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# Inhibition of sulfate-forming activity in rat liver mitochondria by (aminooxy)acetate

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**Summary.** The effect of (aminooxy)acetate, an inhibitor of aminotransferases, on the sulfate formation from L-cysteine and L-cysteinesulfinate in rat liver mitochondria was studied. Incubation of 10 mM L-cysteine with rat liver mitochondria at 37°C in the presence of 10 mM 2-oxoglutarate and 10 mM glutathione resulted in the formation of 4.60 and 1.52 μmol of sulfate and thiosulfate, respectively, per 60 min per mitochondria obtained from 1 g of liver. Under the same conditions sulfate formation from L-cysteinesulfinate was 24.96 μmol, but thiosulfate was not formed. The addition of (aminooxy)acetate at 2 mM or more completely inhibited the sulfate and thiosulfate formation from L-cysteine and the sulfate formation from L-cysteinesulfinate. These findings support our previous conclusion that cysteine transamination and 3-mercaptopyruvate pathway (MP pathway) are involved in the sulfate formation from L-cysteine in rat liver mitochondria (Ubuka et al., 1992).

**Keywords:** Amino acids – Cysteine metabolism – MP pathway – Sulfate formation – (Aminooxy)acetate – Rat liver mitochondria

## Introduction

Oxidation of cysteine to cysteinesulfinate is believed to be the major pathway of cysteine catabolism in mammals, particularly when cysteine availability is high (Griffith, 1987). Main final products of this oxidation pathway are sulfate and taurine.

Wainer suggested the presence of a second system of sulfate formation from L-cysteine in rat liver mitochondria which was different from the above oxidation pathway (Wainer, 1964, 1967). However, the responsible enzyme(s) and mechanism of this system have been remained unknown for many years (Griffith, 1987; Singer, 1975).

We have studied 3-mercaptopyruvate pathway (MP pathway) of L-cysteine metabolism and have reported that L-cysteine aminotransferase (EC 2.6.1.3) from rat liver mitochondria is identical with mitochondrial aspartate aminotransferase (EC 2.6.1.1) (Ubuka et al., 1978). In our previous papers (Ubuka et al., 1990, 1992), we reported evidence that sulfate is formed from L-cysteine via MP pathway in rat liver mitochondria, and discussed that this mitochondrial sulfate-forming system seemed to explain most questions concerning Wainer's reports (Griffith, 1987; Singer, 1975). The present study was undertaken to produce further evidence for the involvement of cysteine transamination and MP pathway in the mitochondrial sulfate-forming system using (aminooxy)acetate, a potent inhibitor of various aminotransferases (Hopper and Segal, 1964).

## Materials and methods

### *Materials*

Male Wistar rats weighing 350–400 g were used. Liver mitochondrial fraction was prepared at 0–4°C according to the method of Hogeboom (Ubuka et al., 1978). The mitochondrial fraction obtained was washed once with cold 0.14 M KCl solution containing 10 mM Tris chloride and 0.1 mM EDTA (pH 7.4) in order to eliminate sulfate contained in sucrose as a contaminant and suspended in the same solution at a concentration of mitochondrial fraction obtained from 2 g of liver per ml of the suspension (Ubuka et al., 1992).

L-Cysteine and (aminooxy)acetate were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Glutathione (GSH) was from Bohringer-Mannheim GmbH, Mannheim, Germany. 2-Oxoglutarate was purchased from Wako Pure Chemical Ind., Inc., Osaka, Japan. L-Cysteinesulfinate was prepared according to the method reported previously (Ubuka et al. 1982). L-Cysteinesulfinate, 2-oxoglutarate, GSH and (aminooxy)acetate were neutralized with sodium hydroxide solution just before use.

### *Enzyme reactions*

The standard reaction mixture contained, in a final volume of 1.0 ml, 200 μmol of Tris chloride (pH 8.40), 10 μmol of L-cysteine, 10 μmol of 2-oxoglutarate, 10 μmol of GSH and mitochondrial fraction prepared from 0.5 g of liver. (Aminooxy)acetate was added to the reaction mixture before the addition of the mitochondrial fraction, at various concentrations as described below. Most reactions with L-cysteinesulfinate were performed under the same conditions as those with L-cysteine for the comparison of results with these substrates. In some experiments, 1.0 ml of a reaction mixture containing 20 mM L-cysteinesulfinate, 20 mM 2-oxoglutarate and mitochondrial fraction from 0.5 g of liver was incubated at 37°C for 10 min without or with various concentrations of (aminooxy)acetate. The reaction was initiated by the addition of the mitochondrial suspension and shaken at 130 strokes per min at 37°C for 30 or 10 min. The reaction was terminated by the addition of 0.3 ml of glacial acetic acid. Control reaction was run without mitochondrial suspension, which was added after the reaction was terminated with glacial acetic acid.

