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Abstract

Metabolism of 3-mercaptopyruvate was investigated using homogenates of rat heart, liver and kidney. When 3-mercaptopyruvate was incubated with heart homogenate, L-cysteine, L-alanine, S-(2-hydroxy-2-carboxyethylthio)-L-cysteine and 3-mercaptolactate were produced. At the same time, a decrease in the amounts of L-glutamate and L-aspartate was demonstrated. These results indicate that 3-mercaptopyruvate was converted to L-cysteine by cysteine aminotransferase (EC 2.6.1.3), to 3-mercaptolactate by lactate dehydrogenase (EC 1.1.1.27), and to pyruvate by 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2), and that HCETC and L-alanine were formed from these products. In the presence of liver homogenate, 3-mercaptopyruvate was mainly metabolized by 3-mercaptopyruvate sulfurtransferase; production of L-cysteine was small and HCETC was not formed. The metabolism of 3-mercaptopyruvate in the presence of kidney homogenate was intermediate between heart and liver: a fair amount of L-cysteine was formed, but HCETC was not produced. A peak which corresponds to L-cysteine-glutathione disulfide on the chromatogram of amino acid analysis was present when 3-mercaptopyruvate was incubated with heart or liver homogenate, but not with kidney homogenate.

KEYWORDS: 3-mercaptopyruvate, L-cysteine, 3-mercaptolactate, S-(2-hydroxy-2-carboxyethylthio)-L-cysteine, L-cysteine-glutathione disulfide

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METABOLISM OF 3-MERCAPTOPYRUVATE IN RAT TISSUES

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Abstract. Metabolism of 3-mercaptopyruvate was investigated using homogenates of rat heart, liver and kidney. When 3-mercaptopyruvate was incubated with heart homogenate, L-cysteine, L-alanine, S-(2-hydroxy-2-carboxyethylthio)-L-cysteine and 3-mercaptolactate were produced. At the same time, a decrease in the amounts of L-glutamate and L-aspartate was demonstrated. These results indicate that 3-mercaptopyruvate was converted to L-cysteine by cysteine aminotransferase (EC 2.6.1.3), to 3-mercaptolactate by lactate dehydrogenase (EC 1.1.1.27), and to pyruvate by 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2), and that HCETC and L-alanine were formed from these products. In the presence of liver homogenate, 3-mercaptopyruvate was mainly metabolized by 3-mercaptopyruvate sulfurtransferase; production of L-cysteine was small and HCETC was not formed. The metabolism of 3-mercaptopyruvate in the presence of kidney homogenate was intermediate between heart and liver: a fair amount of L-cysteine was formed, but HCETC was not produced. A peak which corresponds to L-cysteine-glutathione disulfide on the chromatogram of amino acid analysis was present when 3-mercaptopyruvate was incubated with heart or liver homogenate, but not with kidney homogenate.

Key words: 3-mercaptopyruvate, L-cysteine, 3-mercaptolactate, S-(2-hydroxy-2-carboxyethylthio)-L-cysteine, L-cysteine-glutathione disulfide.

The transaminative pathway of cysteine metabolism was first suggested by Meister as an alternative pathway of cysteine degradation (1). This pathway belongs to one of two forms of cysteine catabolism, in which the sulfur atom is split off without oxidation (2). In another form of cysteine degradation, the sulfur atom is oxidized first by cysteine dioxygenase (EC 1.13.11.20). The latter form is considered to be the main route of cysteine metabolism in mammals (2, 3).

The transaminative pathway involves the formation of 3-mercaptopyruvate catalyzed by cysteine aminotransferase (EC 2.6.1.3) and transsulfuration of 3-mercaptopyruvate catalyzed by 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2). It has been reported from our laboratory that the transaminative pathway operates in various rat tissues (4, 5). Cysteine aminotransferase has been purified from rat liver (6, 7) and it was suggested that the enzyme was identical to aspartate aminotransferase (EC 2.6.1.1) (6, 7).

The formation of 3-mercaptopyruvate by transamination has been studied and applied to the assay of cysteine aminotransferase (6). The chemical and

biochemical nature of 3-mercaptopyruvate has also been reported (8). The present paper describes the metabolism of 3-mercaptopyruvate when it was incubated with homogenates of various rat tissues.

