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L-Cysteine metabolism via 3-mercaptopyruvate pathway and sulfate formation in rat liver mitochondria

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Summary. We have studied the 3-mercaptopyruvate pathway (transamination pathway) of L-cysteine metabolism in rat liver mitochondria. L-Cysteine and other substrates at 10 mM concentration were incubated with mitochondrial fraction at pH 8.4, and sulfate and thiosulfate were determined by ion chromatography. When L-cysteine alone was incubated, sulfate formed was 0.7 μmol per mitochondria from one g of liver per 60 min. Addition of 2-oxoglutarate and GSH resulted in more than 3-fold increase in sulfate formation, and thiosulfate was formed besides sulfate. The sum (A + 2B) of sulfate (A) and thiosulfate (B) formed was approximately 7-times that with L-cysteine alone. Incubation with 3-mercaptopyruvate resulted in sulfate and thiosulfate formation, and sulfate was formed with thiosulfate. These reactions were stimulated with glutathione. Sulfate formation from L-cysteinesulfinate and 2-oxoglutarate was not enhanced by glutathione and thiosulfate was not formed. These findings indicate that L-cysteine was metabolized and sulfate was formed through 3-mercaptopyruvate pathway in mitochondria.

Keywords: Amino acids – Cysteine metabolism – 3-Mercaptopyruvate pathway – Sulfate formation – Mitochondria – Glutathione

Introduction

β-Mercaptolactate-cysteine disulfiduria (Crawhall, 1978) is an inborn error of L-cysteine metabolism, in which 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2) is deficient (Shie et al., 1977; Hannestad et al., 1981). Patients with this disorder excrete in the urine more than 250 μmol per day of 3-mercaptolactate-cysteine mixed disulfide [S-(2-hydroxy-2-carboxyethylthio)cysteine]. This mixed disulfide and its related compound, mercaptoacetate-cysteine mixed disulfide [S-(carboxymethylthio)cysteine] are excreted in normal human urine in small amounts (Ubuka et al., 1968). These findings prompted us to study L-cysteine metabolism in mammals, and we have shown some evidence that 3-mercapto-

pyruvate pathway (transamination pathway of L-cysteine metabolism) (Meister et al., 1954; Cooper, 1983) is functioning in the rat liver (Ubuka et al., 1977, 1990).

Inorganic sulfate is the major end product of sulfur metabolism in mammals. Sulfur taken mainly in the form of sulfur-containing amino acids in proteins is ultimately oxidized to sulfate (Jocelyn, 1972). Metabolic pathways of cysteine sulfur to sulfate may be divided into two types. One is the oxidation pathway in which sulfur atom is oxidized without removal from the carbon skeleton and which proceeds through L-cysteinesulfinate as an intermediate. In the other type, sulfur atom is removed from the carbon skeleton before oxidation. The 3-mercaptopyruvate pathway belongs to the latter type.

Wainer studied mitochondrial oxidation of L-cysteine and proposed an alternative pathway of sulfate formation from L-cysteine, in which L-cysteinesulfinate was not an intermediate (Wainer, 1964, 1967). However, the mechanism of mitochondrial L-cysteine oxidation system has not been elucidated (Singer, 1975; Griffith, 1987). The present study has been undertaken to clarify questions concerning the sulfate formation from L-cysteine in mitochondria. In addition to the previous report (Ubuka et al., 1990), we have accumulated further evidence that, in rat liver mitochondria, L-cysteine is metabolized through 3-mercaptopyruvate pathway and this pathway is involved in the sulfate formation.

Materials and methods

Materials

Mitochondrial fraction was prepared according to the method of Hogeboom (Ubuka et al., 1978) from the liver of male Wistar rats weighing 250–350 g. The mitochondrial preparation obtained was washed once with 0.14 M potassium chloride 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 finally suspended in the same solution at a concentration of mitochondrial fraction obtained from 2 g of liver per ml. When cysteine contents were determined, mitochondrial fraction was prepared according to Griffith and Meister (1985).

