THE PRODUCTION OF SULFATE FROM CYSTEINE WITHOUT THE FORMATION OF FREE CYSTEINESULFINIC ACID*

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It has generally been accepted that the production of sulfate from cysteine (or cystine) occurs through the intermediate formation of cysteinesulfinic acid (CSA) as first postulated by Pirie (1934). The evidence for the central role of CSA has been largely based on the demonstration that various tissue preparations readily oxidize it (Fromageot et al., 1948; Medes and Floyd, 1942; and Singer and Kearney, 1956). Also, CSA has been demonstrated in normal rat brain (Begeret and Chatnagner, 1954) and S^{35} CSA has been isolated from tissues of rats injected with S^{35} cysteine (Chapeville and Fromageot, 1955). However, no enzyme system has been described which is capable of producing CSA from cysteine, and CSA has not been isolated in the in vitro studies of cystine oxidation to sulfate.

The present study involves a system in which S^{35} cysteine was oxidized to $S^{35}O_4^=$ by rat liver mitochondria under conditions where added CSA remained essentially unmetabolized. Also, there was no formation of S^{35} CSA while $S^{35}O_4^=$ was being actively produced. The present system seems to be of quantitative importance for the metabolism of cystine in vivo.

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METHODS

S^{35} cysteine was produced by preincubation of S^{35} cystine (40 mc/mM (Schwarz Bioresearch) with non-labeled cysteine at pH 7.4. After two minutes at room temperature, essentially all the radioactivity was found in cysteine due to the disulfide interchange and the excess amount of cysteine.

Cysteinesulfinic acid was obtained from Calbiochem and tested for purity by paper chromatography in three solvent systems. Also, a functional test of the transamination of CSA with α-ketoglutarate was performed (Leinweber and Monty, 1962).

Mitochondria were prepared from rat liver by a standard procedure (Hogeboom, 1955).

After incubation, samples were deproteinized with sulfosalicylic acid and aliquots were used for: (1) isolation and analysis of CSA by separation on Dowex 50 columns as described by Singer and Kearney (1956), (2) amino acid analysis on an autoanalyzer and (3) determination of $S^{35}O_4^=$ produced. The last was accomplished by adding a known amount of carrier $SO_4^=$ to the aliquot, followed by precipitation of $BaS^{35}O_4$. The radioactivity of the $BaS^{35}O_4$ derived from samples was compared to that of a standard which contained equivalent amounts of carrier sulfate and S^{35} cysteine which had been chemically oxidized completely to sulfate. This method was feasible since less than 1% of the added activity was precipitated with the protein and thus the $S^{35}O_4$ produced (usually more than 10% of added substrate) was in solution. Also, the cysteine added was completely recovered as either unreacted cysteine or as sulfate.

RESULTS AND DISCUSSION

The results of a typical experiment are summarized in Table I. It can be seen that in the absence of α-ketoglutarate most of the added CSA is recovered unchanged. Also, the production of sulfate from cysteine

Table I. The Oxidation of Cysteine to Sulfate in the Presence of Cysteine Sulfinic Acid.

All flasks contained 8.0 mg of mitochondrial protein (equivalent to the mitochondria present in 0.2 g of rat liver). 5 μeq of glutathione, 10 μeq of S^{35} cysteine (Cys) and where indicated 15 μeq of cysteinesulfinic acid (CSA), 20 μeq of α-ketoglutarate (α-KG). All flasks were incubated for 30 min at 37°, pH 7.4 in 0.05 M phosphate buffer. Final volume 3.0 ml.

Sample	μmoles $S^{35}O_4^=$ produced	μmoles CSA recovered	μmoles glutamic acid recovered
S^{35} Cys	1.40	0	0.2
S^{35} Cys + CSA	1.04	13.4	0.2
S^{35} Cys + CSA + α-KG	0.69	0.5	15.4
S^{35} Cys + α-KG	1.10	-	-
S^{35} Cys (Heat treated mitochondria)	0.03	-	-

continues in the presence of CSA at an only slightly reduced rate. The addition of α-ketoglutarate led to transamination of CSA and formation of glutamic acid but did not increase the rate of sulfate production from cysteine; in fact, the rate was decreased. In addition, the specific activity of the sulfate produced was 858 cpm/μeq whereas the activity of the isolated CSA was less than 7 cpm/μeq.

Experiments performed at a higher substrate concentration of 40 μeq/flask led to the production of 3.9 μeq/30 min/flask (19.5 μeq/gm wet liver). Again, the rate was not increased upon addition of α-ketoglutarate.

From these results it seems evident that CSA is not a free intermediate in the oxidation of cysteine to sulfate (this system). It is possible that CSA is a tightly bound intermediate, but most of the evidence for CSA as an intermediate has been based on the metabolism of the

free compound. The role of cysteinesulfinic acid as an intermediate in the oxidation of cysteine seems now to be uncertain.

REFERENCES

Bergeret, B., and Chatagner, F. Biochim. Biophys. Acta, 14, 297 (1954).
Chapeville, F., and Fromageot, P. Biochim. Biophys. Acta, 17, 275 (1955).
Fromageot, C., Chatagner, F., and Bergeret, B. Biochim. Biophys. Acta, 2, 294 (1948).
Hogeboom, G.H. in Methods in Enzymology, 1, 16 (1955).
Leinweber, F.J., and Monty, K.J. Anal. Biochem., 4, 252 (1962).
Medes, G., and Floyd, N. Biochem. J., 36, 259 (1942).
Pirie, N.W. Biochem. J., 28, 305 (1934).
Singer, T.P., and Kearnery, E.B. Arch. Biochem. and Biophys., 61, 397 (1956)