### *Determination of reaction products*

Ion chromatography was employed to determine the reaction products as described previously (Ubuka et al., 1992) with some modifications. As shown below, sulfate formation was inhibited in the reaction with (aminooxy)acetate. Determination of minute amounts of sulfate formed in these inhibited reactions was found to be interfered with the presence of minute amounts of succinate or unknown tiny peak. In order to separate sulfate and succinate, organic solvents contained in the elution buffer (Ubuka et al., 1992) were omitted.

Under this condition, phosphate, sulfate, succinate, 2-oxoglutarate and thiosulfate were eluted at 13.1, 18.2, 20.1, 27.0 and 35.8 min, respectively.

In some experiments, the determination of minute amounts of sulfate was still interfered with a tiny peak of unknown substance(s). Therefore, an ion chromatography column, Shodex IC I-524A (4.6 mm × 100 mm) and a precolumn, Shodex IC I-524P (4.6 mm × 10 mm) (Showa Denko Co., Ltd., Tokyo, Japan) were used. Buffer solution was 2.5 mM phthalic acid-15% methanol, of which pH was adjusted to 3.8 with Tris. Chromatography was performed at 40°C and at a flow rate of 1.5 ml per min. Range was 500 $\mu$S and sensitivity was 0.002. Sulfate and thiosulfate were eluted at 13.9 and 21.9 min, respectively. This method seems to be most satisfactory at present for the determinations of sulfate and thiosulfate in the mitochondrial sulfate-forming system.

## Results and discussion

*Reaction with fresh or frozen-thawed mitochondria without (aminooxy)acetate*

Table 1 shows sulfate formation from L-cysteine in rat liver mitochondria. Incubation of 10 mM L-cysteine with mitochondrial fraction at pH 8.4 and 37°C resulted in the formation of 1.26 $\mu$mol of sulfate per 60 min per mitochondria from 1 g of liver. By the addition of 10 mM 2-oxoglutarate, the sulfate formation increased to 3.20 $\mu$mol, and 1.92 $\mu$mol of thiosulfate was formed besides sulfate. The increase in the sulfate formation by the addition of 2-oxoglutarate indicates the involvement of mitochondrial aspartate aminotransferase in the sulfate formation from L-cysteine in mitochondria. Formation of thiosulfate indicates the involvement of 3-mercaptopyruvate sulfurtransferase reaction in the mitochondrial sulfate-forming system. As shown in Table 1, the sulfate formation was further increased by the addition of 10 mM GSH. Thus, the addition of

**Table 1.** Sulfate and thiosulfate formation from L-cysteine in rat liver mitochondria and its inhibition by (aminooxy)acetate

| Substrates and inhibitor | Products ($\mu$mol/60 min/mt from 1 g liver) | | | |
|---|---|---|---|---|
| | Sulfate | | Thiosulfate | |
| (Aminooxy)acetate | − | + | − | + |
| L-Cysteine<br>2-Oxoglutarate<br>GSH | 4.60 ± 1.13 | 0.56 ± 0.36 | 1.52 ± 0.28 | 0.00 ± 0.00 |
| L-Cysteine<br>2-Oxoglutarate | 3.20 ± 1.09 | 0.50 ± 0.29 | 1.92 ± 0.94 | 0.00 ± 0.00 |
| L-Cysteine<br>GSH | 1.72 ± 0.47 | 0.53 ± 0.37 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| L-Cysteine | 1.26 ± 0.42 | 0.40 ± 0.29 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Substrates and inhibitor at a concentration of 10 mM were incubated with fresh mitochondrial fraction (mt) from rat liver at 37°C for 30 min. Sulfate and thiosulfate were determined by ion chromatography with a TSKgel IC-Anion-PW column and borate-gluconate buffer, pH 8.5. Results are expressed as mean ± SD of 4 separate experiments

2-oxoglutarate or 2-oxoglutarate and GSH to the reaction mixture increased the formation of the sum (A + 2B) of sulfate (A) and thiosulfate (B) to 5.5- or 6-fold the amount formed in the presence of L-cysteine alone. These results are in agreement with the previous reports (Ubuka et al., 1990, 1992).