L-Cysteine was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Glutathione (GSH) was purchased from Boehringer-Mannheim GmbH, Mannheim, Germany, and oxidized glutathione (GSSG) from Kohjin Co., Tokyo, Japan. Sodium thiosulfate was obtained from E. Merck, Darmstadt, Germany. L-Cysteinesulfinic acid (Ubuka et al., 1982), ammonium 3-mercaptopyruvate (Kun, 1957) and cysteine-glutathione mixed disulfide (Cys-SG) (Ericksson and Ericksson, 1967) were prepared according to the methods reported.

Determination of cysteine and GSH contents in rat liver mitochondria

Cysteine contents were determined by acidic ninhydrin reaction (Gaitonde, 1967) and with an amino acid analyzer (Ubuka et al., 1983) using perchlorate and sulfosalicylate extracts of the mitochondrial fraction, respectively. GSH and GSSG were determined with an amino acid analyzer (Hosaki et al.,1985). These extracts were prepared by sonication of mitochondrial pellet suspended in 4 volumes of 5% perchloric acid or in an equal volume of 6% sulfosalicylic acid at 20 KHz for 10 min and centrifugation at 10,000 xg for 10 min.

When cysteine contents were determined with an amino acid analyzer, the sulfosalicylate extract was brought to pH 8 with sodium hydroxide and stood for 4 h at room temperature. During this treatment, GSH was oxidized to GSSG and most of cysteine was converted to Cys-SG. Formation of cystine was far less than Cys-SG. After adjusting pH to 2.0 with

hydrochloric acid, 0.5 ml the solution was applied to a Hitachi KLA-5 amino acid analyzer, and cysteine contents were calculated from the amounts of Cys-SG and cystine.

Enzyme reaction

The standard incubation 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 suspension prepared from 0.5–0.6 g of rat liver. Sodium thiosulfate, GSSG or L-cysteinesulfinate were used as substrates at a concentration of 10 mM as indicated below. GSH, GSSG, 2-oxoglutarate and L-cysteinesulfinate were neutralized with sodium hydroxide before addition to the reaction mixture. The reaction was initiated by the addition of the mitochondrial suspension, and the mixture was shaken at 130 strokes per min at 37°C for 30 min. The reaction was terminated by the addition of 0.3 ml of glacial acetic acid. Then, 0.3 ml of the mitochondrial suspension was added to the control tubes.

Determination of sulfate and thiosulfate by ion chromatography

After the enzyme reaction was terminated by the addition of acetic acid as described above, the reaction mixture was heated in a boiling water bath for 5 min in order to denature and precipitate proteins. After cooling, 3.7 ml of isopropanol was added, and the mixture was centrifuged at 1,200 xg for 10 min. The resulting supernatant was 1:10 diluted with water, and 100 μl of the diluted solution was injected to a Tosoh ion chromatograph (Tosoh Co., Tokyo), which was consisted of a CCPD pump, a TSKgel IC-Anion-PW column (4.6 × 50 mm), a C-8010 detector and an integrator. One liter of the elution buffer, pH 8.5, contained 360 mg of boric acid, 500 mg of sodium tetraborate, 300 mg of potassium gluconate, 5.0 g of glycerol, 120 ml of acetonitrile and 30 ml of *n*-butanol. Analysis was performed at a flow rate of 1.0 ml per min at 40°C. Detector range was 500 or 1,000 μS and sensitivity was 0.005 μS. Inorganic sulfate and thiosulfate were eluted at 22.6 and 43.0 min, respectively.

In some experiments, the enzyme reaction was terminated by the addition of 3.0 ml of 10% trichloroacetic acid and centrifuged. Trichloroacetic acid in the resulting supernatant was extracted with diethyl ether, because it was eluted at 18.4 min and disturbed sulfate determination by ion chromatography. After the precipitated protein was centrifuged off, the resulting supernatant was placed in a test tube (16 × 100 mm) with a Teflon-lined screw cap and extracted 3 times with 8 ml each of diethyl ether using a mechanical shaker at 250 strokes per min for 5 min. The water layer was diluted and applied to the ion chromatograph as above.

