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Miyabi Taniguchi* Yasuhiro Hosaki†

Toshihiko Ubuka‡

*Okayama University,

†Okayama University,

‡Okayama University,

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Miyabi Taniguchi, Yasuhiro Hosaki, and Toshihiko Ubuka

Abstract

Transaminative metabolism of L-cysteine was investigated using homogenates of guinea pig liver and kidney. L-Cysteine was transaminated in the presence of 2-oxoglutarate and the homogenate of either liver or kidney. S-(2-Hydroxy-2-carboxyethylthio)cysteine (HCETC) (3-mercaptolactate-cysteine disulfide) was formed by liver homogenate, but the amount was very small. On the other hand, a relatively large amount of HCETC was formed in the presence of kidney homogenate. Transamination between 3-mercaptopyruvate and certain amino acids was catalyzed actively by both liver and kidney homogenates in the presence of L-glutamate. However, more half-cysteine was formed by liver than kidney, and more HCETC was produced by kidney than liver. L-Glutamate was the most potent amino donor, and L-aspartate strongly inhibited the reaction. Results indicate that L-cysteine can be transaminated both in liver and kidney of the guinea pig, and that kidney is more active than liver. 2-Oxoglutarate is the most active 2-oxo acid for cysteine transamination. Oxaloacetate (and aspartate in the reverse reaction) is inhibitory to the reaction. These results are in agreement with the previous conclusion that cysteine aminotransferase is identical with aspartate aminotransferase.

KEYWORDS: cysteine, transamination, guinea pig, mercaptopyruvate, mercaptolactate-cysteine disulfide

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TRANSAMINATIVE METABOLISM OF L-CYSTEINE IN GUINEA PIG LIVER AND KIDNEY

Miyabi TANIGUCHI, Yasuhiro HOSAKI and Toshihiko UBUKA

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

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Abstract. Transaminative metabolism of L-cysteine was investigated using homogenates of guinea pig liver and kidney. L-Cysteine was transaminated in the presence of 2-oxoglutarate and the homogenate of either liver or kidney. S-(2-Hydroxy-2-carboxyethylthio)cysteine (HCETC) (3-mercaptolactate-cysteine disulfide) was formed by liver homogenate, but the amount was very small. On the other hand, a relatively large amount of HCETC was formed in the presence of kidney homogenate. Transamination between 3-mercaptopyruvate and certain amino acids was catalyzed actively by both liver and kidney homogenates in the presence of L-glutamate. However, more half-cystine was formed by liver than kidney, and more HCETC was produced by kidney than liver. L-Glutamate was the most potent amino donor, and L-aspartate strongly inhibited the reaction. Results indicate that L-cysteine can be transaminated both in liver and kidney of the guinea pig, and that kidney is more active than liver. 2-Oxoglutarate is the most active 2-oxo acid for cysteine transamination. Oxaloacetate (and aspartate in the reverse reaction) is inhibitory to the reaction. These results are in agreement with the previous conclusion that cysteine aminotransferase is identical with aspartate aminotransferase.

Key words : cysteine, transamination, guinea pig, mercaptopyruvate, mercaptolactate-cysteine disulfide.

In 1968 it was reported that S-(2-hydroxy-2-carboxyethylthio)cysteine (HCETC) (3-mercaptolactate-cysteine disulfide) was excreted in the urine of a mentally retarded patient (1) and of normal human subjects (2).

Studies on the transaminative pathway of cysteine metabolism have been performed in our laboratory in order to elucidate the biosynthesis of HCETC (3-10). The pathway was first suggested by Meister *et al.* in 1954 as an alternative pathway of cysteine degradation (11). It has been reported that cysteine is partly metabolized through this pathway in rat tissues (3, 4, 6), and that HCETC is formed through side reactions of the pathway (9). Although the pathway appears to be minor compared to the oxidative pathway (12, 13), its importance has become recognized (14).

The present paper reports that L-cysteine can be metabolized actively in guinea pig liver and kidney through the transaminative pathway and that a considerable amount of HCETC can be formed in guinea pig kidney.

MATERIALS AND METHODS

Materials. Male guinea pigs of the Hartley strain weighing 490-500 g were used. Before experiments, the animals were fed ad libitum on the RC-4 diet of Oriental Yeast Company, Tokyo, Japan, for one month.

L-Cysteine was obtained from Sigma Chemical Company, U.S.A. Ammonium 3-mercaptopyruvate was prepared according to Kun (15). L-Glutamic acid, L-aspartic acid, L-alanine, 2-oxoglutaric acid, oxaloacetic acid and sodium pyruvate were obtained from Wako Pure Chemical Ind., Ltd., Osaka, Japan. L-Glutamic acid and L-aspartic acid were dissolved in water and titrated with sodium hydroxide solution to pH 8.0 before use in enzyme reactions.

Methods. (a) Preparation of tissue homogenate. Guinea pigs were killed by decapitation. The liver and kidney were removed, washed with 0.9 % sodium chloride containing 0.1 mM EDTA (pH 7.40) (solution A) chilled in an ice-water bath, and then homogenized with two volumes of solution A using a Potter-Elvehjem homogenizer equipped with a Teflon pestle. Homogenates thus obtained were used as enzyme sources.

