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Toshihiko Ubuka[*] Shigeki Yuasa[†] Jun Ohta[‡]

Noriyoshi Masuoka[**] Kenzaburoh Yao[††] Masahiro Kinuta[‡‡]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,

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Toshihiko Ubuka, Shigeki Yuasa, Jun Ohta, Noriyoshi Masuoka, Kenzaburoh Yao, and Masahiro Kinuta

Abstract

Formation of sulfate in rat liver mitochondria was studied. About 0.1 mumol of sulfate was formed in mitochondria from 1 g of liver in 60 min when 10 mM L-cysteine was used as the substrate. Addition of either 10 mM 2-oxoglutarate or 10 mM glutathione to this system increased sulfate formation 3 to 4 times. The addition of both 2-oxoglutarate and glutathione resulted in a 20-fold increase in sulfate formation. Sulfate formation in the presence of 5 mM L-cysteine was 58% of that with 10 mM L-cysteine. L-Cysteine-glutathione mixed disulfide was not a good substrate, indicating that this mixed disulfide was not an intermediate of sulfate formation in the present system. Incubation of 3-mercaptopyruvate with rat liver mitochondria also resulted in sulfate formation, and the addition of glutathione accelerated it. Formation of sulfite and thiosulfate was also detected. These results indicate that sulfate is produced in mitochondria, at least in part, from L-cysteine through the transamination pathway (3-mercaptopyruvate pathway).

KEYWORDS: sulfate formatioon, cysteine metabolism, glutathione, 3-mercaptopyruvate, mitochondria

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Toshihiko Ubuka*, Shigeki Yuasa[a], Jun Ohta, Noriyoshi Masuoka, Kenzaburoh Yao and Masahiro Kinuta

Department of Biochemistry, Okayama University Medical School, Okayama 700, Japan

Formation of sulfate in rat liver mitochondria was studied. About 0.1 μmol of sulfate was formed in mitochondria from 1 g of liver in 60 min when 10 mM L-cysteine was used as the substrate. Addition of either 10 mM 2-oxoglutarate or 10 mM glutathione to this system increased sulfate formation 3 to 4 times. The addition of both 2-oxoglutarate and glutathione resulted in a 20-fold increase in sulfate formation. Sulfate formation in the presence of 5 mM L-cysteine was 58% of that with 10 mM L-cysteine. L-Cysteine-glutathione mixed disulfide was not a good substrate, indicating that this mixed disulfide was not an intermediate of sulfate formation in the present system. Incubation of 3-mercaptopyruvate with rat liver mitochondria also resulted in sulfate formation, and the addition of glutathione accelerated it. Formation of sulfite and thiosulfate was also detected. These results indicate that sulfate is produced in mitochondria, at least in part, from L-cysteine through the transamination pathway (3-mercaptopyruvate pathway).

Key words : sulfate formation, cysteine metabolism, glutathione, 3-mercaptopyruvate, mitochondria

Inorganic sulfate is one of the major end products of sulfur metabolism in mammals (1, 2). Sulfur is taken in mainly as L-methionine and L-cysteine in proteins (1), and most of the sulfur of these sulfur-containing amino acids is ultimately oxidized to sulfate (3). Metabolic pathways of cysteine sulfur to sulfate may be divided into two types. The first type is the oxidation pathway in which sulfur is oxidized before removal from the carbon skeleton and which proceeds through the intermediate L-cysteinesulfinate. In the second type, the sulfur is removed from the carbon skeleton before oxidation. The transamination pathway of cysteine metabolism (4) belongs to the latter type, but its participation in sulfate formation has not been studied. This pathway proceeds through 3-mercaptopyruvate, and thus it may be referred to as the 3-mercaptopyruvate pathway (5).

Wainer has proposed that an alternative pathway of sulfate formation from L-cysteine exists in rat liver mitochondria (6, 7), and claimed that neither L-cysteinesulfinate, 3-mercaptopyruvate nor free H_2S was an intermediate in the mitochondrial system. However, the mechanism of sulfate formation from L-cysteine in mitochondria is poorly understood (8).

* To whom correspondence should be addressed.

a: Present address: Department of Anatomy, School of Medicine, Keio University, Shinjuku, Tokyo 160, Japan.