MATERIALS AND METHODS

Materials. Male Wistar rats weighing 250-300 g were used. Rats were fed ad libitum on a laboratory diet (MF of Oriental Yeast Co., Tokyo). Ammonium 3-mercaptopyruvate was synthesized according to Kun (9). As reported by Cooper *et al.* (8), this compound is unstable in solution; therefore it was dissolved in water just before use. S-(2-Amino-2-carboxyethylsulfonyl)-L-cysteine (ACESC; trivial name, cystine disulfoxide, CDS) (12) was synthesized according to the alternative method of Lavine (10).

Enzyme reactions. Homogenates of rat tissues were prepared at 0℃ as follows and used as enzyme sources. After decapitation, the heart, liver and kidney were removed and chilled in 0.14 M potassium chloride containing 10 mM potassium phosphate and 1 mM EDTA (pH 7.40) (solution A). Ventricles of hearts were cut into small pieces with scissors, and homogenized with two volumes of solution A using a Potter-Elvehjem glass homogenizer. Homogenization was further continued using an Ultra-Turrax homogenizer (Janke & Kunkel KG, Germany) at medium speed for 2 min. Homogenates of liver and kidney were prepared by homogenizing with two volumes of solution A using a Potter-Elvehjem homogenizer equipped with a Teflon pestle.

Two types of enzyme reactions were performed: reaction A and reaction B. Incubation mixture of reaction A (6.0 ml), placed in a 20 ml Erlenmeyer flask, contained 18 μmoles of 3-mercaptopyruvate, 4.0 ml of one of the above homogenates, 600 μmoles of potassium phosphate (pH 7.40) and 60 μmoles of EDTA. A control flask lacking 3-mercaptopyruvate was incubated at the same time. The mixture was shaken at about 100 strokes per min at 37℃ for 30 min. The reaction was stopped by addition of 1.2 ml of 18 % sulfosalicylic acid. Then the mixture was centrifuged at 1200×g for 15 min and the resulting supernatant was separated.

The incubation mixture of reaction B (20.0 ml), placed in a 100 ml Erlenmeyer flask, contained 60 μmoles of 3-mercaptopyruvate, 10.0 ml of heart homogenate, 2 mmoles of potassium phosphate (pH 7.40) and 200 μmoles of EDTA. A flask lacking 3-mercaptopyruvate was incubated at the same time as a control. The incubation was performed as above. The reaction was stopped by the addition of 10.0 ml of 0.3 M formic acid. The mixture was centrifuged at 1200×g for 15 min, and then the resulting supernatant was separated.

Determination of amino acids. Determination of amino acids was performed with a Hitachi KLA-5 amino acid analyzer using 0.2 N sodium citrate pH 3.19 at 55℃. The deproteinized supernatant (0.5 ml) of reaction A obtained above was directly applied to the analyzer.

The supernatant of reaction B, after stopping the reaction by the addition of 0.3 M formic acid as described above, was further deproteinized by picric acid according to Moore and Stein (11) as follows. To 10.0 ml of the supernatant, 50.0 ml of 1 % picric acid was admixed; the mixture was centrifuged at 1200×g for 15 min and the resulting supernatant was separated. Picric acid in the supernatant was eliminated by Diaion SA # 100, a strongly basic anion exchanger (Mitsubishi Chemical Industries Ltd., Tokyo), 2 × 2 cm, Cl^- form. The effluent and washings with 0.02 N hydrochloric acid were combined and evaporated to dryness at 50℃ under a reduced pressure. The residue was dissolved in 4.0 ml of 1 M acetic acid, and 0.5 ml of the solution was applied to the amino acid analyzer.

Determination of free 3-mercaptolactate. Determination of free 3-mercaptolactate in the reaction mixture was performed by using S-(2-amino-2-carboxyethylsulfonyl)-L-cysteine (ACESC) as follows. Five hundred μmoles of solid ACESC and 10.0 ml of the supernatant of reaction B after stopping the reaction by 0.3 M formic acid were put in a test tube (16 × 100 mm) with a Teflon-lined screw cap. The tube was shaken longitudinally at about 170 strokes per min at room temperature for 30 min. Then the mixture was centrifuged at 1200×g for 15 min and the resulting supernatant was separated. By this procedure 3-mercaptolactate was converted quantitatively to HCETC (12). Experimental details of this method will be reported elsewhere. HCETC thus formed was determined with an amino acid analyzer as described above. The amount of free 3-mercaptolactate was calculated by subtracting HCETC in the original supernatant from HCETC after ACESC treatment.