Determination of sulfate with barium chloranilate

This method (Ubuka et. al., 1990) was used when many samples were handled in a short time. To 4.0 ml of the supernatant obtained by centrifugation of a mixture of the heated extract and isopropanol as described above, 1.0 ml of 10% acetic acid in isopropanol and about 75 mg of barium chloranilate were mixed. After shaking in a test tube (16 × 100 mm) with a Teflon-lined screw cap using a mechanical shaker at 160 strokes per min for 30 min, the tube was centrifuged as above. Absorbance of the supernatant was measured at 530 nm using a cuvette with a light path of 10 mm. The absorbance at 530 nm of 5 ml of a standard solution containing 1.0 μmol of potassium sulfate was 0.200 under the present conditions.

Determination of protein concentration

Protein concentration in the mitochondrial fraction was determined by biuret method (Jacobs et al., 1956).

Results

Cysteine contents in rat liver mitochondria

Cysteine contents in rat liver mitochondria were determined by two methods in the present study. As shown in Table 1 average values of cysteine contents determined by acidic ninhydrin reaction was 0.79 ± 0.23 and that with amino acid analysis was 0.56 ± 0.32 nmol per mg of protein, respectively. Cystine contents were negligible. Cysteine contents determined by the latter method were calculated from Cys-SG and cystine which were formed when sulfosalicylate extract was stood at alkaline pH. By this treatment, GSH was oxidized to GSSG and cysteine to Cys-SG and cystine. Cystine formed was less than 10% of Cys-SG formed. The recovery experiment showed that 30 to 70% of the added cysteine at various concentrations was recovered as Cys-SG and cystine. The value shown in Table 1 is the corrected one according to the recovery rate. This method was not completely accurate. However, it is of value because cysteine itself is detected as Cys-SG and cystine by chromatography. GSH and GSSG contents determined by amino acid analyses were 8.18 ± 1.30 and 0.32 ± 0.09 nmol per mg of protein. Concentrations of cysteine and GSH in rat liver mitochondrial matrix calculated from these results according to Wahlländer et al. (1979) were: cysteine, 0.99 ± 0.29 mM (acidic ninhydrin method) and 0.70 ± 0.40 mM (amino acid analysis); GSH, 10.23 ± 1.69 and GSSG, 0.40 ± 0.12 mM (amino acid analysis). Thus, the average cysteine concentration in rat liver mitochondria was close to 1.0 mM. This value is several times higher than that reported for the cysteine concentration in the whole tissue of rat liver (Finkelstein et al., 1982). GSH concentration agreed well with those reported for rat liver mitochondria (Wahlländer et al., 1979; Griffith and Meister, 1985).

Table 1. Cysteine and GSH contents in rat liver mitochondria[a]

Method	Cysteine		GSH	
	nmol/mg of protein	mM	nmol/mg of protein	mM
Acidic ninhydrin reaction	0.79 ± 0.23	0.99 ± 0.29	—	—
Amino acid analysis	0.56 ± 0.32	0.70 ± 0.40	8.18 ± 1.30 (0.32 ± 0.09)	10.23 ± 1.69 (0.40 ± 0.12)

[a] Perchlorate and sulfosalicylate extracts were prepared by sonication of mitochondrial suspension. Cysteine contents were determined by acidic ninhydrin reaction and by amino acid analysis using perchlorate and sulfosalicylate extract, respectively. In the latter method, cysteine was determined after oxidation to cysteine-glutathione mixed disulfide and cystine. GSH and GSSG contents were determined by amino acid analysis of the sulfosalicylate extract. GSSG contents were shown in parentheses. Values are expressed as mean ± SD obtained from 5 to 7 separate experiments

Determination of sulfate and thiosulfate after enzyme reaction

As reported previously (Ubuka et al., 1990), sulfate could not be determined without denaturation of proteins by heating the incubation mixture after the

reaction was terminated with acetic acid. In the present study, termination of the reaction and denaturation of proteins were performed by acidification with acetic acid followed by heat denaturation or by denaturation with trichloroacetic acid. Sulfate in the heated extracts was determined by ion chromatography or colorimetrically with barium chloranilate and that in trichloroacetate extracts was by ion chromatography. The trichloroacetate extracts were used in order to exclude possible change of the reaction products during the heating step. Values obtained by these methods agreed well. In the present study, ion chromatography was used preferentially because sulfate and thiosulfate could be determined simultaneously, and small amount of sulfate could be determined more accurately by ion chromatography than by the barium chloranilate method. However, the colorimetric method was convenient because no special instrument was needed and many samples could be handled in a short time.