The present study was undertaken to clarify problems concerning the formation of sulfate from L-cysteine by rat liver mitochondria. Possible involvement of the transamination pathway of cysteine metabolism was examined. In this study, besides L-cysteine, some related compounds such as L-cystine, S-(2-amino-2-carboxyethylsulfonyl)-L-cysteine (ACESC), L-cysteine-glutathione mixed disulfide (Cys-SG), L-cysteic acid and 3-mercaptopyruvate were also examined as substrates.

Materials and Methods

Materials. L-Cysteine (free base), L-cystine and L-cysteic acid were obtained from Sigma Chemical Co., St. Louis, MO, USA. Glutathione (GSH), oxidized glutathione (GSSG), dithiothreitol and L-glutamate dehydrogenase (EC 1. 4. 1. 3) from beef liver were purchased from Böhringer-Mannheim GmbH, Mannheim, FRG. 2-Oxoglutarate, sucrose and isopropanol were from Wako Pure Chemical Ind., Ltd., Osaka, Japan. ACESC was synthesized as described elsewhere (9). Ammonium 3-mercaptopyruvate was prepared according to Kun (10). Cys-SG was synthesized by the method of Eriksson and Eriksson (11). L-Cysteinesulfinic acid was prepared as reported previously (12).

Preparation of mitochondria. Rat liver mitochondria were prepared according to the method of Hogeboom (13) from male Wistar rats weighing 250–300 g. It was found that the sucrose used contained an appreciable amount of sulfate as a contaminant and interfered with the present experiment because of the high background level. Therefore, mitochondrial preparations were given a final wash with 0.14 M potassium chloride containing 10 mM Tris chloride and 0.1 mM EDTA (final pH 7.40), suspended in the same solution, and used as the enzyme source. Sonication of the mitochondrial suspension was performed at 20 kHz using Sonifier B-12 (Branson Ultrasonic Corp., Danbury, USA) at 0°C for the time indicated below. Freeze-thaw treatment of the mitochondrial suspension was performed by freezing at −20°C and thawing in a water bath at 20°C. In some experiments, a preparation (step 6 of purification) of cysteine aminotransferase (EC 2. 6. 1. 3) purified from rat liver mitochondria according to a previous report (14) was used.

Enzyme reaction. The standard incubation mixture for the sulfate formation from L-cysteine by rat liver mitochondria contained, in a final volume of 1.0 ml, 200 μmol of Tris-hydrochloride (pH 8.20), 10 μmol of L-cysteine, 10 μmol of 2-oxoglutarate, 10 μmol of GSH and mitochondrial preparation obtained from 0.3 g of rat liver. Other sulfur-containing substrates and GSSG were used as described below. The incubation mixture was shaken at 130 strokes per min at 37°C for 30 min. The reaction was stopped by the addition of 0.3 ml of glacial acetic acid. Reactions without enzyme were taken as controls.

In experiments with cysteine aminotransferase, 5 of L-cysteinesulfinate or ACESC and 3 μmol of 2-oxoglutarate were incubated with cysteine aminotransferase preparation (1.2 μg of protein) at pH 8.2 for 10 min. The reaction was terminated with 0.5 M perchloric acid or by heating in a boiling water bath for 2 min.

Determination of sulfate. Sulfate was determined with barium chloranilate (15). In the present study, the method was modified in order to determine sulfate in enzyme reactions with mitochondria as follows. After the enzyme reaction, 0.3 ml of glacial acetic acid was added to the reaction mixture as described above, and the mixture was heated for 5 min in a boiling water bath. After cooling to room temperature, 3.7 ml of isopropanol was admixed, and the mixture was allowed to stand for 10 min. It was then centrifuged at 1,200 × *g* for 10 min, and the resulting supernatant was separated. To 4.0 ml of the supernatant placed in a test tube with a Teflon-lined screw cap, 1.0 ml of 10% acetic acid in isopropanol and about 75 mg of barium chloranilate were added. The tube was shaken vigorously at 160 strokes per min for 30 min. Then it was centrifuged as above, and the absorbance at 530 nm of the resulting supernatant was measured. A solution containing 1 to 3 μmol of potassium sulfate was run in parallel as the standard for the color development. Sulfate formed was expressed as μmol per mitochondria from 1 g of liver per h.