RESULTS AND DISCUSSION

Table 1 summarizes the amino acid analyses of reaction A using heart, liver and kidney homogenates as enzyme sources. The table contains only aspartic acid, glutamic acid, cysteine, HCETC, alanine, threonine, serine, and glycine, because the changes in amino acid concentrations were observed in the regions of these amino acids on the chromatogram. The table shows that, when 3-mercaptopyruvate was incubated with heart homogenate, 0.49 μmole of HCETC and 0.58 μmole of L-cysteine were formed with a concomitant decrease in L-aspartic acid

TABLE 1. CHANGES IN AMINO ACID CONCENTRATIONS AFTER THE INCUBATION OF 3-MERCAPTOPYRUVATE WITH RAT TISSUE HOMOGENATES*

Enzyme Source	Mercapto-pyruvate	Asp	Glu	Cys**	HCETC	Ala	Thr	Ser	Gly
Heart	(a)+	0.91	3.93	0.58	0.49	2.33	2.33	0.60	0.63
	(b)−	1.58	6.93	0.00	0.00	2.03	2.52	0.75	0.76
	(a)−(b)	−0.67	−3.00	0.58	0.49	0.30	−0.19	−0.15	−0.13
Liver	(a)+	0.24	6.55	trace	0.00	13.00	***	***	3.17
	(b)−	2.60	7.89	0.00	0.00	6.52	***	***	3.34
	(a)−(b)	−2.36	−1.34	trace	0.00	6.48	***	***	−0.17
Kidney	(a)+	2.04	10.58	0.96	0.00	7.16	2.39	5.29	6.96
	(b)−	4.49	13.10	0.48	0.00	6.92	2.29	5.99	7.78
	(a)−(b)	−2.45	−2.52	0.48	0.00	0.24	−0.53	−0.70	−0.82

* Eighteen μmoles of 3-mercaptopyruvate was incubated with 4 ml each of homogenates of rat heart, liver or kidney at 37° C for 30 min (Reaction A). After deproteinization with 3% sulfosalicylic acid, amino acids were determined by an amino acid analyzer. Experimental details are given under MATERIALS AND METHODS. 3-Mercaptopyruvate was not contained in reaction mixture (b). Results are expressed as μmoles per flask.

** Cysteine

*** Could not be calculated exactly because of overlapping of peaks.

and L-glutamic acid. The increase in L-alanine was 0.30 μmole. When 3-mercaptopyruvate was incubated with liver homogenate, HCETC was not produced and the formation of L-cysteine was negligible. But the increase in L-alanine was very high compared to the reactions with heart and kidney homogenates. When the reaction was performed with kidney homogenate, HCETC was not produced and 0.48 μmole of L-cysteine was formed. The decreases in L-aspartic acid and L-glutamic acid were 2.45 and 2.52 μmoles, respectively. The results of the reactions with kidney were similar to those of the reaction with heart homogenate except for the formation of HCETC.

The results of reaction B, in which 3-mercaptopyruvate was incubated with rat heart homogenate, are shown in Fig. 1 and Table 2. Fig. 1 illustrates typical chromatograms of the reactions with (b) and without (a) 3-mercaptopyruvate. The absence of HCETC and the presence of only a trace amount of L-cysteine in rat heart homogenate are shown in Fig. 1a. Fig. 1b shows the production of HCETC, L-cysteine (detected as L-cystine on the chromatogram) and L-alanine, and decreases in L-aspartic acid and L-glutamic acid after the incubation of 3-mercaptopyruvate with rat heart homogenate. Fig. 1b also shows the presence of a peak (X) at about 103 ml of the effluent. This peak was tentatively identified

