PATHWAYS OF L-CYSTEINESULFINATE METABOLISM IN ANIMAL TISSUES*

by

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The authors have previously shown[1,2] that in ultrasonic extracts and in intact cells of *Proteus vulgaris* the metabolism of L-cysteinesulfinate (L-CSA) proceeds by way of (a) a rapid transamination with α-ketoglutarate to glutamate and β-sulfinylpyruvate, followed by cleavage of the latter to pyruvate and sulfite and oxidation to sulfate, and (b) a competing pathway of lesser quantitative importance, which involves a slow dehydrogenation of CSA to cysteic acid.

Studies in FROMAGEOT's laboratory[3,4] demonstrated that rabbit liver extracts slowly decarboxylate L-CSA to hypotaurine and rapidly convert it to alanine and sulfite. The latter reaction was recently shown[5] to involve preliminary transamination of CSA with α-ketoglutarate as in *Proteus* extracts.

During the past 2 years the authors have made an extensive study of the fate of L-CSA in a variety of animal tissues, particularly in mitochondrial preparations, wherein most of the metabolic activity toward this amino acid is located. The high rate of L-CSA metabolism in animal tissues leaves little doubt that this metabolite is a major connecting link between sulfur-containing amino acids and the tricarboxylic acid cycle. In all cases studied, the oxidation of CSA is initiated by a rapid transamination with either α-ketoglutarate or oxaloacetate, as in *P. vulgaris*[2]. The rapidity and completeness** of the transamination of CSA with oxaloacetate makes possible the efficient oxidation of precursors of oxaloacetate, such as fumarate and malate (Table I, exp. 1), which alone are not appreciably oxidized by these preparations because of equilibrium considerations. The overall reactions of L-CSA in mitochondrial acetone powders and in soluble extracts thereof is described by equations (1) to (2a) and their stoichiometry is documented in Table I (exps. 1 and 2).

$$\text{L-CSA} + \alpha\text{-Ketoglutarate} + 0.5\ O_2 + H_2O \longrightarrow \text{Pyruvate} + SO_4^{=} + \text{L-Glutamate} + H^+ \quad (1)$$

$$\text{L-CSA} + \text{Oxaloacetate} + 0.5\ O_2 + H_2O \longrightarrow \text{Pyruvate} + SO_4^{=} + \text{L-Aspartate} + H^+ \quad (2)$$

$$\text{L-CSA} + \text{L-Malate (or Fumarate} + H_2O) + O_2 + H_2O \xrightarrow{\text{DPN}} \text{Pyruvate} + SO_4^{=} + \text{L-Aspartate} + H^+ \quad (2a)$$

The possible role of the classical aspartic-α-ketoglutaric transaminase in the above reactions is suggested by the observation that this enzyme, prepared by the method of CAMMARATA AND COHEN[6] to the highest stage of purification, catalyzes the transamination of L-CSA with α-ketoglutarate faster than the corresponding reaction with L-aspartate. (We are grateful to Dr. S. GRISOLIA for a preparation of this enzyme.)

β-Sulfinylpyruvate, the primary product of transaminations involving L-CSA, does not accumulate in the mitochondrial extracts, even in the presence of ethylenediaminetetraacetate (EDTA) to bind traces of Mn^{++}. Instead, pyruvate and sulfite accumulate in beef heart mitochondrial extracts, whereas in rat liver preparations pyruvate and sulfate are the only demonstrable products.

The question of whether $SO_4^{=}$ arises from β-sulfinylpyruvate by oxidation of the latter to β-sulfonylpyruvate and cleavage to sulfate or by desulfination followed by oxidation of the sulfite to sulfate has been answered as follows. An analytically pure sample of β-sulfonylpyruvate (kindly provided by Dr. A. MEISTER) was not cleaved to pyruvate by any of the enzyme preparations from animal tissues or *Proteus*, nor was the accumulation of this compound observed in reactions (1) and (2). This rules out β-sulfonylpyruvate as an intermediate in the oxidation of L-CSA and implicates cleavage of β-sulfinylpyruvate to SO_2 and pyruvate as the reaction mechanism, as in *Proteus*[2] and

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** With excess L-CSA and limiting α-ketoglutarate transamination comes to a halt when approximately 40 to 50% of the α-ketoglutarate is converted to glutamate (Table I, line 2), even though β-sulfinylpyruvate is removed as fast as it is formed. Failure of the α-ketoglutarate-aspartate reaction to proceed to completion when α-ketoglutarate is limiting, despite rapid and continuous removal of oxaloacetate, has been independently observed by GRISOLIA[9]. In contrast, the transamination of L-CSA with oxaloacetate proceeds to completion without difficulty (Table I, line 1).
rabbit liver[5]. Furthermore, the characteristics and extent of the O_2 uptake in reactions (1) and (2) parallel exactly those of the enzymic oxidation of sulfite in liver preparations.