In experiments with the cysteine aminotransferase preparation described above, sulfate was determined directly after the reaction as above or determined after the treatment of the reaction mixture at room temperature with 1% hydrogen peroxide for 10 min and/or 4 mM dithiothreitol for 30 min.

Determination of sulfite, thiosulfate and L-glutamate. Sulfite and thiosulfate were determined using ACESC as reported (9, 12, 16). L-Glutamate was determined with L-glutamate dehydrogenase as reported previously (13).

Results

Analytical methods. In the present study, the enzymatically produced inorganic sulfate in mitochondria was determined by a colorimetric method with barium chloranilate (15). However, it was found that the presence of mitochondria reduced the recovery of sulfate even in the presence of 80% isopropanol (14) and 1 to 8% acetic acid. Therefore, it was assumed that the sulfate formed was retained in mitochondria, or protein inhibited the reaction between sulfate and barium chloranilate. The recovery improved when the reaction mixture was heated in a boiling water bath for 5 min in the presence of 23% acetic acid. When the heat extract was reacted with barium chloranilate in 80% isopropanol, the presence of acetic acid between 2 and 6% gave the maximum recovery, but the recovery decreased in the presence of more than 8% acetic acid. The addition of isopropanol to the heat extract at a final concentration of 80% gave the most satisfactory results. The blank value increased when isopropanol was less than 75%. The conditions described under Materials and Methods were the most satisfactory for the determination of sulfate formed in mitochondria.

Table 1 Sulfate formation from L-cysteine in rat liver mitochondria[a]

Substrate (mM)	Sulfate formed (μmol/Mt from 1 g of liver/60 min)
L-Cysteine (10), 2-Oxoglutarate (10) and GSH (10)	1.70
L-Cysteine (10), 2-Oxoglutarate (10) and GSSG (5)	1.47
L-Cysteine (10) and 2-Oxoglutarate (10)	0.40
L-Cysteine (10) and GSH (10)	0.33
L-Cysteine (10)	0.09

a: Rat liver mitochondria were incubated with L-cysteine with or without other substrates at pH 8.2 for 30 min at 37°C while shaking. Reactions without substrates were taken as the control. Sulfate formed was determined with barium chloranilate and expressed as μmol per mitochondria prepared from 1 g of rat liver per 60 min. Details are described under Materials and Methods. Abbreviations: GSH, glutathione; GSSG, oxidized glutathione; Mt, mitochondria.

Sulfite and thiosulfate were determined by converting them to L-cysteine derivatives with ACESC (9, 12, 16). This method is specific to these compounds, which can be determined simultaneously.

Formation of sulfate from L-cysteine in rat liver mitochondria. Formation of inorganic sulfate was examined using rat liver mitochondria as the enzyme source. As shown in Table 1, sulfate was formed in the presence of L-cysteine, 2-oxoglutarate and GSH.

The sulfate formation was proportional to the incubation time and to the amount of mitochondrial suspension as shown in Figs. 1 and 2. Heating the mitochondrial suspension at 85°C for 7 min abolished the sulfate formation.

Fig. 3 shows the effect of pH on the sulfate formation under the present conditions. Sulfate formation was high between pH 8.2 and pH 8.6.

Effect of some factors on the sulfate formation from L-cysteine in rat liver mitochondria. As shown in Table 1, sulfate formation was 0.09 μmol per mitochondria from 1 g of liver per 60 min when L-cysteine alone was used as a substrate. Addition of either GSH or 2-oxoglutarate to the incubation mixture increased sulfate formation 3 to 4 times. The addition of both GSH and 2-oxoglutarate increased sulfate formation almost 20-fold. GSSG also had an accelerating effect on sulfate formation, but to a lesser extent than GSH.

The effect of some sulfhydryl compounds other than GSH on sulfate formation was examined. At a concentration of 10 mM, dithiothreitol, 2-mercaptoethanol, L-cysteamine and D, L-homocysteine exhibited no accelerating effect on sulfate formation from L-cysteine in rat liver mitochondria.