TABLE 2. CHANGES IN AMINO ACID CONCENTRATIONS AFTER THE INCUBATION OF 3-MERCAPTOPYRUVATE WITH RAT HEART HOMOGENATE*

Exp.	Mercapto-pyruvate	Asp	Glu	Cys**	HCETC	Ala	Ser	Thr	Gly
1	(a)+	4.52	11.63	2.26	2.10	6.03	1.76	4.77	1.35
	(b)−	7.10	18.35	trace	0.00	4.27	1.90	4.84	1.61
	(a)−(b)	−2.58	−6.72	2.26	2.10	1.76	−0.14	−0.07	−0.26
2	(a)+	5.93	6.32	2.40	2.27	6.74	2.78	1.65	1.55
	(b)−	8.00	12.21	0.00	0.00	4.01	3.22	1.69	1.66
	(a)−(b)	−2.07	−5.89	2.40	2.27	2.73	−0.44	−0.04	−0.11
3	(a)+	5.86	4.75	3.16	3.13	5.51	2.26	3.34	1.47
	(b)−	8.74	13.70	trace	0.00	4.02	2.74	3.54	1.70
	(a)−(b)	−2.88	−8.95	3.16	3.13	1.49	−0.48	−0.20	−0.23
	Mean±SD of (a)−(b)	−2.51 ±0.33	−7.19 ±1.29	2.60 ±0.40	2.50 ±0.45	1.99 ±0.53	−0.35 ±0.15	−0.10 ±0.07	−0.20 ±0.06

* Sixty μmoles of 3-mercaptopyruvate was incubated with rat heart homogenate at 37° C for 30 min (Reaction B). Experimental details are given under MATERIALS AND METHODS. After deproteinization, amino acids were determined using an amino acid analyzer. Reaction mixture (b) contained no 3-mercaptopyruvate. Results are expressed as μmoles per flask.

** Cysteine

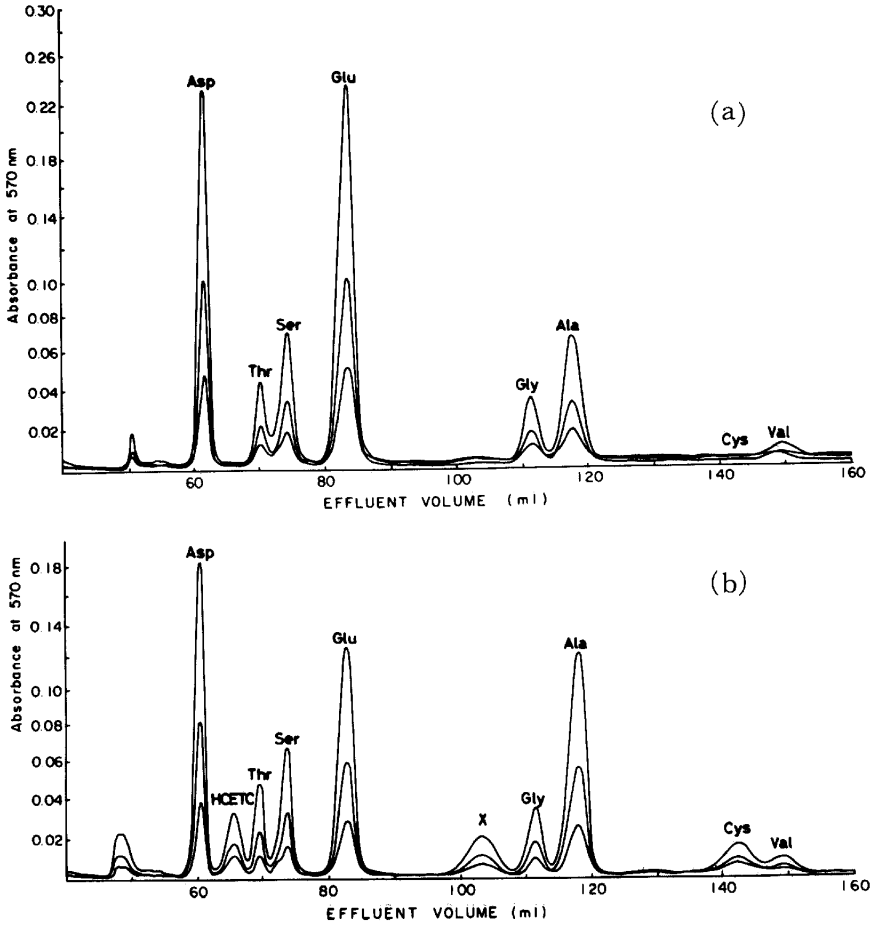

Fig. 1. Chromatograms of amino acid analyses after incubation of rat heart homogenate (Reaction B) with (b) or without (a) 3-mercaptopyruvate. Experimental details are described under MATERIALS AND METHODS.

as L-cysteine-glutathione disulfide (13).

