiocyanate by chromatography on Whatman no. 1 paper developed with butan-1-ol-pyridine-water-aq. NH_3 soln. (sp.gr. 0.88) (80:40:80:5; upper phase) (McOmie & Pollard, 1953).

RESULTS

Reaction of thiosulphate-oxidizing enzyme with ferricyanide

Effect of pH. This is shown in Fig. 1. The activity increased markedly as the pH was lowered from neutrality to pH 4.5. Below pH 4.5 a rapid chemical reaction between thiosulphate and ferricyanide, which produced Prussian blue, prevented study of the enzymic reaction. This chemical reaction also occurred at pH 4.5, though at a much slower rate than at pH 4.0, and for this reason pH 5 was chosen for assays of enzyme activity. At pH 6.5 and above a lag occurred in the reduction of ferricyanide; the reason for this is not known. At pH 5, with 5 μ -moles of ferricyanide, the reaction was essentially linear until about 25% of the ferricyanide was reduced.

Effect of thiosulphate concentration. Maximum initial rates of ferricyanide reduction were obtained with concentrations of thiosulphate above about 5 mM (Fig. 2).

Effect of enzyme concentration. The initial rate of ferricyanide reduction increased linearly with enzyme concentration over a 20-fold range (Fig. 3).

Product of thiosulphate oxidation. The product of oxidation of thiosulphate by thiosulphate-oxidizing enzyme could not be separated from authentic tetrathionate by rear-phase chromatography or by paper electrophoresis. Table 1 shows the results of analyses of the product of oxidation of thiosulphate labelled with ^{35}S . When the compound formed from doubly labelled thiosulphate was degraded with HgCl_2 the ratio of ^{35}S in the supernatant to that in the precipitate was 1:1:1 compared with the theoretical ratio for uniformly labelled tetrathionate of 1:1. The product of doubly labelled thiosulphate oxidation also reacted with KCN to give sulphate, thiocyanate and thiosulphate. The

ratio of ^{35}S in this case was sulphate:thiocyanate:thiosulphate = 1:0.93:2 (theoretical ratio for uniformly labelled tetrathionate, 1:1:2). No

Table 1. Analysis of the end product of thiosulphate oxidation

Sodium thiosulphate labelled with ^{35}S was oxidized to completion under similar conditions to those described in Fig. 2. The end product was analysed by reaction with HgCl_2 or KCN. Results are expressed as ^{35}S counts/min.

(A) HgCl_2 reaction				
Substrate	End product	Supernatant (SO_4^{2-})	Precipitate ($\text{HgCl}_2 \cdot 2\text{HgS} + \text{S}$)	
$^{35}\text{S} - ^{35}\text{SO}_3$	63 000	31 000	34 000	
$^{35}\text{S} - \text{SO}_3$	82 000	50	80 000	
$\text{S} - ^{35}\text{SO}_3$	46 000	47 000	20	

(B) KCN reaction				
Substrate	End product	Sulphate	Thiocyanate	Thiosulphate
$^{35}\text{S} - ^{35}\text{SO}_3$	63 000	15 000	14 000	30 000
$^{35}\text{S} - \text{SO}_3$	82 000	0	41 000	39 000
$\text{S} - ^{35}\text{SO}_3$	46 000	22 000	50	23 000

Fig. 1. Effect of pH on the thiosulphate–ferricyanide reaction. The reaction mixtures contained 5 μmoles of $\text{K}_3\text{Fe}(\text{CN})_6$, 40 μmoles of $\text{Na}_2\text{S}_2\text{O}_3$ and 2.5 units of thiosulphate-oxidizing enzyme in 5 ml. of 0.12M-buffer (1, phthalate, pH 4.5; 2, phthalate, pH 5.0; 3, phthalate, pH 5.5; 4, phosphate, pH 5.5; 5, phosphate, pH 6.0; 6, phosphate, pH 6.5; 7, phosphate, pH 7.1). Reduction of $\text{K}_3\text{Fe}(\text{CN})_6$ was followed by changes in E at 420 m μ .

appreciable exchange occurred between the inner and outer sulphur atoms, which is in agreement with the results obtained with intact cells (Trudinger, 1959).

Stoichiometry of the thiosulphate–ferricyanide reaction. This is shown in Table 2. The results are in agreement with equation 3.