Sulfate formation in rat liver mitochondria in the presence of L-cysteine, 2-oxoglutarate and GSH

As shown in Table 2, 10 mM L-cysteine was incubated with rat liver mitochondria, 0.7 μmol of sulfate was formed per mitochondria obtained from one

Table 2. Formation of sulfate and thiosulfate in rat liver mitochondria in the presence of L-cysteine, 2-oxoglutarate and GSH[a]

Substrates	Products (μmol/mt from one g of liver per 60 min)		
	Sulfate (A)	Thiosulfate (B)	A + 2B[b]
L-Cysteine 2-Oxoglutarate GSH	1.92 ± 0.30	1.35 ± 0.37	4.62
L-Cysteine 2-Oxoglutarate GSSG	1.61 ± 1.11	1.51 ± 0.61	4.63
L-Cysteine 2-Oxoglutarate	1.06 ± 0.55	0.87 ± 0.51	2.80
L-Cysteine GSH	0.63 ± 0.58	0.00	0.63
L-Cysteine GSSG	0.75 ± 0.3	0.00	0.75
L-Cysteine	0.70 ± 0.14	0.00	0.70

[a] Ten μmol each of L-cysteine and other substrates in a final volume of 1.0 ml was incubated with mitochondria (mt) obtained from 0.5 g of rat liver at 37°C for 30 min. Sulfate and thiosulfate formed were determined by ion chromatography after deproteinization. Values are expressed as mean ± SD obtained from 3 separate experiments

[b] Obtained from mean values of A and B

g of liver per 60 min. Although this value was higher than that obtained in the previous experiment (Ubuka et al., 1990), the sulfate formation was low when L-cysteine was the only substrate. However, the sulfate formation nearly doubled when 10 mM 2-oxoglutarate was added to the reaction mixture, and thiosulfate was also formed besides sulfate. Sulfate formation further increased when both 10 mM 2-oxoglutarate and 10 mM GSH were added to the incubation mixture. The sum (A + 2B) of sulfate (A) and thiosulfate (B) was approximately 7 times the amount formed in the presence of L-cysteine alone. Because thiosulfate contains 2 sulfur atoms in one molecule, its amount was doubled in the calculation of the sum of the products. GSSG exhibited similar stimulating effect on sulfate and thiosulfate formation. There was a tendency that the effect of GSSG on the sulfate formation was slightly lower than that of GSH, and that thiosulfate formed in the presence of GSSG was higher than that in the presence of GSH.

Sulfate formation in the incubation mixture with L-cysteine alone in the present study was higher than that in the previous report (Ubuka et al., 1990), and stimulation by GSH was not as profound as that in the previous one. This might have derived from the difference in endogenous GSH contents in mitochondria.

Fig. 1 shows the effect of L-cysteine concentration on the sulfate and thiosulfate formation in rat liver mitochondria in the presence of 10 mM 2-oxoglutarate and 10 mM GSH. Sulfate formation was maximum at 5 mM cysteine, and even at 2 mM L-cysteine 0.9 μmol of sulfate was formed per mitochondria from one g of liver per 60 min. The maximum amount of the sum (A + 2B) of sulfate (A) and thiosulfate (B) formed was at 10 mM.

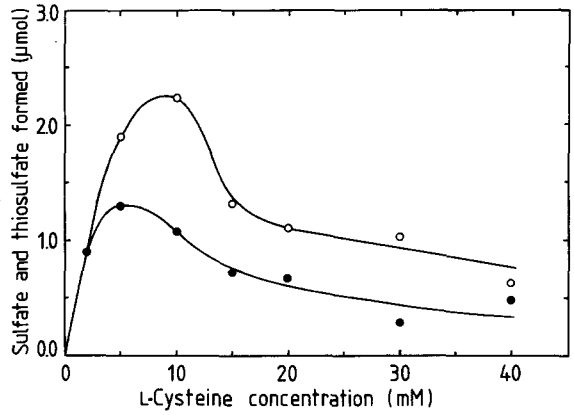

Fig. 1. Effect of L-cysteine concentration on sulfate and thiosulfate formation in rat liver mitochondria. One ml of reaction mixture containing various concentration of L-cysteine, 10 mM 2-oxoglutarate and 10 mM GSH was incubated with mitochondrial fraction obtained from 0.6 g of liver at 37°C for 30 min. Sulfate (●—●) and thiosulfate formed were determined by ion chromatography. The sum (A + 2B) of sulfate (A) and thiosulfate (B) is shown with open circles (○—○)

