Short Communications and Preliminary Notes

INTERMEDIARY METABOLISM OF CYSTEINESULFINIC ACID IN CELL-FREE EXTRACTS OF *PROTEUS VULGARIS**

by

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Apart from the early observations of MEDES[1] and of MEDES AND FLOYD[2] on the oxidation of cysteinesulfinic acid by washed particles from rat liver, little appears to be known about the aerobic metabolism of this compound, although physiological evidence[3] and the presence of highly active enzymes for its oxidation in bacteria suggest that it is an important intermediate in sulfur metabolism. Under anaerobic conditions cysteinesulfinic acid is known to be desulfinicated[4] and decarboxylated[5]. We have undertaken a systematic investigation of the intermediary metabolism of this compound in bacteria and the results hitherto obtained are summarized below.

Proteus vulgaris, strain OX-19 (from the collection of the Pasteur Institute) was grown on a tryptone-beef extract agar medium for 18–20 hours at 37° and the harvested cells were thrice washed with distilled water. The resulting cell suspension rapidly oxidizes L-cysteinesulfinic acid and L-cysteic acid at 35°; the Q_{O_2} of a typical preparation on the former substrate is 41 (corrected for endogenous respiration) and optimal rate is obtained in tris(hydroxymethyl)aminomethane buffer between pH 8 and 8.5. Addition of 2×10^{-3} *M* Mn^{++} greatly increases the rate.

While L-cysteinesulfinic acid appears to be essentially oxidized to CO_2 and H_2O, the initially rapid oxidation of L-cysteic acid comes to a halt after 1 atom of O_2 has been consumed, indicating conversion to β-sulfonylpyruvic acid.

Cell-free extracts of *Proteus vulgaris* were obtained by ultrasonic disintegration of the washed cells at 960 kilocycles for 13 to 15 minutes at 0°, followed by centrifugation at 13,000 r.p.m. The clear supernatant solution was preserved in the lyophilized state at 0°. Such enzyme preparations are completely inert in manometric measurements toward both cysteinesulfinic and cysteic acids. A rapid and linear oxidation of cysteinesulfinic acid is observed, however, when the enzyme is supplemented with boiled bakers' yeast extract and brilliant cresyl blue. The Q_{O_2} under these conditions exceeds that of the whole cells, indicating that the dehydrogenases are intact in the preparation and that only cofactors and a suitable electron transfer system are missing.

Detailed analysis of the reaction products, possible intermediates and O_2 yields per mole of substrate revealed the existence in our enzyme preparation of two competing pathways for the metabolism of cysteinesulfinic acid. This amino acid is in part oxidized to cysteic acid with the uptake of 1 atom of O_2 (pathway *A*). Cysteic acid is not further oxidized. The second pathway (*B*) is via transamination of cysteinesulfinic acid with α-ketoglutaric acid and oxaloacetic acid to give β-sulfinylpyruvic acid. A close analogue of oxaloacetic acid, β-sulfinylpyruvic acid is similarly unstable and, in the presence of Mn^{++}, it breaks down to pyruvate and sulfite and the latter is oxidized to sulfate. While the desulfinication of the keto-acid and the oxidation of sulfite are rapid, non-enzymatic reactions in our extracts, it is conceivable that, as with oxaloacetic acid, these reactions may be enzymatic in the bacterial cell.

No evidence has been found for aerobic deamination, anaerobic decarboxylation or desulfinication of cysteinesulfinic acid, and only a very slight anaerobic deamination has been ascertained. Cysteic acid is slowly transaminated with α-ketoglutaric acid, but the resulting β-sulfonylpyruvate is not metabolized.

It has been established that the boiled yeast extract supplies for these reactions the following components: for pathway (*A*) a new coenzyme of pyridine nucleotide nature (coenzyme III, described in the succeeding paper[6]), as well as an additional cofactor for the diaphorase involved in hydrogen

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transport; for pathway (*B*) a limited amount of α-keto acids, needed in the transamination step, and Mn^{++} for the subsequent reactions. In addition, in the presence of the small quantities of α-keto acids present in yeast extract, TPN is supplied by the latter for a TPN-specific L-glutamic dehydrogenase, which slowly regenerates the α-ketoglutaric acid from the glutamic acid formed by transamination. These relations are summarized in Fig. 1.

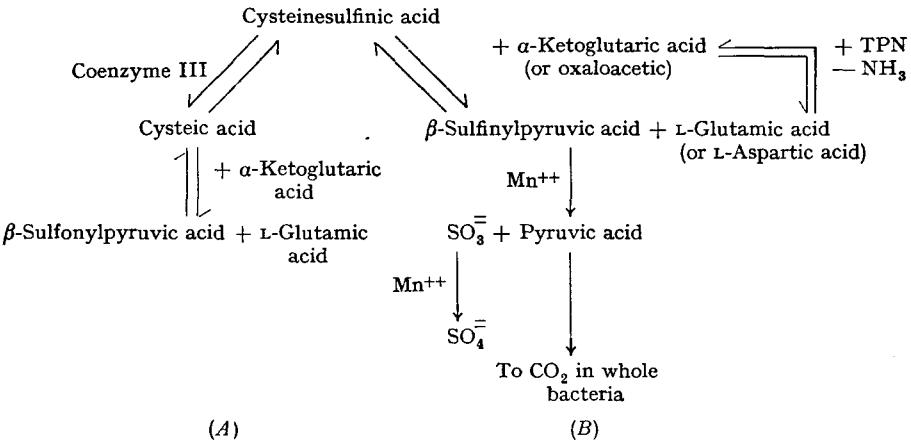

Fig. 1. Pathway of metabolism of cysteinesulfinic acid in *Proteus vulgaris*

Pathway (*B*) is completely eliminated by 2×10^{-2} *M* semicarbazide, which binds the keto acids needed for transamination. Under these conditions the enzyme system fortified with yeast extract takes up only 1 atom of O_2 and the formation of cysteic acid can be demonstrated by filter paper chromatography. The coenzyme III activity of the crude yeast extract is retained throughout extensive purification (cf. next paper) and such purified preparations can be used to demonstrate the oxidation to cysteic acid both manometrically and spectrophotometrically.

Pathway (*B*) may be studied in the absence of (*A*) by treatment of crude yeast extracts with highly purified nucleotide pyrophosphatase[7], which destroys coenzyme III. In the absence of yeast extract, pathway (*B*); i.e., transamination to β-sulfinylpyruvic acid, desulfinication, and oxidation of sulfite can be studied in a simplified system consisting of enzyme, α-ketoglutaric acid, Mn^{++}, and L-cysteinesulfinic acid. Under these conditions the accumulation of glutamate can be demonstrated by paper chromatography. Aerobically, with brilliant cresyl blue as carrier, 1 atom of O_2 is taken up per mole of L-cysteinesulfinic acid oxidized, while anaerobically with ferricyanide as oxidant in $NaHCO_3$–CO_2 buffer 3 moles of CO_2 are liberated, indicating the transfer of 2 electrons and the formation of 1 acid group (inorganic sulfate). Pyruvic acid is not oxidized by the enzyme preparation.

The kinetics and the characteristics of the individual enzymatic reactions involved will be reported in detailed communications. Future work is aimed at establishing whether similar pathways play a role in the sulfur metabolism of other microorganisms and of animal tissues.

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