Formation of sulfate from cysteine-related compounds. Table 2 shows the sulfate formation from L-cysteine and related compounds in rat liver mitochondria in the presence of 5 mM 2-oxoglutarate and 5 mM GSH. When 5 mM

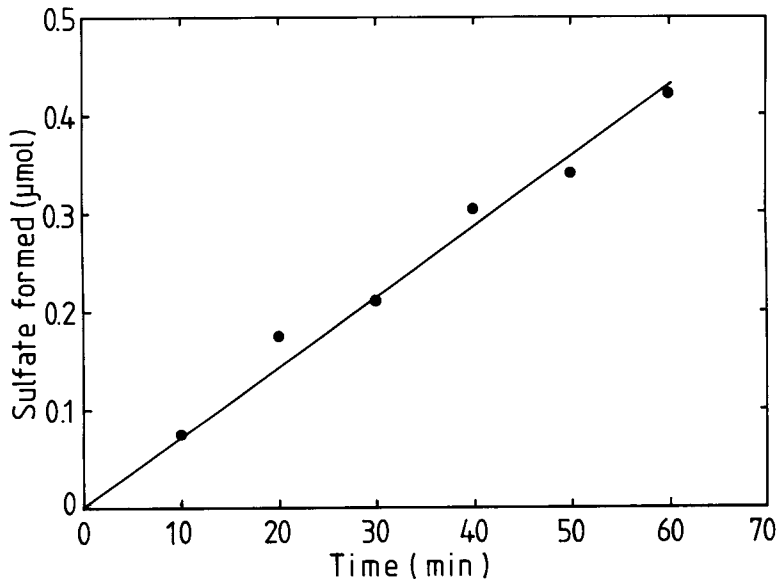

Fig. 1 Relationship of sulfate formation from L-cysteine and the reaction time. A mixture of 10 μmol each of L-cysteine, 2-oxoglutarate and glutathione was incubated with rat liver mitochondria prepared from 0.5 g of rat liver in a final volume of 1 ml at pH 8.4 and 37°C for the time indicated.

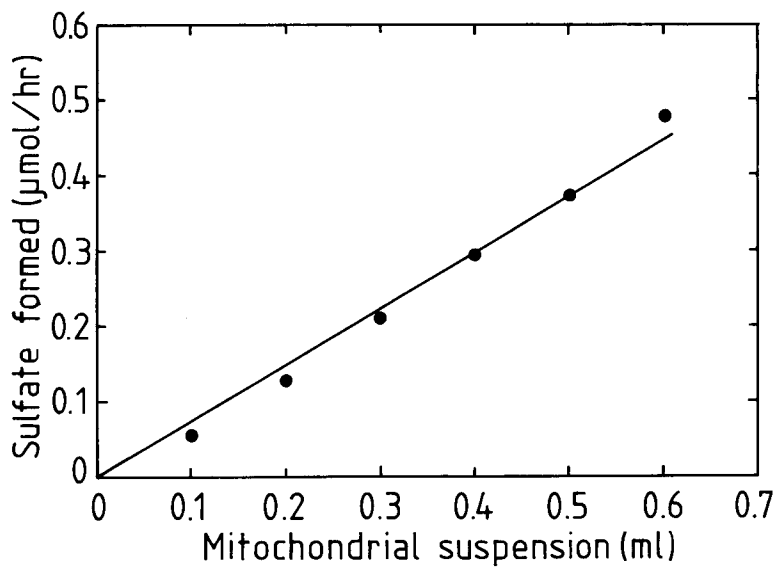

Fig. 2 Relationship of sulfate formation from L-cysteine and the amount of mitochondria. A mixture of 10 μmol each of L-cysteine, 2-oxoglutarate and glutathione was incubated at pH 8.4 and 37°C for 30 min with various amounts of mitochondrial suspension prepared from rat liver.