Fig. 2. Effect of thiosulphate concentration on the thiosulphate–ferricyanide reaction. The reaction mixtures contained $\text{Na}_2\text{S}_2\text{O}_3$, 5 μmoles of $\text{K}_3\text{Fe}(\text{CN})_6$ and 0.6 unit of thiosulphate-oxidizing enzyme in 5 ml. of 0.12 M-potassium phthalate, pH 5.

Reversibility. The thiosulphate–ferricyanide reaction appeared to be irreversible. No thiosulphate was formed when a large excess of tetrathionate was incubated with enzyme and ferrocyanide. Attempts to demonstrate an enzymic exchange of ^{35}S between labelled tetrathionate and thiosulphate in the presence of a catalytic amount of ferricyanide were not successful because of the rapid exchange which occurs spontaneously (cf. Fava, 1953).

Substrate specificity. No reduction of ferricyanide was observed when tetrathionate, trithionate, dithionate or colloidal sulphur replaced thiosulphate under the standard conditions for enzyme assay. Sulphite reduced ferricyanide at the same rate in the presence as in the absence of enzyme.

Fig. 3. Effect of enzyme concentration on the thiosulphate-ferricyanide reaction. The reaction mixtures contained thiosulphate-oxidizing enzyme and 40 μmoles of $\text{Na}_2\text{S}_2\text{O}_3$, with other conditions as shown in Fig. 2.

Table 2. *Stoichiometry of the thiosulphate-ferricyanide reaction*

Enzyme (3.2 units) was incubated with $\text{Na}_2\text{S}_2\text{O}_3$ and $\text{K}_3\text{Fe}(\text{CN})_6$ in 0.12M-phthalate, pH 5, until the reaction was complete (no further change in E_{430}). Results are expressed as μmoles.

Expt. no.	Initial		Final		
	$\text{S}_2\text{O}_3^{2-}$	$\text{Fe}(\text{CN})_6^{3-}$	$\text{S}_2\text{O}_3^{2-}$	$\text{Fe}(\text{CN})_6^{3-}$	$\text{S}_4\text{O}_6^{2-}$
1	5	10	0	5.0	2.55
2	10	5	4.8	0	2.50

Electron acceptors. Methylene blue, phenol blue, diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN) and flavinadenine dinucleotide were not reduced under N_2 by thiosulphate and oxidizing enzyme. Some reduction of dyes of the indophenol series appeared to occur but a chemical reaction between the dyes and thiosulphate masked the reduction process. The reduction of cytochromes will be discussed below.

Effect of some reagents on enzyme activity. Reduction of ferricyanide was not affected by the addition of Mg^{2+} (0.01M), Mn^{2+} (0.01M), quinine hydrochloride (2 mM), CN^- (0.01M), F^- (0.01M), N_3^- (0.01M), diethyl dithiocarbamate (1 mM), iodoacetate (1 mM), 8-hydroxyquinoline (1 mM), glutathione (1 mM), pyridoxal phosphate (10 μg./ml.), coenzyme A (CoA; 10 μg./ml.), cocarboxylase (50 μg./ml.) or a boiled extract of *Thiobacillus X*.

Stability of enzyme. Enzyme activity was completely destroyed by heating at 60° for 3 min. at pH 7. About 40% of its activity was lost after 2 months at -25° in 0.02M-phosphate.

Reaction of thiosulphate-oxidizing enzyme with soluble cytochromes of Thiobacillus X

As only small amounts of material were available, cytochrome reduction was studied with the Beck microspectroscope and the approximate relative rates of reduction were based upon the time taken to reach complete reduction.

Reduction of cytochrome 553.5. Cytochrome 553.5 was reduced by thiosulphate in the presence of the thiosulphate-oxidizing enzyme but not by thiosulphate alone (Table 3). The reduction was more rapid under N_2 than in air, presumably because of the relatively rapid auto-oxidation of this cytochrome (Trudinger, 1961). Potassium cyanide did not increase the rate of cytochrome reduction in air. The anaerobic reaction was not inhibited by CO (saturated) nor by quinine hydrochloride, CN^- , N_3^- , 8-hydroxyquinoline and iodoacetate at the concentrations used previously (see above). No reduction of cytochrome 553.5 occurred when trithionate, tetrathionate or dithionate replaced thiosulphate. Whereas the enzymic reaction between thiosulphate and ferricyanide was maximal in the region of pH 5 (Fig. 1), the rate of reduction of cytochrome 553.5 at this pH was about 10% of the rate at pH 7 (Table 3).