Three experiments of reaction B are summarized in Table 2. On the average 2.50 μmoles of HCETC, 2.60 μmoles of free L-cysteine (detected as 1.30 μmoles of L-cystine) and 1.99 μmoles of L-alanine were produced during the incubation. On the other hand, the levels of L-aspartic acid and L-glutamic acid decreased by 2.51 μmoles and 7.19 μmoles, respectively, during the incubation. The mean increase in X calculated as L-cysteine-glutathione disulfide was 0.68 μmole. HCETC seems to be produced from L-cysteine and 3-mercaptolactate which were both formed from 3-mercaptopyruvate in the presence of rat heart homogenate (13). Therefore the sum of L-cysteine formed from 3-mercaptopyruvate by transamina-

tion amounts to at least 5.78 μmoles. The increase in L-alanine may be explained as follows: 3-Mercaptopyruvate was metabolized by 3-mercaptopyruvate sulfurtransferase to produce pyruvate, which was then converted to L-alanine by transamination. The sum (7.77 μmoles) of L-cysteine and L-alanine produced corresponds roughly to the sum (9.70 μmoles) of the decreases in L-glutamic acid and L-aspartic acid.

Free 3-mercaptolactate present at the end of the incubation was found to be 3.64 μmoles in experiment 1 of reaction B. As reported (6, 8, 9, 14), 3-mercaptopyruvate is a good substrate for lactate dehydrogenase, which is active in heart tissue (15). Thus it appears that 3-mercaptopyruvate was converted by lactate dehydrogenase to 3-mercaptolactate, a part of which was utilized for the formation of HCETC. Therefore 3-mercaptolactate formed in experiment 1 should be equal at least to the sum of HCETC and free 3-mercaptolactate, *i.e.*, 5.74 μmoles.

Fig. 2 illustrates the reaction scheme of 3-mercaptopyruvate metabolism. It may be concluded from the above results that, in heart tissue, 3-mercaptopyruvate was converted to L-cysteine, 3-mercaptolactate and pyruvate; from these products HCETC, L-alanine and probably L-cysteine-glutathione disulfide were produced. In liver, in which 3-mercaptopyruvate sulfurtransferase is very active (4, 5), the main route of 3-mercaptopyruvate metabolism was pyruvate formation, and hence HCETC was not formed under the present conditions. A part of 3-mercaptopyruvate was converted to L-cysteine, which was used for the formation of L-cysteine-glutathione disulfide. Metabolism of 3-mercaptopyruvate in kidney appears to be intermediate between that in heart and liver. HCETC was not produced in kidney.

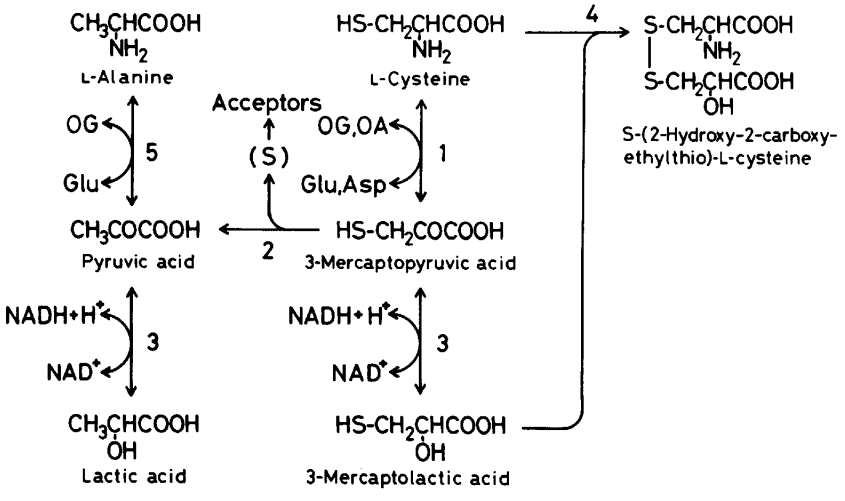

Fig. 2. Reaction scheme of the metabolism of 3-mercaptopyruvate in rat tissues. 1: Cysteine aminotransferase. 2: 3-Mercaptopyruvate sulfurtransferase. 3: Lactate dehydrogenase. 4: Disulfide formation. 5: Alanine aminotransferase.

Thus these results indicate that 3-mercaptopyruvate can be actively metabolized in these tissues in different ways.

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