Fig. 2 illustrates the effect of GSH concentration added to the reaction mixture on the sulfate and thiosulfate formation in rat liver mitochondria in the

presence of 10 mM L-cysteine and 10 mM 2-oxoglutarate. The accelerating effect of GSH was maximum at 10 mM.

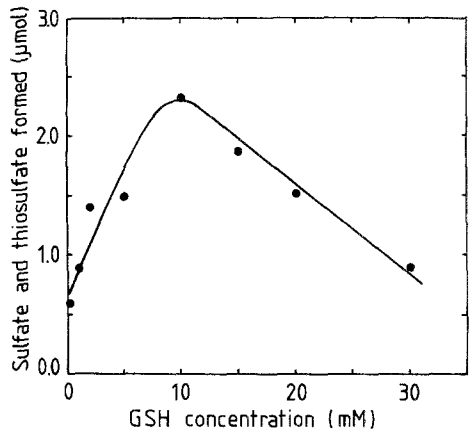

Fig. 2. Effect of GSH concentration on sulfate and thiosulfate formation in rat liver mitochondria. One ml of reaction mixture containing various concentration of GSH, 10 mM L-cysteine and 10 mM 2-oxoglutarate was incubated with mitochondrial fraction obtained from 0.6 g of liver at 37°C for 30 min. The sum (A + 2B) of sulfate (A) and thiosulfate (B) formed is shown

Sulfate formation in rat liver mitochondria in the presence of 3-mercaptopyruvate

As shown in Table 3, approximately 0.7 μmol of sulfate and 0.3 μmol of thiosulfate were formed per mitochondria from one g of liver per 60 min when 10 mM 3-mercaptopyruvate was incubated with rat liver mitochondria. Again GSH accelerated the sulfate and thiosulfate formation as that in the reaction with L-cysteine. GSSG exhibited also the accelerating effect, but it was less effective than GSH.

Table 3. Effect of GSH on the formation of sulfate and thiosulfate in rat liver mitochondira in the presence of 3-mercaptopyruvate[a]

Substrates	Products (μmol/mt from one g of liver per 60 min)		
	Sulfate (A)	Thiosulfate (B)	A + 2B
3-Mercaptopyruvate	0.67	0.30	1.27
3-Mercaptopyruvate GSH	1.91	1.37	4.65
3-Mercaptopyruvate GSSG	1.19	0.92	3.03

[a] Ten μmol of ammonium 3-mercaptopyruvate with or without GSH or GSSG in a final volume of 1.0 ml was incubated with mitochondria (mt) obtained from 0.6 g of rat liver at 37°C for 30 min. Sulfate and thiosulfate were determined by ion chromatography after deproteinization. Values are expressed as the mean of duplicate experiments

Sulfate formation in rat liver mitochondria in the presence of thiosulfate

Table 4 shows sulfate formation in rat liver mitochondria when 10 mM thiosulfate was incubated with or without 10 mM GSH. The presence of GSH enhanced sulfate formation as that with L-cysteine or 3-mercaptopyruvate. GSSG also enhanced sulfate formation.

