THE MODE OF UTILIZATION OF CYSTEINE SULFINIC ACID BY BACTERIA[1]

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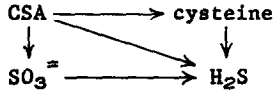
Cysteine sulfinic acid (CSA) serves efficiently as a source of sulfur for a wide variety of microorganisms. While it is clear that CSA may be an intermediate in the oxidative catabolism of both higher animals and lower life forms (Singer and Kearney, 1955), the sequence of reactions leading to the utilization of the sulfur atom of CSA for the synthesis of cysteine has not been clearly established. The results of the present investigations indicate that the sole pathway for CSA metabolism in Escherichia coli may be described as follows:

$$CSA \longrightarrow SO_3^{=} \longrightarrow S^{=}$$

Furthermore, the organism lacks an enzyme for the specific purpose of releasing sulfite from CSA. This process is catalyzed by an enzyme which may be referred to as a glutamate-aspartate transaminase. Preliminary evidence indicates that the utilization of CSA in Salmonella typhimurium (strain LT-2) follows this same pattern.

Establishment of the pathway for CSA metabolism in E. coli was based on considerations of the following diagram describing possible pathways for the formation of H_2S from CSA:



The maximum rates of formation of H_2S from the indicated precursors were

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measured with cells of E. coli strain B harvested in the logarithmic phase of aerobic growth on a minimal medium in which sulfate served as sole sulfur source. The data of Table 1 demonstrate that exactly additive rates of H_2S formation were obtained from CSA and cysteine, and that the rate obtained from sulfite alone could not be increased by the addition of CSA to the incubation medium. Failure to obtain additive rates from the combination of sulfite and cysteine was explained by the finding that sulfite inhibits the cysteine desulfhydrase activity of E. coli in cell-free preparations.

Table 1

Rates of H_2S Formation by E. coli in vivo.

Substrate	Maximum rate of sulfide formation (mμmoles/mg. protein/15 min.)		
Experiment	1	2	3
Cysteine	97.8		147
CSA	26.9		29.3
Cysteine + CSA	122		176
CSA	24.9	22.2	
Sulfite	41.7	38.0	
CSA + Sulfite	41.4	36.9	
Cysteine		101	118
Sulfite		35.3	45.6
Cysteine + Sulfite		97	108

Incubation mixtures contained 5 ml. of minimal medium, an aliquot of bacterial culture, and the sulfur-containing substrate at saturating concentration (7.5×10^{-4} M) in a volume of 12.6 ml.

Incubation was at 37° C for 15 min. H_2S was removed continuously with a stream of N_2 and determined by the method of Fogo and Popowsky (1949).

From the foregoing it is concluded that sulfite is an obligate intermediate in the formation of H_2S from CSA. If any alternate pathway exists, seemingly it would be of negligible quantitative significance. That this situation obtains in Salmonella was demonstrated in like fashion, but using cell-free preparations. Permeability restrictions apparently interfered with whole-cell experiments with this organism.

Reconstruction of the pathway for the formation of H_2S from CSA was achieved with two protein fractions obtained from E. coli disrupted by sonic

oscillation. One of these, collected by 35-50% saturation with $(NH_4)_2SO_4$, appeared identical with the sulfite reductase obtained from this organism by Mager (1960). This fraction required TPNH, but no other apparent factors, for the formation of H_2S from sulfite. The second fraction, obtained by 50-80% saturation with $(NH_4)_2SO_4$, would release sulfite from CSA, the maximum rate being achieved in the presence of pyridoxal phosphate and α-ketoglutarate, although oxalacetate would satisfactorily replace the latter. The reaction proceeded at near optimum rate over the range of pH 7-9, and would proceed to the complete disruption of CSA over this range. The kinetics remained first order with respect to CSA concentration throughout the course of this reaction.

The assignment of the "desulfinase" activity of E. coli to a glutamate-aspartate transaminase is based upon considerations of the competitive relationships between amino acids in the pyridoxal phosphate dependent reactions catalyzed by the 50-80% $(NH_4)_2SO_4$ fraction. These are summarized in Table 2. In each case, the K_I for an amino acid was found to agree very favorably with the K_M for that same amino acid. Advantage was taken of the effects of both temperature and pH on the magnitude of the dissociation constants in establishing the validity of the latter statement.

Table 2

Competitive Relations Between Glutamate, Aspartate and CSA.

Reaction Observed	Competitively Inhibited By
CSA + α-ketoglutarate → $SO_3^=$	aspartate
CSA + oxalacetate → $SO_3^=$	glutamate
aspartate + α-ketoglutarate → oxalacetate	CSA

The foregoing data indicate that glutamate, aspartate and CSA are accepted as substrates, not only by a common enzyme, but at a common reactive site. Recognition of the structural similarities between these compounds

(Meister, 1957) makes this finding somewhat less surprising. Preliminary investigations have revealed that aspartate and glutamate inhibit competitively the release of sulfite from CSA in extracts of Salmonella as well. The lack of a unique pathway for CSA metabolism in this organism was suggested by the nutritional behavior of cysteine-less mutants (Clowes, 1958).

The earlier findings of Cobey and Handler (1956) on the formation of sulfite from CSA in extracts of E. coli are undoubtedly closely related to the present data. Support is also gained for the suggestion that the desulfination of CSA by preparations from pig heart and from Proteus vulgaris is catalyzed by a glutamate-aspartate transaminase (Singer and Kearney, 1955). Furthermore, the present findings justify the expediency of substituting CSA for sulfite in growth media for E. coli and S. typhimurium, thereby avoiding problems introduced by the relatively great instability and toxicity of sulfite. Through the use of experiments like those described above, criteria for establishing the equation of CSA and sulfite in the nutrition of other organisms may be easily fulfilled.

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