TABLE I

Additions	*O_2 uptake* μ atoms*	*CSA removed μM*	*Aspartate formed μM*	*Glutamate formed μM*	*Pyruvate formed μM*	*NH_3 formed μM*
1. 10 μM Fumarate + 15 μM L-CSA	16.5	10.2	9.1	—	9.9	0
2. 10 μM α-Ketoglutarate + 15 μM L-CSA	4.6	—	—	4.4	4.2	0
3. 15 μM L-CSA	12.6	15	0	0	12.5	12.3

* At cessation of O_2 uptake. Note that in the presence of methylene blue, as used here, the oxidation of sulfite to sulfate by the enzyme system is incomplete, giving rise to only 0.6–0.8 atoms O_2 per mole. L-CSA and aspartate were determined, following chromatography on Dowex-50 columns, by means of ninhydrin and chloramine T, respectively. Glutamate was determined by the purified decarboxylase from *E. coli*, pyruvate by means of 2,4-dinitrophenylhydrazine and also with lactic dehydrogenase, and NH_3 by aeration, followed by Nesslerization. In exps. 1 and 2, the slight O_2 uptake, CSA removal, and pyruvate formation due to reactions (3–4) have been subtracted.

Conditions, 12 mg rat liver mitochondrial acetone powder in experiments 1 and 2, 24 mg in exp. 3; 0.05 *M* phosphate buffer, pH 7.6, 1.2 μM DPN (exps. 1 and 3), and 1 mg methylene blue; temperature, 38°.

HEIMBERG *et al.*[7] have reported that the enzymic oxidation of sulfite to sulfate in partially purified preparations (Q_{O_2} = 44)[7] of whole rat liver has a pH optimum of 9.3, shows a pronounced induction period, and is inhibited by EDTA[8]. In the authors' laboratory the oxidation of sulfite was studied in extracts of rat liver mitochondria (Q_{O_2} = 60), wherein the enzyme appears to be localized. The pH optimum was observed to be about 7.3; EDTA was not inhibitory; and there was no indication of a lag period in sulfite oxidation. Despite these apparent differences, in view of the complex nature of sulfite oxidation, one cannot state definitely as yet that different enzymes are involved. In beef heart mitochondrial acetone powders sulfite oxidase is weak or absent and *Proteus vulgaris* extracts possess only a very feeble oxidase, which, unlike the animal enzyme, requires methylene blue.

In addition to the reactions above, a non-transaminative type of oxidation of CSA has also been observed in rat liver mitochondrial acetone powders. The reaction requires only DPN and an autooxidizable dye (diaphorase is present in the preparation). The reaction products are pyruvate, $SO_4^=$, and NH_3; no amino acid accumulates in the reaction (paper chromatography). The reaction has been observed only in rat liver mitochondria; it is relatively slow compared with reactions (1) and (2), and the activity of the enzyme shows considerable variation in different preparations. The simplest interpretation of its stoichiometry (Table I, exp. 3) is that it is an anaerobic pyridine nucleotide dehydrogenase, analogous to glutamic dehydrogenase, and that the reaction sequence involved is:

$$\text{L-CSA} + \text{DPN} \longrightarrow \beta\text{-Sulfinylpyruvate} + NH_3 + \text{DPNH} + H^+ \quad (3)$$

$$\beta\text{-Sulfinylpyruvate} \longrightarrow \text{Pyruvate} + SO_3^= \xrightarrow{0.5\ O_2} SO_4^= \quad (4)$$

Only traces of cysteic acid were detected when L-CSA was oxidized by rat liver mitochondrial acetone powder, and no hypotaurine accumulated under the experimental conditions.

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