Table 4. Effect of GSH on the sulfate formation in rat liver mitochondria in the presence of thiosulfate[a]

Substrates	Sulfate formed (μmol/mt from one g of liver per 60 min)
Thiosulfate	0.93
Thiosulfate GSH	2.61
Thiosulfate GSSG	2.62

[a] Ten μmol of thiosulfate in a final volume of 1.0 ml was incubated with mitochondria (mt) obtained from 0.5 g of rat liver at 37°C for 30 min in the absence or presence of 10 μmol of GSH or GSSG. After deproteinization, sulfate formed was determined by ion chromatography. Values are expressed as mean obtained from 2 separate experiments

Sulfate formation in rat liver mitochondria in the presence of L-cysteinesulfinate

Sulfate formation from L-cysteinesulfinate in rat liver mitochondria was studied. As shown in Table 5, about 12 μmol of sulfate was formed per mitochondria obtained from one g of liver per 60 min when 10 mM L-cysteinesulfinate and 10 mM 2-oxoglutarate were incubated under the same conditions. As shown in the table, sulfate formation was accelerated about 8-fold by the addition of 2-oxoglutarate than that with L-cysteinesulfinate alone. However, GSH and GSSG did not exhibit any accelerating effect which was observed in experiments with L-cysteine, 3-mercaptopyruvate or thiosulfate. Moreover, thiosulfate formation was not detected.

Discussion

Major pathway of L-cysteine metabolism in rat liver is the oxidation pathway, in which L-cysteine is first oxidized to L-cysteinesulfinate by cysteine dioxygenase (EC 1.13.11.20) present in the cytosol (Yamaguchi et al., 1987). L-Cysteinesulfinate thus formed may be decarboxylated to form hypotaurine by L-cysteinesulfinate decarboxylase (EC 4.1.1.29) or transaminated to form sulfinopyruvate by aspartate aminotransferase (EC 2.6.1.1) (Griffith, 1987). Some of the L-cysteinesulfinate may be transported to mitochondria (Palmieri et al., 1979), where it is further metabolized to sulfate.

Table 5. Effect of GSH on the sulfate formation in rat liver mitochondria in the presence of L-cysteinesulfinic acid[a]

Substrates	Sulfate formed (μmol/mt from one g of liver per 60 min)
L-Cysteinesulfinate	1.28
L-Cysteinesulfinate 2-Oxoglutarate	12.26
L-Cysteinesulfinate 2-Oxoglutarate GSH	12.55
L-Cysteinesulfinate 2-Oxoglutarate GSSG	8.92
L-Cysteinesulfinate GSH	0.95
L-Cysteinesulfinate GSSG	0.70

[a] Ten μmol each of L-cysteinesulfinate and other substrates in a final volume of 1.0 ml was incubated with mitochondria (mt) obtained from 0.5 g of rat liver at 37°C for 30 min. After deproteinization, sulfate formed was determined by ion chromatography. Values are expressed as mean obtained from 2 separate experiments

Wainer proposed an alternative pathway of sulfate formation in rat liver mitochondria and claimed that only L-cysteine was the substrate and neither L-cysteinesulfinate, 3-mercaptopyruvate nor free hydrogen sulfide was an intermediate in his system, and that GSH stimulated the sulfate formation in damaged mitochondria (Wainer, 1967). Singer (1975) noted that sulfate was actively formed from L-cysteine in rat liver mitochondria and the stimulation by GSH occurred in fresh mitochondria as well as in damaged ones. However, the mechanism of the mitochondrial system has not been fully studied.

We have shown in the present study and in the previous report (Ubuka et al., 1990) that incubation of L-cysteine, 2-oxoglutarate and GSH or GSSG resulted in the formation of substantial amount of inorganic sulfate and thiosulfate. Even at 2 mM of L-cysteine concentration appreciable amount of sulfate was formed. As reported previously, mitochondrial L-cysteine aminotransferase (EC 2.6.1.3) is suggested to be identical with mitochondrial aspartate aminotransferase (Ubuka et al., 1978). 3-Mercaptopyruvate sulfurtransferase activity is present in mitochondria (Koj et al., 1975 and Ubuka et al., 1977). The only known sulfur donor of 3-mercaptopyruvate sulfurtransferase is 3-mercaptopyruvate (Sörbo, 1975) and the reaction seems to proceed irreversibly (Hylin, 1962). 3-Mercaptopyruvate is toxic to the cell (Jackson and Lindahl-Kiessling, 1963) and it has not been detected in tissues. These facts seem to suggest that 3-mercaptopyruvate is effectively metabolized by lactate dehydrogenase (Ohta and Ubuka, 1989) and/or 3-mercaptopyruvate sulfurtransferase reactions. As lactate dehydrogenase is a cytosolic enzyme, 3-mercaptopyruvate formed in

mitochondria may be metabolized by 3-mercaptopyruvate sulfurtransferase in mitochondria or might be transported to the cytosol.