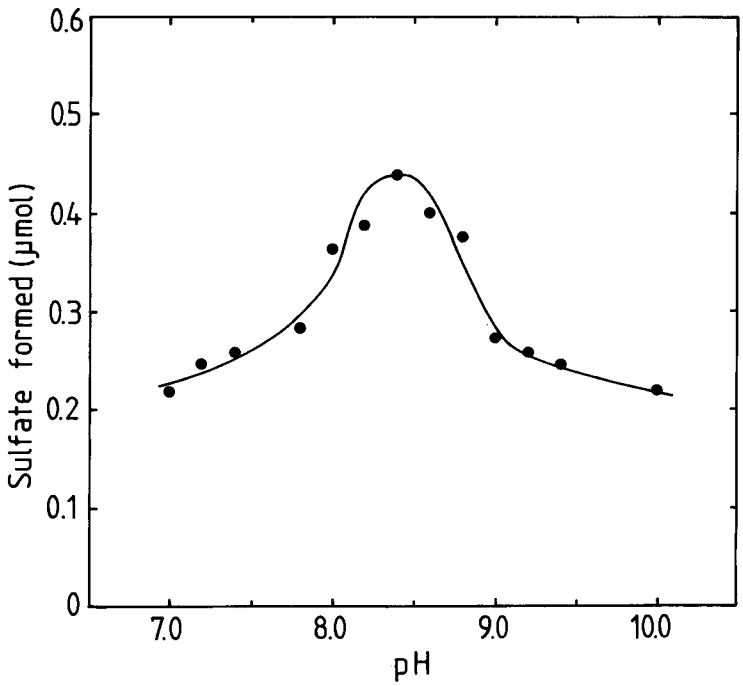

Fig. 3 Relationship of sulfate formation from L-cysteine and pH. A mixture of 10 μmol each of L-cysteine, 2-oxoglutarate and glutathione was incubated at various pH with mitochondrial suspension prepared from 0.3 g of rat liver in a final volume of 1 ml at 37 °C for 30 min. Sulfate formed is expressed as μmol per mitochondria from 1 g of liver.

Table 2 Sulfate formation from L-cysteine and related compounds in rat liver mitochondria in the presence of 5 mM 2-oxoglutarate and 5 mM GSH[a]

Substrate (mM)	Sulfate formed (μmol/Mt from 1 g of liver/60 min)
L-Cysteine (10)	1.46
L-Cystine (5)	0.84
L-Cys-SG (10)	0.13
L-Cysteinesulfinate (10)	3.44
ACESC (10)	8.24
L-Cysteic acid (10)	0.19

a: Substrates were incubated in the presence of 5 mM 2-oxoglutarate, 5 mM GSH and rat liver mitochondria at pH 8.2 for 30 min at 37 °C. Sulfate formed was determined with barium chloranilate and expressed as μmol per mitochondria prepared from 1 g of liver per 60 min. Values are the means of 2 to 4 experiments. Abbreviations: GSH, glutathione; Cys-SG, cysteine-glutathione mixed disulfide; ACESC, S-(2-amino-2-carboxyethylsulfonyl)-L-cysteine; Mt, mitochondria. Details are described under Materials and Methods.

L-cystine (equivalent to 10 mM L-cysteine when reduced) was used instead of 10 mM L-cysteine, sulfate formation was 58 % of that with L-cysteine. Production of sulfate in the presence of Cys-SG or L-cysteic acid was very low. On the contrary, sulfate formation was very high when L-cysteinesulfinate or ACESC was used as a substrate.

As shown in Table 3, when L-cysteinesulfinate was incubated with cysteine aminotransferase preparation purified from rat liver mitochondria and 2-oxoglutarate, L-glutamate was formed, but little sulfate was detected. However, incubation of the reaction mixture with 1 % hydrogen peroxide solution resulted in the formation of sulfate in an amount equivalent to that of L-glutamate. These results indicated that sulfinopyruvate was formed, and that the sulfite liberated was oxidized to sulfate by hydrogen peroxide.

When ACESC was incubated with the same cysteine aminotransferase preparation as above,

Table 3 Products of the transamination reaction of L-cysteinesulfinate and ACESC[a]

Substrate	Products (μmol/reaction tube)				
	L-Glu	Sulfate[b]	Sulfate after H_2O_2 treatment[c]	Sulfate after DTT treatment[d]	Sulfate after DTT and H_2O_2 treatments[e]
L-Cysteinesulfinate	1.69	0.04	1.41	—	—
ACESC	1.27	0.00	0.00	—	—
ACESC	—	0.00	—	0.06	0.77

a: Five μmol each of L-cysteinesulfinate or ACESC was incubated with 3μmol of 2-oxoglutarate and cysteine aminotransferase preparation (1.2μg of protein) purified from rat liver mitochondria in a final volume of 1 ml at pH 8.2 for 10 min at 37°C. Three tubes were incubated in parallel, and each tube was used for the determination of L-Glu or sulfate. Tubes lacking the enzyme were run in parallel as controls. Sulfate was determined with barium chloranilate directly after the reaction (*b*) or after the treatment with H_2O_2 (*c*), DTT (*d*) or DTT and H_2O_2 (*e*). Details are described under Materials and Methods. Abbreviations: L-Glu, L-glutamate; DTT, dithiothreitol; GSH and ACESC, see the legend to Table 2.