Table 2 shows sulfate and thiosulfate formation from L-cysteine by frozen-thawed mitochondria. Sulfate formation decreased to 42–64% of that in the reaction with fresh mitochondria. These results agree with the previous results obtained with sonicated or frozen-thawed mitochondria (Ubuka et al., 1990). Formation of thiosulfate in the reaction with the standard reaction mixture and frozen-thawed mitochondria decreased to 70% of that formed in the reaction with fresh mitochondria.

**Table 2.** Sulfate and thiosulfate formation from L-cysteine in frozen-thawed mitochondria and its inhibition by (aminooxy)acetate

| Substrates and inhibitor | Products (μmol/60 min/mt from 1 g liver) | | | |
|---|---|---|---|---|
| | Sulfate | | Thiosulfate | |
| (Aminooxy)acetate | − | + | − | + |
| L-Cysteine 2-Oxoglutarate GSH | 2.93 ± 0.62 | 0.76 ± 0.16 | 1.07 ± 0.65 | 0.00 ± 0.00 |
| L-Cysteine 2-Oxoglutarate | 1.43 ± 0.18 | 0.97 ± 0.28 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| L-Cysteine GSH | 0.73 ± 0.19 | 0.63 ± 0.19 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| L-Cysteine | 0.68 ± 0.37 | 0.49 ± 0.22 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Substrates and inhibitor at a concentration of 10 mM were incubated with frozen-thawed mitochondrial fraction (mt) from rat liver at 37°C for 30 min. Sulfate and thiosulfate were determined by ion chromatography with a TSKgel IC-Anion-PW column and borate-gluconate buffer, pH 8.5. Results are expressed as mean ± SD of two separate experiments

The sulfate formation in the presence of L-cysteinesulfinate was accelerated greatly by the addition of 2-oxoglutarate as shown in Table 3. However, thiosulfate was not formed in these reactions. In contrast to the reaction with L-cysteine, the sulfate formation from L-cysteinesulfinate was not enhanced by the addition of GSH. These findings indicate that the sulfate formation from L-cysteinesulfinate does not involve thiosulfate metabolism as discussed previously (Ubuka et al., 1992).

As shown in Table 4, sulfate formation in the reactions with frozen-thawed mitochondria, L-cysteinesulfinate and 2-oxoglutarate decreased to about 70%. GSH did not exhibit any accelerating effect on the sulfate formation, and thiosulfate was not formed in these reactions with frozen-thawed mitochondria as those with fresh mitochondria.

**Table 3.** Sulfate and thiosulfate formation from L-cysteinesulfinate in rat liver mitochondria and its inhibition by (aminooxy)acetate

| Substrates and inhibitor | Products (μmol/60 min/mt from 1 g liver) | | | |
|---|---|---|---|---|
| | Sulfate | | Thiosulfate | |
| (Aminooxy)acetate | − | + | − | + |
| L-Cysteinesulfinate 2-Oxoglutarate GSH | 24.96 ± 1.85 | 0.70 ± 0.57 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| L-Cysteinesulfinate 2-Oxoglutarate | 23.02 ± 0.80 | 0.90 ± 0.89 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| L-Cysteinesulfinate GSH | 0.81 ± 0.11 | 0.53 ± 0.35 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| L-Cysteinesulfinate | 1.57 ± 0.49 | 1.14 ± 0.38 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Substrates and inhibitor at a concentration of 10 mM were incubated with mitochondrial fraction (mt) from rat liver at 37°C for 30 min. Sulfate and thiosulfate were determined by ion chromatography with a TSKgel IC-Anion PW column and borate-gluconate buffer, pH 8.5. Results are expressed as mean ± SD of 4 separate experiments

*Reaction with (aminooxy)acetate*

Although the sulfate-forming pathways from L-cysteine and from L-cysteinesulfinate are different (Ubuka et al., 1992), the transamination reaction constitutes the key reaction in both pathways, and this reaction is catalyzed by a common enzyme, aspartate aminotransferase (Ubuka et al., 1992). Therefore, we examined the effect of (aminooxy)acetate, a potent inhibitor of various aminotransferases (Hopper and Segal, 1964). As shown in Tables 1 and 2, the sulfate and thiosulfate formation from L-cysteine was strongly inhibited by (aminooxy)acetate.

