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Abstract

Free amino acid contents in various guinea pig tissues were determined with an amino acid analyzer. The most abundant amino acids in these tissues were: Gly and Glu in the liver and kidney, Gln, Glu and Ala in the heart, Glu and Gln in the brain, Gly in the blood plasma and Lys in erythrocytes. Glutathione was present as the reduced form in these tissues. Cystine was not detected except in the blood plasma, but cysteine was present in these tissues. These results indicate that most thiols are present in the reduced form in these guinea pig tissues. Taurine contents were low compared with those in rat tissues. The results were discussed in relation to the metabolism of sulfur-containing amino acids, and it was suggested that the oxidative metabolism of L-cysteine was lower in guinea pig tissues than in rat tissues.

KEYWORDS: free amino acids, guinea pig, cysteine

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FREE AMINO ACID CONTENTS IN GUINEA PIG TISSUES

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Abstract. Free amino acid contents in various guinea pig tissues were determined with an amino acid analyzer. The most abundant amino acids in these tissues were: Gly and Glu in the liver and kidney, Gln, Glu and Ala in the heart, Glu and Gln in the brain, Gly in the blood plasma and Lys in erythrocytes. Glutathione was present as the reduced form in these tissues. Cystine was not detected except in the blood plasma, but cysteine was present in these tissues. These results indicate that most thiols are present in the reduced form in these guinea pig tissues. Taurine contents were low compared with those in rat tissues. The results were discussed in relation to the metabolism of sulfur-containing amino acids, and it was suggested that the oxidative metabolism of L-cysteine was lower in guinea pig tissues than in rat tissues.

Key words: free amino acids, guinea pig, cysteine.

In the course of our study on the metabolism of L-cysteine (1-5), we have found that L-cysteine can be metabolized through the transaminative pathway in guinea pig tissues (2) as in rat tissues (3-5). 3-Mercaptolactate-cysteine disulfide (S-(2-hydroxy-2-carboxyethylthio)cysteine, HCETC) is formed more actively in guinea pig tissues than in rat tissues (2, 6). 3-Mercaptopyruvate sulfurtransferase (EC 2. 8. 1. 2), an enzyme in the transaminative metabolism of L-cysteine, catalyzes the transfer of the sulfur of 3-mercaptopyruvate to sulfur acceptors such as sulfite and sulfinates (7). It has been found that the activity of this enzyme is very low in various guinea pig tissues compared with rat tissues (8). These results seem to indicate a difference in the metabolism of L-cysteine between these two animal species.

The present paper reports the results of amino acid analyses of various guinea pig tissues with emphasis on sulfur-containing amino acids.

MATERIALS AND METHODS

Six male guinea pigs of the Hartley strain weighing 500-600 g were used. Animals were maintained on laboratory diet RC4 (Oriental Yeast Company Ltd., Tokyo, Japan) and water *ad libitum*.

Preparation of tissue extracts was performed at 0-4°C. Animals were killed by decapitation, and blood was collected in a beaker coated with 200 units of heparin. Blood plasma was obtained by centrifuging the heparinized blood at 1200 × g for 10 min. Erythrocytes were washed three times with 0.9 % sodium chloride containing 1 mM EDTA, pH 7.4 (solution A).

The liver, kidney, heart and whole brain were removed as soon as possible and chilled in ice-cold solution A. Tissues were homogenized with one volume of solution A and two volumes of 6.0 % sulfosalicylic acid solution using a Potter-Elvehjem glass homogenizer. Homogenates were centrifuged at $1200 \times g$ for 20 min and the resulting supernatants were filtered through Whatman No 1 filter paper.

Amino acid analyses were performed with a Hitachi KLA-5 amino acid analyzer (Hitachi Ltd., Tokyo, Japan). Acidic and neutral amino acids were analyzed using custom ion-exchange resin No 2613 and 0.2 N sodium citrate buffers, pH 3.19 and 4.25. Analyses of basic amino acids were performed with No 2615 ion-exchange resin and 0.35 N sodium citrate buffer containing 0.57 M sodium chloride, pH 5.28. Five-tenths ml of one of the tissue extracts obtained above was applied, and elution was performed at a speed of 60 ml per 60 min at 55 °C. Results were expressed as the mean $\pm$ SD (standard deviation) of the amino acid contents (μ moles per g) of fresh tissue obtained from four to six animals.

RESULTS AND DISCUSSION

Table 1 summarizes the results of analyses of free amino acid contents in various tissues obtained from four to six normal guinea pigs. Reduced glutathione (GSH) was eluted at 50 ml of effluent with a sharp peak under the present conditions, and oxidized glutathione (GSSG) at 68 ml with a broad peak. Thus, reduced glutathione could be determined by the present method along with amino acids.

The most abundant amino acid in the liver was Gly followed by Glu. The average content of GSH was $7.94 \pm 0.508 \mu$ moles per g of fresh liver. GSSG was not detected by the present method.

In the kidney Gly and Glu were the most abundant amino acids, as in the liver. The GSH content was $2.871 \pm 0.420 \mu$ moles per g of kidney. GSSG was not detected as in the liver.

In the heart and brain, however, the Gly content was much lower than in the liver and kidney. Gln, Glu and Ala were the main amino acids in the heart, and Glu and Gln were the most abundant in the brain.