(b) Reactions with L-cysteine and 2-oxo acids. The reaction mixture contained, in a final volume of 3.0 ml placed in a 20-ml Erlenmeyer flask, 500 μmol potassium phosphate (pH 8.00), 200 μmol L-cysteine, 50 μmol sodium 2-oxo acid and 1.0 ml of the homogenate. The flask was shaken at about 100 strokes per min at 37 ℃ for 60 min. The reaction was stopped by the addition of 3.0 ml of 6 % sulfosalicylic acid. The mixture was centrifuged at 1200 $\times$ *g* for 20 min., and the resulting supernatant was filtered through Whatman No. 1 filter paper, Amino acids in the filtrate were determined as described below.

(c) Reactions with 3-mercaptopyruvate and L-glutamate, L-aspartate or L-alanine. The reaction mixture contained, in a final volume of 2.0 ml placed in a 10 ml-Erlenmeyer flask, 200 μmol potassium phosphate (pH 8.00), 20 μmol ammonium 3-mercaptopyruvate, 20 μmol sodium L-glutamate, sodium L-aspartate or L-alanine and 1.0 ml of the homogenate. The mixture was shaken at about 100 strokes per min at 37 ℃ for 30 min. The reaction was terminated by the addition of 2.0 ml of 6 % sulfosalicylic acid, and centrifuged and filtered as above.

(d) Determination of amino acids. Acidic and neutral amino acids in 0.5 ml of the clear filtrate obtained above were determined with a Hitachi KLA-5 amino acid analyzer using 0.2 N sodium citrate (pH 3.19) at 55 ℃. The analyses were performed without buffer change until valine was eluted.

RESULTS AND DISCUSSION

Table 1A summarizes the results of the reactions in which L-cysteine and a 2-oxo acid were incubated with liver homogenate. Transamination of L-cysteine occured when it was incubated with 2-oxoglutarate, but not with oxaloacetate, as judged by the increase in glutamate and aspartate. Formation of alanine from pyruvate was strongly inhibited by the addition of L-cysteine. There was no big difference in glutathione content among these reactions with different 2-oxo acids. However, a small amount of HCETC was detected when liver homogenate was incubated with cysteine together with any of the 2-oxo acids. In all four flasks containing L-cysteine, a peak which corresponded with cysteine-glutathione disulfide (9, 10) was detected on the chromatograms of the amino acid analyses. The amount of the peak calculated as cysteine-glutathione disulfide was about 0.6 μmol in all these flasks as shown in Table 1A.

TABLE 1. AMINO ACID CONCENTRATIONS AFTER INCUBATION OF L-CYSTEINE AND 2-OXO ACIDS WITH HOMOGENATE OF GUINEA PIG LIVER OR KIDNEY*

Tissue	Substrates	Amino acid concentrations (μmol/flask, 60 min)						
		Glu	Asp	Ala	Half-cystine	GSH	Cys-SG	HCETC
A. Liver	Cys + OG	5.724	tr	0.516	a	0.168	0.624	tr
	Cys + OA	1.200	3.528	0.660	a	0.372	0.648	tr
	Cys + Pyr	2.124	0.336	0.924	a	0.216	0.624	tr
	Cys	2.124	0.324	0.636	a	0.168	0.756	nd
	OG	4.896	tr	0.084	0.264	0.264	tr	nd
	OA	0.660	3.828	1.788	0.192	0.348	tr	nd
	Pyr	0.276	tr	5.088	tr	0.396	tr	nd
	none	2.592	0.108	0.600	0.072	0.312	tr	nd
B. Kidney	Cys + OG	6.276	0.096	0.432	a	tr	tr	0.924
	Cys + OA	1.188	3.312	0.408	a	tr	tr	tr
	Cys + Pyr	2.784	0.504	0.552	a	tr	tr	0.312
	Cys	2.604	0.792	0.432	a	tr	tr	0.204
	OG	4.932	0.036	0.072	0.168	tr	tr	nd
	OA	0.852	4.332	0.780	0.072	tr	nd	nd
	Pyr	1.776	0.060	2.676	tr	tr	nd	nd
	none	1.644	0.768	0.648	0.336	tr	nd	nd

* L-Cysteine (200 μmol) and/or one of 2-oxo acids (50 μmol) were incubated with liver or kidney homogenate at 37° C for 60 min. [a]Exceeded the limit of exact determination.

Abbreviations: Cys-SG, cysteine-glutathione disulfide; Cys, cysteine; OG, 2-oxoglutarate; OA, oxaloacetate; Pyr, pyruvate; tr, trace; nd, not detected.