As shown previouly (Ubuka et al., 1977), incubation of L-cysteine, 2-oxoglutarate and potassium cyanide with rat liver mitochondria resulted in the formation of appreciable amount of thiocyanate. The present study shows incubation of L-cysteine, 2-oxoglutarate and GSH with rat liver mitochondria resulted in the formation of substantial amount of sulfate and thiosulfate. Therefore, it seems likely that L-cysteine is effectively metabolized by the coupled reaction of aspartate aminotransferase and 3-mercaptopyruvate sulfurtransferase in mitochondria although the Km value of the former enzyme for L-cysteine was as high as 22 mM (Ubuka et al., 1978). This is reminiscent of L-cysteinesulfinate metabolism by aspartate aminotransferase reaction. Sulfinopyruvate formed decomposes spontaneously and rapidly (Ubuka et al., 1982), and the enzyme reaction proceeds faster than that with L-aspartate, although the Km value for L-cysteinesulfinate is much higher than that for L-aspartate (Stipanuk, 1986).

L-Cysteine at a concentration more than 10 mM was inhibitory to the sulfate and thiosulfate formation as shown in Fig. 1. The reason is unknown, but it may suggest that L-cysteine affected as an SH reagent on enzyme proteins.

Incubation of 3-mercaptopyruvate also resulted in sulfate and thiosulfate formation (Table 3) and that of thiosulfate in sulfate formation (Table 4), and these were also accelerated by the addition of GSH or GSSG. In contrast, the sulfate formation was not accelerated by GSH and GSSG when L-cysteinesulfinate and 2-oxoglutarate were incubated with rat liver mitochondria.

It has been reported that one of the physiological sulfur acceptor of 3-mercaptopyruvate sulfurtransferase reaction is sulfite (Sörbo, 1957) and that the activity of thiosulfate oxidation to sulfate in the presence of GSH is associated with rat liver mitochondria (Koj et al., 1967). It has been proposed that thiosulfate is the most important intermediate in sulfate formation and the thiosulfate cycle is participated in sulfate formation in animal tissues (Koj et al., 1967). However, the mechanism of sulfate formation from thiosulfate has been obscure. It has been reported that thiosulfate is reduced in the presence of GSH to hydrogen sulfide and sulfite by thiosulfate reductase (EC 2.8.1.3) (Sörbo, 1964), and this enzyme is present in mitochondrial matrix (Koj et al., 1975). In contrast to this enzyme, GSH is not a substrate of thiosulfate sulfurtransferase (EC 2.8.1.1) (Sörbo, 1962), which is also present in mitochondrial matrix (Koj et al., 1975). Thus, the accelerating effect of GSH on sulfate formation in the present study and in Wainer's report seems to be explained by the action of thiosulfate reductase (Ubuka et al., 1990). The accelerating effect of GSSG, at lesser extent than that of GSH, seems to suggest that GSSG acted after it was reduced to GSH by glutathione reductase (EC 1.6.4.2) which was present in mitochondria (Flohé and Schlegel, 1971).

The effect of GSH concentration on the sulfate formation in the present system was examined. As shown in Fig. 2, the maximum amount of the sum of sulfate and thiosulfate was formed at 10 mM GSH. This concentration coincided with the physiological GSH concentration in rat liver mitochondria as shown in Table 1 and that reported (Wahlländer et al., 1979). Dithiothreitol and

2-mercaptoethanol at 10 mM concentration did not exhibit any accelerating effect as reported by Wainer (1967).

Wainer (1967) reported that 3-mercaptopyruvate was not the substrate for sulfate formation in his system. In the present study, however, substantial amounts of sulfate and thiosulfate were formed when 3-mercaptopyruvate and GSH were incubated with rat liver mitochondria as shown in Table 3. Although the reason(s) of the discrepancy between these two results is not clear, it might be one of the reason(s) that GSH was not contained in Wainer's experiments.