no sulfate was detected, even after hydrogen peroxide treatment. However, sulfate was detected when the reaction mixture was incubated with 4 mM dithiothreitol followed by 1% hydrogen peroxide treatment. These results indicate that S-sulfocysteine was formed from ACESC as reported (17), from which sulfite was split by dithiothreitol and then oxidized to sulfate by hydrogen peroxide.

Effect of sonication and of freezing and thawing on sulfate formation in rat liver mitochondria. As shown in Table 2, Cys-SG was not an active substrate for sulfate formation when intact mitochondria were used as the enzyme source. In order to examine the possibility that Cys-SG could not be transported into mitochondria where sulfate formation took place, mitochondria were disrupted by sonication or by freezing and thawing. As shown in Fig. 4, sonication decreased sulfate formation from L-cysteine. The activity remaining after mitochondria were sonicated longer than 5 min or when mitochondria were treated by freezing and thawing was about 40% of that of intact mitochondria (Fig. 4 and Table 4). Sulfate formation from Cys-SG did not increase due to sonication or freezing and thawing, indicating that low sulfate production from Cys-SG was not due to the failure of it being transported into mitochondria and that Cys-SG was not a good substrate for sulfate formation in mitochondria.

Formation of sulfate from 3-mercaptopyruvate. Table 5 shows that sulfate was formed from 3-mercaptopyruvate in rat liver mitochondria. GSH doubled the sulfate formation. Sulfate and thiosulfate were produced in addition to sulfate. The sum of sulfate, sulfite and thiosulfate (value doubled because of there being two sulfur atoms in one molecule) formed from 3-mercaptopyruvate was also doubled in the presence of GSH.

Discussion

Major metabolism of L-cysteine in rat liver is its oxidation to cysteinesulfinic acid by cysteine dioxygenase (EC 1. 13. 11. 20) in the cytosol (18). Some of the cysteinesulfinic acid thus formed is transported to mitochondria and further metabolized (19).

Wainer has reported that an alternative pathway of cysteine oxidation resulting in the formation of sulfate is present in rat liver mitochondria

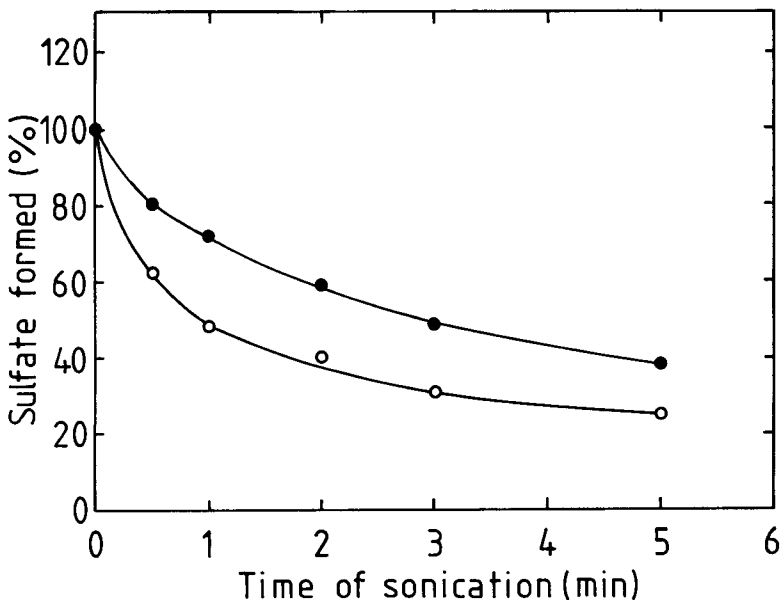

Fig. 4 Effect of the sonication of the mitochondrial suspension on sulfate formation from L-cysteine (●) or L-cystine (○) in rat liver mitochondria. L-Cysteine (10 mM) or L-cystine (5 mM) was incubated with 2-oxoglutarate (10 mM), glutathione (10 mM) and fresh or sonicated mitochondria.

