SOME ASPECTS OF INORGANIC SULPHUR METABOLISM

Part I. Production and Degradation of Sulphur Nucleotides in Microorganisms and Plants

Part II. Uptake and Utilization of Inorganic Sulphur Compounds in *Thiobacillus ferrooxidans*

by

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SUMMARY

PART I. Production and Degradation of Sulphur Nucleotides in Microorganisms and Plants

1. A rapid, sensitive, bioluminescence technique has been developed for detecting PAPS (adenosine 3'-phosphate 5'-phosphosulphate) in biological materials. PAPS is first hydrolysed in 0.2 N HCl to PAP (adenosine 3'-phosphate 5'-phosphate) and is then assayed by the luciferin-luciferase system of the sea pansy, *Renilla reniformis*, which is specific for PAP. This bioluminescence system produces light at a rate that is proportional to the amount of PAP present. Light emission is measured in a liquid scintillation spectrometer with the two photomultipliers out of coincidence. Amounts as low as 10 pmoles PAP may be determined by this procedure.

2. The method has been used to follow PAPS formation from sulphate and ATP in a variety of biological materials. Thus, PAPS produced was detected in very small amounts (50-180 pmoles/min/mg protein) in extracts of green plants. On adding 5'-AMP to the reaction mixture, the production of PAPS was enhanced about ten-fold.

PAPS produced in extracts of baker's yeast from (a) sulphate and ATP and (b) APS and ATP was readily monitored by the bioluminescence method.

3. PAPS was rapidly degraded in extracts of young wheat leaves and baker's yeast via APS to sulphate whereas, in cell-free extracts of *Thiobacillus ferrooxidans*, sulphate was cleaved first from PAPS. The

fate of APS was also investigated in extracts of this bacterium. The stabilizing effects of nucleotides, phosphate and pyrophosphate on the degradation of these sulphur nucleotides were examined.

PART 2. Uptake and Utilization of Inorganic Sulphur Compounds in *Thiobacillus ferrooxidans*

1. Differentially labelled ^{35}S-thiosulphate [$^{35}S.SO_3$, $S.^{35}SO_3$] was taken up by washed cells of *Thiobacillus ferrooxidans* grown on thiosulphate. The uptake, which was proportional to the biomass over the range 0.5-4.0 mg dry weight, showed typical saturation kinetics, with an estimated K_m value of 0.5 mM for thiosulphate. Dithionate and Group VI anions inhibited the uptake, which was under pH control and had a temperature optimum of 50°.

In the absence of thiosulphate, the cells rapidly bound ^{35}S-sulphate (within 15 seconds) but this effect did not increase on incubating further. Moreover, the label was removed completely by washing with dilute sulphuric acid, indicating that sulphate was not assimilated.

Over a 60 minute period, increasing amounts of the label were incorporated into cellular materials from the [outer-^{35}S]thiosulphate. There was little or no incorporation of the label from the [inner-^{35}S]thiosulphate.

2. The following enzymes which mediate the oxidation of thiosulphate to sulphate and the assimilation of sulphate to sulphide were assayed in various cell-free fractions of *Thiobacillus ferrooxidans* grown autotrophically with either ferrous-iron or thiosulphate, or

heterotrophically with glucose: Thiosulphate-oxidizing enzyme, rhodanese, AMP-independent sulphite oxidase, APS-reductase, ATP-sulphurylase, ADP-sulphurylase, adenylate kinase, and NADPH-linked sulphite reductase. Thiosulphate-oxidizing enzyme was not detected in extracts of bacteria grown with ferrous-iron. Comparable activities for ATP-sulphurylase, ADP-sulphurylase and adenylate kinase were found in bacteria grown autotrophically with either ferrous-iron or thiosulphate and heterotrophically with glucose.

3. The kinetic properties of partially purified ATP-sulphurylase from bacteria grown with thiosulphate and ferrous-iron were similar. This enzyme has an assimilatory function only when the bacteria are grown with ferrous-iron. The possible function of the enzyme in thiosulphate-grown cells is discussed.