Catalysis of ferricyanide reduction. At pH 7, cytochrome 553.5 catalysed the reduction of ferricyanide by thiosulphate and the thiosulphate-oxidizing enzyme (Fig. 4). The end product was established as tetrathionate by the tests described elsewhere. The reduction of ferricyanide by this system was not affected by the addition of DPN (1 mM), TPN (1 mM), CoA (10 μg./ml.), pyridoxal phosphate (10 μg./ml.) or thiamine pyrophosphate (50 μg./ml.).

In the experiment described in Fig. 4 ferricyanide was reduced at the rate of 0.9 μmole/min./unit of thiosulphate-oxidizing enzyme. This presumably corresponds to the rate of turnover of cytochrome 553.5. The amount of thiosulphate-oxidizing enzyme extracted from 1 mg. of *Thiobacillus X* was about 0.8 unit (Trudinger, 1961), which is equivalent to a rate of cytochrome 553.5 reduction at pH 7 of about 0.7 μmole/min. This is comparable with the rate of oxygen uptake by fresh cells utilizing thiosulphate, which is in the order of 0.3 μg. atom of oxygen/mg. dry wt./min. The unit of thiosulphate-oxidizing enzyme is based on the rate of enzymic reduction of ferricyanide at pH 5 by thiosulphate in the absence of cytochrome (Trudinger, 1961). In the above-mentioned calculation it has been

Table 3. *Reduction of soluble cytochromes by thiosulphate*

Cytochromes were dissolved in 0.1 M-phosphate, pH 7, to give a value for E_{1cm} at α_{max} of approx. 0.2. In the experimental mixtures 0.3 ml. of such a solution was used for the major cytochrome component and 0.02 ml. for the minor component. The additions (0.03 ml.) were 0.2 M- $Na_2S_2O_3$ and thiosulphate-oxidizing enzyme (1 unit/ml.). Oxygen-free nitrogen was bubbled through all solutions before and after mixing. The approximate times (min.), for complete reduction of the major cytochrome component, are recorded. No reduction of cytochromes occurred in the absence of $Na_2S_2O_3$.

Cytochrome		Additions	
Major	Minor	$Na_2S_2O_3$	$Na_2S_2O_3$ and enzyme
557	—	∞ *	∞
557	553.5	∞	1
557	550	8-9	8-9
553.5	—	∞	< 1
553.5†	—	∞	10-11
553.5	550	7-8	< 1
550	—	8-9	8-10
550	553.5	8-9	1
Acidic fraction	—	7-8‡	8-9‡
Acidic fraction	553.5	7-8‡	1-2‡
Mammalian c	—	> 60	15-20
Mammalian c	553.5	> 60	15-20

* No observable reduction in 60 min.

† 0.1 M-Phthalate, pH 5, in place of phosphate.

‡ Both components of the acidic fraction were reduced.

Fig. 4. Catalysis of ferricyanide reduction by cytochrome 553.5. The complete system contained 40 μ moles of $Na_2S_2O_3$, 5 μ moles of $K_3Fe(CN)_6$, 3 units of thiosulphate-oxidizing enzyme and cytochrome 553.5 (E_{1cm} in the reduced form, 0.023 at 553.5 m μ) in 0.05 M-potassium phosphate, pH 7. (1) Complete system; (2) cytochrome omitted; (3) thiosulphate omitted; (4) thiosulphate-oxidizing enzyme omitted.

assumed that the relation between this rate and that of enzymic cytochrome reduction at pH 7 is the same for both crude and purified preparations of the thiosulphate-oxidizing enzyme. Spectral interference by other cytochromes of *Thiobacillus X* prevented a direct determination of cytochrome 553.5 reduction by crude extracts.

Reduction of other soluble cytochromes. Cytochrome 557 (1) became reduced when mixed with thiosulphate, thiosulphate-oxidizing enzyme and a trace of cytochrome 553.5 (Table 3). The omission of either the enzyme or cytochrome 553.5 prevented this reduction.

Cytochrome 550 (1) and the components of the acidic fraction were slowly reduced by thiosulphate alone and the rates of reduction were unchanged on addition of thiosulphate-oxidizing enzyme. However, in the presence of both thiosulphate and oxidizing enzyme, cytochrome 553.5 catalysed the rapid reduction of cytochrome 550 (1) and the acidic fraction components. The rate of reduction of cytochrome 550 (1) by thiosulphate alone was about the same at pH 5, 6 and 7 and was not decreased by additional chromatography of the cytochrome on Amberlite IRC-50. Cytochrome 550 (1) catalysed a slow reduction of cytochromes 553.5 and 557 (1) by thiosulphate in the absence of the thiosulphate-oxidizing enzyme (Table 3).