Formation of sulfate from L-cysteinesulfinate was also strongly inhibited by (aminooxy)acetate as shown in Table 3. Inhibition by (aminooxy)acetate on the sulfate formation in the reaction with frozen-thawed mitochondria was somewhat weaker than that with fresh mitochondria (Table 4), the reason of which is unknown at present.

Figure 1 illustrates the effect of various concentrations of (aminooxy)acetate on the mitochondrial sulfate formation from L-cysteine and L-cysteinesulfinate. The sulfate formation was almost completely inhibited at concentrations more than 2 mM. It was shown that (aminooxy)acetate was a potent inhibitor of various aminotransferases (Hopper and Segal, 1962; 1964; Wallach, 1961). Its inhibitory action on alanine aminotransferase was reported to be stronger than that on aspartate aminotransferase (Hopper and Segal, 1964). The inhibition was competitive as for amino acids and was pH-dependent (Hopper and Segal, 1962, 1964; Wallach, 1961). At higher pH such as pH 8.4, which is the optimum pH for the mitochondrial sulfate-forming activity, the inhibitory action of (aminooxy)acetate is rather weak (Hopper and Segal, 1962, 1964). This seems to be

**Table 4.** Sulfate and thiosulfate formation from L-cysteinesulfinate in frozen-thawed mitochondria and its inhibition by (aminooxy)acetate

| Substrates and inhibitor | Products (μmol/60 min/mt from 1 g liver) | | | |
|---|---|---|---|---|
| | Sulfate | | Thiosulfate | |
| (Aminooxy)acetate | − | + | − | + |
| L-Cysteinesulfinate 2-Oxoglutarate GSH | 17.26 ± 0.54 | 3.76 ± 0.03 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| L-Cysteinesulfinate 2-Oxoglutarate | 16.76 ± 1.88 | 4.13 ± 0.08 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| L-Cysteinesulfinate GSH | 0.90 ± 0.19 | 1.18 ± 0.02 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| L-Cysteinesulfinate | 1.33 ± 0.34 | 1.31 ± 0.33 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Substrates and inhibitor were incubated at a concentration of 10 mM with frozen-thawed mitochondrial fraction (mt) from rat liver at 37°C for 30 min. Sulfate and thiosulfate were determined by ion chromatography with a TSKgel IC-Anion PW column and borate-gluconate buffer, pH 8.5. Results are expressed as mean ± SD of two separate experiments

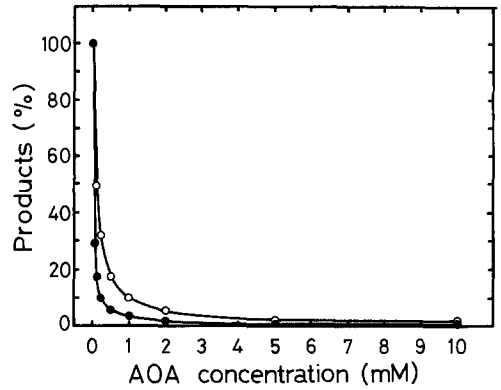


**Fig. 1.** Inhibition of sulfate formation from L-cysteine (●—●) or L-cysteinesulfinate (○—○) in rat liver mitochondria by (aminooxy)acetate (AOA). Reactions with L-cysteine were performed under the standard reaction conditions described under Materials and methods. Reactions with L-cysteinesulfinate were performed in a reaction mixture containing 20 mM L-cysteinesulfinate and 20 mM 2-oxoglutarate at 37°C for 10 min. AOA was added before the addition of the mitochondrial fraction. After the reaction was terminated by the addition of glacial acetic acid, sulfate (A) and thiosulfate (B) formed were determined by ion chromatography with a Shodex IC I-524A column and phthalate-methanol system (pH 3.8) as described under Materials and methods. The amounts of the products (A + 2B in the reactions with L-cysteine and A in those with L-cysteinesulfinate) in the reaction without the inhibitor were taken as 100%

the reason why relatively high concentrations of the inhibitor were needed to produce the complete inhibition of mitochondrial sulfate-forming activity.

In summary, the present finding that the mitochondrial sulfate formation from L-cysteine is inhibited by (aminooxy)acetate indicates that the mitochon-

drial sulfate formation from L-cysteine involves L-cysteine transamination and supports our previous conclusion that this mitochondrial sulfate-forming system is mediated by MP pathway.

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