According to Wainer (1967) free hydrogen sulfide was not a substrate in his system. However, it has been shown that hydrogen sulfide is oxidized to thiosulfate in rat liver by heme compounds (Sörbo, 1958), and metal-protein complexes and ferritin (Baxter and van Reen, 1958b), that the activity was associated with mitochondria (Baxter et al., 1958), and that sulfite may be involved as an intermediate (Baxter and van Reen, 1958a). It is well known that sulfite oxidase (EC 1.8.3.1) is present in mitochondria (Cohen et al., 1972).

The present findings and previous studies seem to indicate that, in mitochondria, L-cysteine is metabolized through 3-mercaptopyruvate pathway and the sulfur atom is transferred to sulfite to form thiosulfate, which is further metabolized to sulfate. Thus, we propose a scheme shown in Fig. 3 for L-cysteine metabolism via 3-mercaptopyruvate pathway and sulfate formation in mitochondria. It seems that some part of L-cysteine in the cell is metabolized in mitochondria through the 3-mercaptopyruvate pathway and thiosulfate formed is reduced to hydrogen sulfide and sulfite. Hydrogen sulfide thus formed may be oxidized to sulfite, and sulfite may be finally oxidized to sulfate by sulfite oxidase. Some of the sulfite may be again utilized to accept sulfur from 3-mercaptopyruvate to produce thiosulfate.

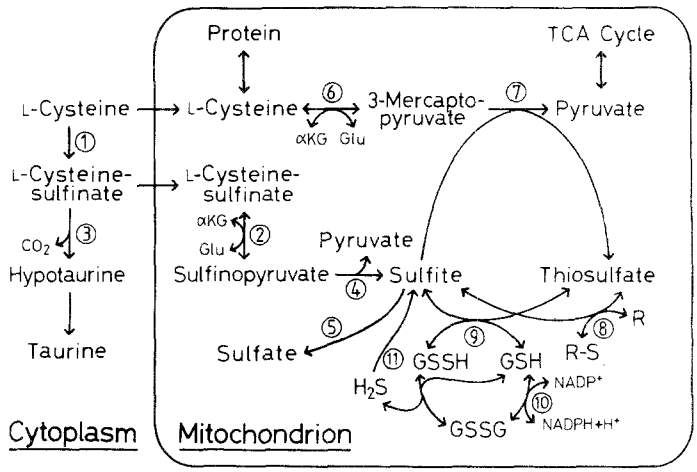

Fig. 3. Scheme for 3-mercaptopyruvate pathway of L-cysteine metabolism and sulfate formation in mitochondria

1 L-Cysteine dioxygenase; *2* L-aspartate aminotransferase; *3* L-cysteinesulfinate decarboxylase; *4* non-enzymatic; *5* sulfite oxidase; *6* L-aspartate aminotransferase; *7* 3-mercaptopyruvate sulfurtransferase; *8* thiosulfate sulfurtransferase; *9* thiosulfate reductase; *10* glutathione reductase; *11* non-enzymatic; *αKG* 2-oxoglutarate; *R* sulfur acceptor; *R–S* sulfur acceptor which received sulfur

As shown in the present study, sulfate formation from L-cysteinesulfinate is not accelerated by GSH. Therefore, L-cysteinesulfinate seems not to be involved in the mitochondrial sulfate-forming system from L-cysteine as reported (Wainer, 1964, 1967; Singer, 1975).

Thiosulfate is the important substrate of thiosulfate sulfurtransferase, which seems to be involved in cyanide detoxication (Sörbo, 1975) and in the formation of iron-sulfur complexes (Cerletti, 1986). Thus, it may be assumed that the physiological significance of the 3-mercaptopyruvate pathway is to supply nonoxidized divalent sulfur atoms from L-cysteine to the thiosulfate sulfurtransferase reaction, and excess sulfur seems to be oxidized to sulfate in the system described above. Therefore, the 3-mercaptopyruvate pathway seems to be physiologically important as suggested (Cooper, 1983), although it may be quantitatively small and has received relatively little attention. In this meaning, GSH seems to play a role in the mitochondrial sulfur metabolism.

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