After denaturation by heating at 60° for 3 min., none of the soluble cytochromes of *Thiobacillus X* was reduced by thiosulphate with or without the oxidizing enzyme.

The thiosulphate-oxidizing enzyme also catalysed a slow reduction of mammalian cytochrome c by thiosulphate (Table 3). This reduction was not stimulated by cytochrome 553.5.

Oxidation of cytochromes and thiosulphate by a particulate system

As described in the preceding paper, the material precipitated from crude extracts of *Thiobacillus X* between 8000 and 145 000 g (fraction P_2) contained several cytochrome components. The cytochromes of fraction P_2 , prepared from fresh cells, were rapidly reoxidized in air after reduction with a trace of $Na_2S_2O_4$. Fraction P_2 from fresh cells also catalysed the oxidation of the reduced soluble cytochromes 550 (1), 553.5 and 557 (1), the components of the acidic fraction and mammalian cytochrome c. In all cases the oxidations were completely inhibited by 0.01 M-KCN.

In the presence of KCN or in the absence of oxygen, the particulate cytochromes became reduced by endogenous substrates within 2-3 min. The particles then showed a spectrum similar to that shown in the presence of $Na_2S_2O_4$ (Trudinger, 1961), with absorption bands in the regions of 550, 552-

Table 4. *Oxidation of thiosulphate by a soluble system and particles*

The manometric flask contained 6.5 units of purified thiosulphate-oxidizing enzyme, cytochrome 553.5 (E_{1cm} at 553.5 m μ , approx. 0.1), 0.5 ml. of fraction P₂* and 40 μ -moles of thiosulphate in 2 ml. of 0.1 M-phosphate, pH 7. The centre well contained 0.2 ml. of 2N-NaOH. Temperature 30°.

System	Oxygen absorbed (μ l./60 min.)
Complete	103
Cytochrome 553.5 replaced by cytochrome 550 (1)	45
Cytochrome 553.5 replaced by cytochrome 557 (1)	36
Cytochrome 553.5 replaced by acidic fraction	39
Complete + cytochrome 550 (1) + cytochrome 557 (1)	110
Complete + 0.01 M-KCN	1
Cytochrome 553.5 omitted	40
Enzyme omitted	37
Thiosulphate omitted	35
Fraction P ₂ omitted	3

* Particles from 100 mg. dry wt. of fresh cells in 5 ml. of buffer: 10 mg. dry wt. of fresh cells oxidized thiosulphate (under similar conditions) at the initial rate of 1780 μ l. of O₂ absorbed/60 min.

558 and 562 m μ . The addition of thiosulphate and thiosulphate-oxidizing enzyme, with or without traces of the soluble cytochromes of *Thiobacillus X*, did not appear to increase the rates of reduction of any of the particulate cytochromes, anaerobically or in the presence of KCN. However, aerobically in the absence of KCN, the 562 m μ -absorption band appeared when fraction P₂ was mixed with thiosulphate, oxidizing enzyme and a trace of cytochrome 553.5.

Thiosulphate was oxidized, with the uptake of oxygen by a system with thiosulphate-oxidizing enzyme, cytochrome 553.5 and fraction P₂ (Table 4). None of the other soluble cytochromes of *Thiobacillus X* could replace cytochrome 553.5 nor did their addition to the complete system increase the rate of oxygen uptake to any marked extent. In the experiment described in Table 4 the complete system oxidized 11.5 μ moles of thiosulphate in 60 min., and 5.6 μ moles of tetrathionate were recovered. The oxygen absorbed, corrected for the amount taken up in the absence of thiosulphate, was 3.0 μ moles, compared with the theoretical value of 2.9 μ moles for oxidation of 11.5 μ moles of thiosulphate to tetrathionate. The rate of oxidation of thiosulphate by this system was low, being about 4% of that of an equivalent amount of intact cells, but no attempt has yet been made to establish optimum conditions for the preparation of active particles.

DISCUSSION

The results presented in this paper indicate that cytochrome 553.5 and the thiosulphate-oxidizing enzyme are involved in thiosulphate oxidation by *Thiobacillus X*. Moreover the amount of enzyme extracted appears to be sufficient to account for the rates of thiosulphate oxidation by intact cells. It is not possible to say whether cytochrome 553.5 reacts directly with thiosulphate-oxidizing enzyme. No evidence was obtained for the presence of an intermediate electron acceptor, but it is probable that both the cytochrome and enzyme preparations were very impure and the possibility of such intermediaries in one or both preparations cannot be excluded.

