THE ENZYMATIC OXIDATION OF CYSTEINE TO CYSTEINESULFINATE IN RAT LIVER

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Cysteinesulfinate is considered to be an important intermediate in the catabolism of cysteine and cystine but very little is known about the mechanism of the in vivo oxidation of cysteine to cysteinesulfinate. We have been interested in the mechanism of biological oxidation of cysteine in connection with studies on the metabolism of sulfur containing radioprotective agents. The present paper reports an assay system for the enzyme system responsible for the oxidation of cysteine to cysteinesulfinate and its co-factor requirements.

MATERIALS AND METHODS

S^{35}-labelled l-cystine (obtained from the Radiochemical Centre, Amersham or Schwartz Biochemicals Inc.) was converted to cysteine by disulfide-thiol exchange with an excess of unlabelled l-cysteine (Jensen, 1959). Rat liver homogenates were prepared in 0.14 M KCl with a Potter-Elvehjem glass homogenizer. Cysteine was incubated with rat liver preparations in Warburg flasks at 37°C and the reaction stopped by the addition of trichloroacetic acid. Cysteinesulfinate was then separated from cysteine and cystine in the deproteinized sample by ion exchange chromatography on Dowex 50 (hydrogen form) as outlined by Singer and Kearney (1954) and determined colorimetrically with ninhydrin. The value thus obtained also included any cysteate formed in the reaction, but cysteinesulfinate could be

specifically determined by an enzymatic method, based on transamination of cysteinesulfinate with α-ketoglutarate in the presence of GOT (Leinweber and Monty, 1962).

RESULTS

When rat liver homogenates were shaken with cysteine in the presence of air, no significant formation of cysteinesulfinate could be detected. Further experiments showed, however, that cysteinesulfinate was very rapidly metabolized by rat liver homogenates, which could explain the failure to observe any conversion of cysteine to cysteinesulfinate. As the latter is transformed in rat liver by desulfination or decarboxylation reactions (Fromageot et al, 1948 and Chatagner and Bergeret, 1951), which are catalysed by enzymes requiring pyridoxal phosphate as a co-factor, we tried to inhibit the disappearance of cysteinesulfinate by carbonyl group reagents.

Table I. Requirements of co-factors for cysteinesulfinate formation.

The test system of final pH 7.0 and final volume 2.0 ml contained 0.50 ml of a centrifuged rat liver homogenate (2 g rat liver +8ml 0.14 M KCl, centrifuged for 15 min at 9.000 g), 10 µmoles of cysteine hydrochloride, 10 µmoles of hydroxylamine hydrochloride, 20 µmoles of nicotinamide, 100 µmoles of sodium phosphate and, when indicated, 4 µmoles of TPNH and 1 µmole of ferrous ammonium sulphate. The samples were shaken for 60 min at 37°C in Warburg flasks containing air. Cysteinesulfinate was then determined by the ninhydrin method. The values were corrected for a blank, which was not incubated.

Co-factor added	Cysteinesulfinate formed
	µmoles
None	0.35
Fe^{2+}	0.58
TPNH	0.99
Fe^{2+} + TPNH	2.07

Hydroxylamine was found to be very effective in this respect, whereas semicarbazide was less effective. When cysteine was incubated with a rat liver homogenate or with the supernatant obtained after centrifugation of the homogenate, the formation of cysteinesulfinate could in fact be demonstrated in the presence of 0.005 M hydroxylamine. The reaction was stimulated by ferrous ions and by TPNH (table I) whereas ATP, cytochrome c, hypoxanthine, hydrogen peroxide, Mg^{2+}, Mn^{2+} and Cu^{2} had no stimulating effect.

No reaction took place anaerobically or with a liver extract heated to 70°C for 5 min. Paper electrophoresis at pH 1.9 (Jacobsen et al, 1964) of a reaction system containing S^{35}-labelled cysteine as substrate, demonstrated the formation of a ninhydrin-positive, labelled product with a migration identical with that of cysteinesulfinate, together with small amounts of a ninhydrin-positive labelled product which migrated as cysteate. The identity of the reaction products was also established by paper chromatography in 3 different solvent systems (phenol-water 4:1, tert-butanol-formic acid-water 4:1:1 and lutidine-ethanol-water 55:35:10, c.f. Chapeville, 1960). Determination of cysteinesulfinate by the enzymatic method gave values which were 85 - 90% of those obtained by the ninhydrin method, which could be expected as the ninhydrin method also included cysteate. As the latter is presumably formed by a secondary oxidation of cysteinesulfinate, we feel that the ninhydrin method gives values more representative for the activity of the enzyme system which forms cysteinesulfinate from cysteine. This enzyme system is confined to the soluble fraction of liver homogenates (table II).

Experiments with crude liver extracts showed that TPN, DPNH and DPN were about as effective as TPNH as co-factors. If the extracts were dialyzed against phosphate buffer, however, only the reduced

Table II. Distribution of cysteinesulfinate forming system in liver homogenates.

8 ml of a 20% liver homogenate in 0.14 M KCl was centrifuged at 144.000 g for 30 min and the precipitate was washed twice on the centrifuge with 2 ml 0.14 M KCl. Both the supernatants were then combined and the precipitate dissolved in 0.14 M KCl to a final volume of 5 ml. The test systems contained either 0.4 ml of homogenate or supernatant or 0.25 ml precipitate as indicated, and TPNH and Fe^{2+} as co-factors. Other reaction conditions as in table I.

Enzyme source	Cysteinesulfinate determined
	µmoles
Homogenate	1.99
Precipitate	0.15
Supernatant	1.62

forms of the pyridine nucleotides were active co-factors (table III) and TPNH was more effective than DPNH.

Table III. Pyridine nucleotides as co-factors for dialyzed extracts.

A rat liver extract, prepared as in table I, was dialyzed for 18 hrs against 0.002 M phosphate buffer pH 6.8 at +4°C. The test system contained Fe^{2+} and different pyridine nucleotides as indicated and was otherwise similar to that of table I.

Co-factor	Cysteinesulfinate formed
	µmoles
TPNH	2.38
TPN	0.26
DPNH	1.02
DPN	0.40

DISCUSSION

The present results demonstrate that cysteine is oxidized to cysteinesulfinate in the presence of rat liver extracts in a reac-

tion requiring oxygen and reduced pyridine nucleotides together with ferrous ions as co-factors. As the active factor in the liver is heat labile and non-dialyzable it is presumably an enzyme or an enzyme system. Its requirements for both oxygen and reduced pyridine nucleotides indicates that it should be classified as a "mixed function oxidase" (Mason, 1957). The present findings are different from those of Awapara and Doctor (1955), who reported a formation of cysteinesulfinate from S^{35}-labelled cysteine in the presence of a washed rat liver residue fortified with Mg^{2+}, ATP, DPN and cytochrome c. However, whereas the present test system gives rates of cysteinesulfinate formation of about 20 μmoles/g liver/hr, it can be calculated from the data of Awapara and Doctor that the rate in their test system was only about 0.1 μmoles/g liver/hr. An important difference between the latter and our test system is the presence of hydroxylamine. Whether this compound functions only as an inhibitor for the destruction of cysteinesulfinate or if it also activates the formation of the latter by other means cannot be decided until the enzyme system has been further purified.

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