THE OXIDATION OF INORGANIC SULPHUR COMPOUNDS
IN RELATION TO DENITRIFICATION IN
THIOBACILLUS DENITRIFICANS

by

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SUMMARY

1. The work described in this thesis is mainly concerned with the metabolism of inorganic sulphur and nitrogen compounds and their inter-relationships in the chemoaautotrophic bacterium, Thiobacillus denitrificans.

2. Cells and crude extracts (S10) catalysed the enzymic oxidation of sulphide, which may be coupled via the respiratory chain to either oxygen, nitrate or nitrite as terminal electron acceptors. Enzyme activity was associated mainly with membrane fraction (P144).

Cell suspensions reduced nitrate and nitrite to NO, N₂O and N₂ gases when sulphide was the electron donor. Sulphide-linked nitrate reductase was detected in crude extracts (S10) but the P144 and S144 fractions catalysed the reaction only when they were recombined. Sulphide linked nitrite reductase activity, located mainly in the P144 fraction, had a pH optimum of 7.5 with one mole of sulphide oxidized per mole of nitrite reduced.

The initial product of sulphide oxidation was a membrane-bound polysulphide. In the absence of either nitrate or nitrite, sulphide was oxidized to polysulphide and sulphite. When nitrate was present sulphide was oxidized to sulphate with
a concomitant reduction of nitrate to nitrite. Under anaerobic conditions and in the presence of nitrite, sulphide was oxidized to polysulphide only. The formation of this membrane-bound polysulphide was inhibited by CO.

3. The oxidation of elemental sulphur was catalysed by an enzyme present in the S144 fraction; GSH and a low molecular weight, heat-stable factor were required for the reaction. Sulphur oxidation may be linked to oxygen uptake in the S144 fraction and to nitrate reduction in the crude extracts (S10). The initial product of sulphur oxidation was sulphite.

4. There are two sulphite oxidizing enzyme systems, namely a soluble APS-reductase and a particulate, AMP-independent sulphite oxidase. The latter enzyme may be linked to either oxygen uptake, the reduction of ferricyanide or nitrate reduction while the former was linked to ferricyanide only.

Oxygen uptake coupled to sulphite oxidation in the P144 fraction was not affected by AMP but was inhibited non-competitively by nitrate.

Sulphite oxidase and APS-reductase were purified and their properties compared.
5. Thiosulphate oxidation located in the membrane fraction may be linked to either oxygen uptake or nitrate reduction. Thiosulphate utilised oxygen only after adding GSH whereas nitrate reduction occurred with thiosulphate alone. 

$^{35}\text{S-SO}_3^-$ was oxidized to $^{35}\text{S-sulphide}$, $^{35}\text{S-polysulphide}$, $^{35}\text{S-tetrathionate}$ and $^{35}\text{S-sulphate}$. In the presence of GSH $^{35}\text{S-SO}_3^-$ was oxidized mainly to $^{35}\text{S-sulphite}$ but when nitrate was substituted for GSH, the main products were $^{35}\text{S-tetrathionate}$ and $^{35}\text{S-sulphate}$.

6. Nitrate was oxidized to nitrite by cell suspensions and crude extracts (S10) when thiosulphate, sulphide, sulphite and NADH were the electron donors. These compounds also reduced nitrite in intact cells but in crude extracts nitrite was reduced by sulphide and NADH only.

Cytochromes of the $\alpha$, $\alpha$ and $\delta$ types were detected in crude extracts. In the membrane fraction (P144) cytochromes of the $\alpha$ and $\delta$ types reduced by sulphide under anaerobic conditions were reoxidized by either oxygen or nitrite. Sulphite, however, reduced only cytochrome $\alpha$ which was reoxidized by nitrate and oxygen but not by nitrite. In the S144 fraction cytochromes of the $\alpha$ and $\alpha$ types reduced by sulphite were reoxidized by oxygen but nitrate and nitrite were ineffective. Cytochromes of the $\alpha$
and d types combined with CO and these effects were reversed by light.

7. Particulate fractions (P144) catalysed the phosphorylation of ADP to ATP during the oxidation of either various inorganic sulphur compounds or NADH. The production of ATP was verified by the firefly luciferin-luciferase enzyme as well as by following the incorporation of $^{32}$P into ATP. During the oxidation of either sulphide, sulphite or NADH, ATP production was inhibited by 2,4-dinitrophenol and oligomycin as well as by compounds that restrict electron transfer. Under anaerobic conditions, intact cells produced ATP during either sulphide oxidation linked to nitrite reduction or the oxidation of sulphite coupled to the reduction of nitrate.

In the S144 fraction, ATP was formed from APS and Pi. The S144 fraction contained high activities of ATP-sulphurylase, inorganic pyrophosphatase and adenylyl kinase but ADP-sulphurylase activity was relatively low. The contribution of these enzymes to substrate level phosphorylation was investigated.

8. ATP-sulphurylase was purified about 250-fold and some of the properties of the enzyme were studied.