Table 4 Effect of sonication and of freezing and thawing on the formation of sulfate from L-cysteine or L-cysteine-glutathione mixed disulfide (Cys-SG)[a]

Treatment	Sulfate formed (μmol/Mt from 1 g of liver/60 min)	
	Substrate	
	Cys-SG and 2-oxoglutarate	L-Cysteine, GSH and 2-oxoglutarate
None	0.06	2.30
Sonication for 10 min	0.08	0.90
Freezing and thawing once	0.02	0.96

a: Substrates (10 mM) were incubated with fresh, sonicated or frozen-thawed mitochondrial preparation at 37 °C for 30 min. Sulfate formed was determined with barium chloranilate and expressed as μmol per mitochondria from 1 g of liver per 60 min. Details are described under Materials and Methods. For abbreviations, see the legends to Tables 1 and 2.

Table 5 Formation of sulfate, sulfite and thiosulfate from 3-mercaptopyruvate in rat liver mitochondria in the presence of GSH[a]

Substrate	Products (μmol/Mt from 1 g of liver/60 min)		
	Sulfate	Sulfite	Thiosulfate
3-Mercaptopyruvate and GSH	2.80	0.45	1.60
3-Mercaptopyruvate	1.55	1.00	0.50
L-Cysteine and GSH	0.30	0.00	0.50
L-Cysteine	0.15	0.00	0.50
GSH	0.00	0.08	0.00

a: Substrates (10 mM) were incubated with fresh mitochondrial preparations at 37 °C for 30 min. Sulfate formed was determined with barium chloranilate, and sulfite and thiosulfate were determined after conversion to cysteine derivatives. Amounts of products were expressed as μmol per mitochondria from 1 g of liver per 60 min. Details are described under Materials and Methods. For abbreviations, see the legend to Table 1.

(6, 7). According to Wainer, the alternative pathway requires only cysteine and oxygen in fresh mitochondria, but either GSH or GSSG stimulate sulfate formation in frozen-thawed mitochondria. It was also reported that L-cysteinesulfinate, hydrogen sulfide and 3-mercaptopyruvic acid were not intermediates in the mitochondrial system. However, the mecha-

nism of this alternative pathway has not been clarified, although Singer also noted that sulfate was actively formed from L-cysteine in rat liver mitochondria (8). The present investigation was undertaken to clarify the mechanism of sulfate formation in mitochondria.

Under the present conditions, 1.7 μmol of sulfate was formed from L-cysteine in 60 min in fresh mitochondria prepared from 1 g of rat liver with 10 mM 2-oxoglutarate and 10 mM GSH added. Contrary to Wainer's report (6, 7), 2-oxoglutarate, added together with GSH, strongly accelerated sulfate formation as shown in Table 1. As already reported, transamination of L-cysteine occurs in mitochondria (13). The responsible enzyme has been purified, and it has been suggested that mitochondrial cysteine aminotransferase is identical with mitochondrial aspartate aminotransferase (14).

Mitochondrial sulfate formation from L-cysteine was also accelerated by the addition of GSH or GSSG as reported by Wainer (6). In contrast to Wainer's results (6, 7), GSH was effective in both fresh and frozen-thawed mitochondria in accordance with the report of Singer (8). GSH was effective in sonicated mitochondria as well.

As shown in Fig. 4, sonication of mitochondrial preparation reduced the sulfate-forming activity. As the time of sonication increased, the rate of the decrease became less, and the activity approached a value of about 40 % of the fresh mitochondria. This result seems to indicate that the effect of sonication is mainly the dilution of enzymes involved in sulfate formation.

In as much as GSH and GSSG are effective in stimulating sulfate formation, it may be assumed that Cys-SG is formed and is an intermediate of sulfate formation. However, as shown in Table 2, the disulfide was not a good substrate. It could have been that Cys-SG added to the fresh mitochondrial preparation did not reach the enzyme system for sulfate formation, but this possibility was ruled out because sulfate formation from this disulfide in sonicated and frozen-thawed mitochondria was very low (Table 4).