In the blood plasma, the most abundant amino acid was Gly, and in erythrocytes, Lys.

In all guinea pig tissues examined in the present study, glutathione was present in the reduced form. GSSG was not detected by the present method except in erythrocytes, in which a large part of the GSH was oxidized during the homogenization of the erythrocytes.

Cystine was detected only in very small amounts, if any, in all guinea pig tissues examined except for the blood plasma, in which about 0.1μ mol per ml of half cystine was present. However, a small peak of cysteine after that of Glu appeared on the chromatograms of the tissues examined. The contents of GSH, GSSG, cysteine and cystine indicate that most thiols in guinea pig tissues are in the reduced state.

Another unique feature of free amino acid profiles of guinea pig tissues is that the taurine content is quite low except for heart and muscle (9-11). In the

TABLE 1. AMINO ACID CONTENTS IN GUINEA PIG TISSUES*

Amino acid	Liver (6)	Kidney (6)	Heart (4)	Brain (4)	Blood plasma (4)	Erythro- cytes (4)
Asp	0.899 ±0.104	0.800 ±0.130	1.135 ±0.457	1.894 ±0.195	0.016 ±0.001	0.178 ±0.012
Thr + Gln	2.220 ±0.359	1.779 ±0.273	4.892 ±0.413	2.664 ±0.166	0.558 ±0.174	a
Ser + Asn	2.855 ±0.761	0.601 ±0.087	0.390 ±0.077	0.526 ±0.063	0.300 ±0.090	0.264 ±0.075
Glu	6.072 ±1.057	4.744 ±0.736	3.732 ±0.781	5.946 ±0.444	0.092 ±0.038	0.148 ±0.010
Gly	7.857 ±1.238	6.492 ±1.130	0.642 ±0.086	0.710 ±0.083	0.632 ±0.149	0.378 ±0.042
Ala	1.721 ±0.331	0.872 ±0.229	3.182 ±0.704	0.496 ±0.028	0.234 ±0.086	0.178 ±0.033
Half Cystine	b	c	c	c	0.098 ±0.054	c
Val	0.367 ±0.058	0.269 ±0.076	0.164 ±0.031	0.072 ±0.009	0.364 ±0.079	0.274 ±0.023
Met	0.099 ±0.028	0.074 ±0.030	b	0.025 ±0.008	d	b
Ile	0.167 ±0.021	0.124 ±0.038	0.070 ±0.008	0.023 ±0.007	0.154 ±0.030	0.106 ±0.014
Leu	0.287 ±0.053	0.197 ±0.072	0.158 ±0.020	0.060 ±0.005	0.191 ±0.048	0.144 ±0.007
Tyr	0.105 ±0.024	0.101 ±0.030	0.128 ±0.037	0.035 ±0.010	0.100 ±0.035	0.076 ±0.019
Phe	0.064 ±0.014	0.079 ±0.028	0.064 ±0.011	b	0.088 ±0.029	0.056 ±0.013
Lys	1.483 ±0.507	0.317 ±0.059	0.418 ±0.064	0.116 ±0.008	0.302 ±0.075	0.510 ±0.053
His	0.063 ±0.124	0.207 ±0.034	0.416 ±0.076	0.054 ±0.014	0.088 ±0.024	0.060 ±0.010
Arg	b	0.198 ±0.039	0.326 ±0.049	0.116 ±0.005	0.150 ±0.035	0.246 ±0.030
GSH	7.974 ±0.508	2.871 ±0.420	1.900 ±0.248	1.450 ±0.123	c	c
HCETC	f	b	c	c	c	c

* Values are the mean ± SD of the amino acid contents (μ moles per g or ml of fresh tissue). The numbers of animals are shown in parentheses.

a : Could not be calculated because of overlapping peaks. b : Trace amounts. c : Not detected. d : Trace amount to 0.032 μ moles per g of fresh tissue. e : Could not be determined because oxidized glutathione was formed during the preparation of samples. f : Trace amount to 0.288 μ moles per g of fresh tissue.

present study, the exact values of taurine contents were not obtained because its peak overlapped with those of urea and phosphoethanolamine. However, estimated taurine concentrations in the guinea pig tissues agree with those reported by others (9, 11). In rat tissues, on the contrary, taurine is one of the most abundant amino acids (9-11). It has been reported that the oxidative metabolism of L-cysteine is very active in the rat (12). Results of the present study and of others (9-11, 13) suggest that the metabolism of L-cysteine in the guinea pig is quite different from that in the rat, and that the oxidative metabolism of L-cysteine in guinea pig tissues is not as active as in rat tissues.

As reported previously, L-cysteine is metabolized actively through the transaminative pathway in guinea pig liver and kidney (2). However, 3-mercaptopyruvate sulfurtransferase activity was found to be very low in various guinea pig tissues compared to rat tissues (8). Therefore, 3-mercaptopyruvate formed by transamination seems to be further metabolized in part by 3-mercaptopyruvate sulfurtransferase and in part by lactate dehydrogenase. The involvement of the latter enzyme in cysteine metabolism was indicated by the formation of HCETC (2, 6). In the present study, HCETC was detected in the liver and kidney, in which the transaminative metabolism of L-cysteine is active.

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