The particles contain a cyanide-sensitive oxidase for the soluble *Thiobacillus X* cytochromes but otherwise little can be said of the electron-transport chain present in this fraction. On reduction with dithionite the particles exhibit absorption bands in the regions of 550 and 552–558 m μ but spectroscopic studies provided no evidence for the participation of these particulate components in thiosulphate oxidation. The particles also contain a component, with an absorption maximum in the reduced state at 562 m μ , which became partly reduced on the addition of thiosulphate, thiosulphate-oxidizing enzyme and cytochrome 553.5. Should this be a cytochrome of the 'b' type, as its spectrum indicates, it is unlikely, in view of current concepts of respiratory chains (Chance & Williams, 1956), that it is part of the electron-transport chain between the 'c' type cytochrome 553.5 and oxygen.

The soluble cytochromes 550 (1), 557 (1) and the components of the acidic fraction equilibrate rapidly with cytochrome 553.5 and are readily oxidized by the particles. They are capable therefore of taking part in electron transfer from thiosulphate to oxygen. The fact that the rate of oxidation of thiosulphate, by the system consisting of thiosulphate-oxidizing enzyme, cytochrome 553.5 and particles, was very much less than the rate of reduction of cytochrome 553.5 indicates that the rate-limiting step was in the particulate electron-transport chain. This probably explains why no stimulation of oxygen uptake was obtained on the addition of cytochromes 550 (1), 557 (1) or the acidic fraction, particularly if a common step is involved in the oxidation of all the soluble cytochromes. The extent to which the various cytochromes are involved in thiosulphate oxidation depends on the relative rates of oxidation and reduction. Such factors cannot be assessed at this stage.

Cytochrome 550 (1) is also reduced by thiosulphate alone but this reaction is too slow to account for much thiosulphate oxidation. It is

Fig. 5. Summary of electron transport between thiosulphate and oxygen. Arrows indicate the routes of electron transfer demonstrated in this work. The summary is over-simplified to the extent that the cytochromes of the acidic fraction are not included.

unlikely that the cytochrome was contaminated with thiosulphate-oxidizing enzyme for the following reasons: (a) the rate of reduction was independent of pH between 5 and 7; (b) addition of the separated enzyme did not increase the rate of cytochrome reduction by thiosulphate; (c) the rate of reduction was not affected by storage nor by further treatment of the cytochrome with IRC-50. Fig. 5 summarizes the results obtained and indicates possible electron paths during the oxidation of thiosulphate.

The term 'thiosulphate-oxidizing enzyme' has been used in this paper in preference to 'tetrathionase', applied by other workers to enzyme systems converting thiosulphate into tetrathionate (Baalsrud, 1954; Lees, 1955), since the latter term has also been used by Knox & Pollock (1944) for the enzyme system in some heterotrophic organisms, that reduces tetrathionate to thiosulphate. Baalsrud & Baalsrud (quoted by van Niel, 1953) have shown that some heterotrophs are capable of carrying out both the oxidation of thiosulphate and reduction of tetrathionate under aerobic and anaerobic conditions respectively, but there is no evidence that the same enzyme system is involved in both reactions. In the present work no evidence was obtained for the reduction of tetrathionate by the thiosulphate-oxidizing enzyme of *Thiobacillus X*. However, anaerobically, intact *Thiobacillus X* catalyses a rapid conversion of tetrathionate into thiosulphate and other products (P. A. Trudinger, unpublished results). It remains to be seen whether thiosulphate-oxidizing enzyme is involved in this reaction.

SUMMARY

1. Some properties of the thiosulphate-oxidizing enzyme from the aerobic sulphur autotroph *Thiobacillus X* are described.

2. The enzyme catalyses the oxidation of thiosulphate to tetrathionate with ferricyanide or

Thiobacillus X cytochrome 553.5 as electron acceptors. Other soluble cytochromes from the same bacterium do not react directly with thiosulphate-oxidizing enzyme but become reduced in the presence of thiosulphate, enzyme and cytochrome 553.5.

3. A particulate preparation from *Thiobacillus X* oxidizes the reduced soluble cytochromes 550 (1), 553.5, 557 (1) and the components of the acidic fraction. In the presence of cytochrome 553.5 and thiosulphate-oxidizing enzyme, the particles oxidize thiosulphate to tetrathionate with the consumption of oxygen.

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