When 3-mercaptopyruvate, the product of transamination of cysteine, was incubated with the mitochondrial preparation, formation of appreciable amounts of sulfate, thiosulfate and sulfite were detected as summarized in Table 5. Total formation of sulfate, thiosulfate and sulfite was doubled by the addition of GSH. As reported (20), rat liver mitochondria contain 3-mercaptopyruvate sulfurtransferase (EC 2. 8. 1. 2) activity. Sörbo proposed that one of the physiological substrates of 3-mercaptopyruvate sulfurtransferase was sulfite and the product was thiosulfate (21). It has been reported that oxidation of thiosulfate to sulfate occurs in rat liver in the presence of GSH (22) and that this activity is associated with mitochondria (23). Sörbo and Koj *et al.* proposed that thiosulfate is the most important intermediate in sulfate formation in mammals, and Koj *et al.* proposed that a thiosulfate cycle participates in sulfate formation in animal tissues (23).

Sörbo (24) and Baxter *et al.* (25–27) studied oxidation of hydrogen sulfide to thiosulfate in rat liver. They concluded that the oxidation was catalyzed by heme compounds (24), metal-protein complexes (27) and ferritin (27), that the activity was associated with mitochondria (25), and that sulfite may be involved as an intermediate (26).

As shown in Table 2, L-cysteinesulfinate is a better substrate than L-cysteine. It seems that L-cysteinesulfinate formed in the cytosol by cysteine dioxygenase is transported to mitochondria (19) and then transaminated to produce sulfinopyruvate, which decomposes spontaneously to pyruvate and sulfite. The sulfite thus formed may join the sulfite pool and may be oxidized to sulfate. This assumption seems to be supported by the original experiment of Wainer (6), in which the addition of cysteinesulfinate together with 2-oxoglutarate to his system halved the production of [^{35}S]sulfate.

The present study and other results mentioned above seem to indicate that both oxidation and transamination pathways participate in intracel-

lular sulfate formation. Thus, we propose that sulfate formation from L-cysteine in rat liver mitochondria may be, at least in part, mediated by the 3-mercaptopyruvate pathway. Namely, L-cysteine is transaminated to give 3-mercaptopyruvate. The sulfur atom of 3-mercaptopyruvate is then transferred by 3-mercaptopyruvate sulfurtransferase to sulfite to give thiosulfate, and finally thiosulfate is oxidized to sulfate.

Thiosulfate seems to be an important intermediate in sulfate formation (22, 23). However, the mechanism of sulfate formation from thiosulfate is obscure at present. It has been reported that thiosulfate is reduced in the presence of GSH to give GSSG and hydrogen sulfide, and that this reaction is catalyzed by thiosulfate reductase (EC 2. 8. 1. 3) and/or thiosulfate sulfurtransferase (EC 2. 8. 1. 1) (22). The latter enzyme is known to be present in mitochondria, but it was reported that GSH was not a substrate of this enzyme (28, 29) and that thiosulfate sulfurtransferase can reduce thiosulfate in the presence of lipoate (29). Therefore, thiosulfate can be reduced by thiosulfate reductase and thiosulfate sulfurtransferase in the presence of GSH and lipoic acid, respectively (22). The accelerating effect of GSH on sulfate formation in the present study and in Wainer's experiments seems to be explained by the action of thiosulfate reductase. The hydrogen sulfide thus formed may be oxidized to sulfite, and sulfite may be oxidized to sulfate by sulfite oxidase (EC 1. 8. 3. 1) which is present in mitochondria (30). Some of the sulfite may be again utilized to accept sulfur in a 3-mercaptopyruvate sulfurtransferase reaction. GSSG formed during thiosulfate reduction may be reduced to GSH by glutathione reductase (EC 1. 6. 4. 2) which is present in mitochondria (31). GSH is present in high concentrations in liver mitochondria (32). Thus, the present study seems to suggest that one of the physiological functions of GSH in mitochondria is the participation in sulfate formation.

It should be mentioned that L-cysteine may be metabolized partly by cystathionine γ-lyase (EC 4. 4. 1. 1) in the cytosol, resulting in the formation of hydrogen sulfide (33). This hydrogen sulfide may also be oxidized to sulfate in the cell.

ACESC was a much better substrate than L-cysteine or L-cysteinesulfinate for sulfate formation in rat liver mitochondria (Table 2). However, the production of ACESC in animal tissues has not been detected.

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