Table 1B is the summary of results obtained by similar reactions using kidney homogenate. Formation of glutamate increased in the presence of L-cysteine, but that of aspartate was inhibited by the addition of L-cysteine. A strong inhibition of alanine formation by the addition of L-cysteine was also observed as with liver homogenate. However, in these reactions, in contrast to the results with liver homogenate, almost all glutathione disappeared, and the peak corresponding to cysteine-glutathione disulfide was negligibly small. Another sharp contrast between reactions with these two tissues was the formation of HCETC. When L-cysteine was incubated with kidney homogenate, 0.204 μmol HCETC was produced per reaction flask in 60 min. The addition of 2-oxoglutarate or pyruvate to this system accelerated HCETC formation. Almost five times as much HCETC was formed by the addition of 2-oxoglutarate, and about a 50 % increase was observed by the addition of pyruvate. However, the addition of oxaloacetate was inhibitory to HCETC formation from L-cysteine.

The metabolism of 3-mercaptopyruvate incubated with homogenates of guinea pig liver or kidney is summarized in Table 2. As reported by Cooper *et al.* (16), 3-mercaptopyruvate is unstable in solution. Therefore, the reaction time

TABLE 2. FORMATION OF HCETC FROM 3-MERCAPTOPYRUVATE BY GUINEA PIG LIVER OR KIDNEY HOMOGENATE*.

Tissue	Substrates	Amino acid concentrations (μmol/flask, 30 min)						
		Glu	Asp	Ala	Half-cystine	GSH	Cys-SG	HCETC
A. Liver	MP + Glu	11.064	0.352	3.544	1.784	0.312	0.056	0.576
	MP + Asp	3.048	10.192	2.792	0.776	0.344	0.032	b
	MP + Ala	2.488	0.120	12.792	1.160	0.496	0.032	0.368
	MP	2.408	0.184	2.488	0.912	0.352	0.040	0.312
	Glu	10.144	0.320	1.880	0.136[a]	0.376	0.016	tr
	Asp	3.552	9.424	1.600	0.192[a]	0.448	0.032	nd
	Ala	3.256	0.024	11.368	0.328[a]	0.576	0.016	tr
	none	2.672	0.032	1.104	0.208[a]	0.440	0.112	tr
B. Kidney	MP + Glu	10.856	0.640	1.168	0.416	tr	nd	1.184
	MP + Asp	2.736	11.264	0.712	0.106	tr	nd	b
	MP + Ala	1.496	0.304	12.056	0.224	tr	tr	0.544
	MP	1.240	0.256	0.504	0.104	tr	tr	0.528
	Glu	10.768	0.688	0.552	tr[a]	tr	tr	tr
	Asp	2.544	11.336	0.784	0.072[a]	tr	tr	tr
	Ala	1.960	0.584	12.016	0.096[a]	tr	tr	tr
	none	1.480	0.480	0.424	0.064[a]	tr	tr	tr

* 3-Mercaptopyruvate (20 μmol) and/or one (20 μmol) of the amino acids indicated were incubated with liver or kidney homogenate at 37° C for 30 min. Flasks lacking 3-mercaptopyruvate were taken as controls.

[a] A small amount of cysteine was also present. [b] A small amount of HCETC was present, but it could not be calculated because of the overlapping of peaks.

Abbreviations : MP, 3-mercaptopyruvate ; tr, trace ; nd, not detected.

was shortened to 30 min. When 3-mercaptopyruvate was incubated with liver homogenate, 0.912 μmol half-cystine was detected by amino acid analysis as shown in Table 2A. The addition of L-glutamate doubled the amount of half-cystine, and that of alanine slightly increased it. On the other hand, the addition of L-aspartate inhibited the formation of half-cystine. HCETC formation from 3-mercaptopyruvate by liver homogenate was 0.312 μmol. The addition of L-glutamate or L-alanine increased HCETC. However, the formation of HCETC was inhibited by the addition of L-aspartate as was that of half-cystine.

Results obtained when 3-mercaptopyruvate was incubated with kidney homogenate are summarized in Table 2B. The formation of half-cystine from 3-mercaptopyruvate increased upon addition of L-glutamate or L-alanine by a factor of 4 and 2, respectively. L-Aspartate did not accelerate half-cystine formation. The incubation of 3-mercaptopyruvate with kidney homogenate resulted in the formation of a relatively large amount of HCETC; 0.528 μmol HCETC per flask in 30 min. The addition of L-glutamate doubled HCETC

formation. L-Alanine slightly increased it. However, as in the case of liver homogenate, HCETC formation was inhibited by the addition of L-aspartate.

Results obtained in the present studies appear to indicate that L-cysteine can be metabolized through the transaminative pathway both in the liver and kidney of guinea pigs as in rats (4, 6, 7). The results also indicate that kidney is more active than liver for the transamination of L-cysteine as judged by the amount of products per *g* wet weight per hour. This was exemplified by the formation of HCETC from L-cysteine or 3-mercaptopyruvate and that of half-cystine from 3-mercaptopyruvate.

For the transamination of L-cysteine, 2-oxoglutarate is the most active amino acceptor of the 2-oxo acids tested, followed by pyruvate. Oxaloacetate inhibited the reaction. In the reverse reaction, L-aspartate inhibited the formation of half-cystine and HCETC from 3-mercaptopyruvate. These results are in agreement with the previous conclusion that cysteine aminotransferase (EC 2, 6, 1, 3) from rat liver mitochondria and cytosol are identical with mitochondrial and soluble aspartate aminotransferase (EC 2, 6, 1, 1), respectively (5, 8).

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