Study of Essential Amino Acid Requirement on the Basis of Sulfur Amino Acid Metabolism in Young Rats

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SUMMARY

Requirement of essential amino acid for growing rats was examined on the basis of hepatic cysteine dioxygenase (CDO) activity, hepatic glutathione, cysteine and taurine contents and urinary taurine excretion.

Young male rats were fed on the semipurified diets limited by sulfur amino acids (methionine+cystine) in the levels of 0.20, 0.30, 0.45, 0.50, 0.55 and 0.60% of requirement recomended by NRC for 18 days. The values hepatic glutathione, cysteine and taurine of 0.50% limitation of sulfur amino acid level were similar to those of controls (0.60%). The values of CDO activity and urinary taurine excretion increased in animals fed on 0.55% limitation of sulfur amino acids diets, and the values showed very low less than 0.45% limitation. It is estimated that level of 0.49% (about 0.50%) limitation of sulfur amino acid meet the minimum requirement. Requirements of lysine and threonine were also studied in animals fed on 0.45% limitation of sulfur amino acids and different level of each test amino acids. Hepatic glutathione, CDO activity and urinary taurine were also used as parameters. It was estimated minimum requirements of lysine and threonine are 0.61~0.66% limitation, and 0.42~0.48% limitation, respectively.

INTRODUCTION

Essential amino acids except sulfur amino acids are finally decomposed to urea and are excreted to urine in mammals. On the other hand, a part of sulfur amino acids is metabolized from cysteine to taurine through cysteine sulfinate and hypotaurine, and taurine is almost excreted to urine[1].

It has been reported that sulfur amino acid metabolism is regulated by the intake of dietary sulfur amino acid level[2,3], and that also altered by limitting amino acid except sulfur amino acids[4]. The purpose of this study is to examine the requirement of essential amino acids on the basis of sulfur amino acid metabolism. Sulfur amino acids, lysine and threonine were firstly examined. Urinary taurine, hepatic cysteine dioxygenase activity and hepatic glutathione, cysteine and taurine were used as possible indicators for the determination of essential amino acids requirement.

EXPERIMENTAL

Animals and diets. Male rats of Sprague-Dawley strain of 4 weeks after birth were used. All rats were fed on 20% casein diet for 5 days before experiments. Composition of experiemntal diets were shown in Table 1. The mixture of amino acids of NRC composition[5] was used as nitrogen source. The ratio of methionine and cystine in the diet was fixed to 7:5. Experiment 1: The contents of sulfur amino acids in the diets were prepared to 0.20, 0.30, 0.45, 0.50, 0.55 and 0.60 g/100g diet respectively. The total calorie was adjusted by the additional sucrose.

Table 1. Composition of experimental diets (g/kg)

Ingredients	Basal diet
AA mixture	108.9[1)]
α-Corn starch	474.1
Sucrose	300
Soybean oil	50
Salt mixture[2)]	35
Cellulose powder	20
Vitamin mixture[2)]	10
Choline HCl	2

1) National Research Council (NRC): Nutrient requirements of domestic animals, No. 10, p23 (1978)
2) AIN-76 mineral and vitamin mixture, J. Nutr., 107: 1340, (1977)

Experiment 2: Results from experiment 1, requirement of lysine and threonine were examined in rats fed on 0.45 g/100g diet of sulfur amino acids and different level of lysine and threonine. Lysine level of experimental diet was adjusted to 0.175, 0.350, 0.450, 0.500, 0.550, 0.650 and 0.700 g/100g diet, and threonine level was adjusted to 0.125, 0.250, 0.300, 0.350, 0.400 and 0.500 g/100g diet, respectively.

Animals were fed on these examined diets for 18 days. The animals were individually caged in stainless steel cages under 23±1°C. Food intake and body weight gain were measured every day. The urine was collected for final three days of experimental period.

Analytical procedure. Animals were killed by decapitation. The liver was removed after perfusion with cold 0.9% NaCl solution through portal vein. Total glutathione was estimated by the DTNB method of Owens and Belcher[6]. Cysteine was reduced to cysteine by the addition of dithiothreitol. Total cysteine was estimated by the acid-ninhydrine method redported by Gaitonde[7]. Taurine was determined by a modification of the methods of Sorbo and Garvin[8,9]. An aliquate of deproteinized urine and liver supernatant were applied to two column that contained 3.0×1.0 cm of Dowex 50-X8, 200–400 mesh, hydrogen form and 2.0×1.0 cm of Dowex 1-X8, 200–400 mesh, chroride form. Ten times water was flowed into the columns. An aliquot of eluate was mixed with fluorescamine acetone solution, and taurine concentration was measured by fluorometric methods. Cysteine dioxygenase activity (CDO) was measured by the method reported by Yamaguchi *et al.* using radiochemical assay[10].

RESULTS AND DISCUSSION

Experiment 1. Food intake and body weight gain were decreased with increasing of limitation of dietary sulfur amino acids (Table 2).

The hepatic total glutathione contents was increased in proportion to level of dietary sulfur amino acid, and the values were reached to plateau over 0.50% (Table 3). These results were considered that hepatic glutathione pool was saturated by the dose of 0.50% of sulfur amino acids in the diet. The values of hepatic cysteine and taurine were also reached to plateau over 0.45% of sulfur amino acids (Table 3). As shown in Fig. 1 CDO activity in liver and urinary taurine excretion of rats fed on less than 0.45% of sulfur amino acids were low, and increased with the added dose of sulfur amino acids from the level of 0.50%. Fig. 2 showed correlation between cysteine dioxygenase activity and urinary taurine contents and dietary sulfur amino acid level. A dotted line shows mean values of rats fed on 0.20, 0.30 and 0.45% of sulfur amino acids in which the CDO activity and urinary taurine excretion were minimum level. The minimum requirement of sulfur amino acids is estimated from an intersection of regression curve to dotted lines by 0.49%.

Experiment 2. When sulfur amino acid metabolism was applied as parameter for study of essential amino acid requirement, it is not proper that the amount of sulfur amino acids in the diet was extreme limitation or was excess. Because these limited and excess amounts of sulfur amino acids did not indicate sensitive response to sulfur amino acid metabolism. It was considered that a slightly lower dose of sulfur amino acids minimum requirement seems to be suitable as indicators for the estimation of essential amino acid requirement, since the changes in CDO activity and urinary taurine should be enlarged. Therefore, 0.45% limitation of sulfur amino acids is proper level from the result of experiment 1, and the minimum requirements of lysine and threonine were estimated for rats fed on 0.45 g/100g diet of sulfur amino acids and varied doses of lysine and threonine.

Food intake and body weight gain were extremely low in rats fed on under 0.35% of lysine (Table 4). As shown in Table 5, significant change in the values of glutathione with the increase of lysine added dose was observed.

Cysteine content was very low in the rats fed on 0.175% of lysine. As shown in Fig. 3, CDO activity and urinary taurine excretion were gradually decreased to the minimum level at 0.550% of lysine. These results suggest that the utilization of sulfur amino acid in the anabolic process is inhibited by the limitation of lysine in rats, resulting in the remarkable increase in CDO activity and urinary taurine mediated by excess sulfur amino acid. Fig. 4 shows that correlation between CDO activity and urinary taurine contents and dietary lysine contents. The intersection of regression curve to the minimum level (dotted line) was 0.603 in CDO activity and 0.557 in urinary taurine.

These value is a possible amount of capture 0.45% of sulfur amino acids. Since the requirement dose of sulfur amino acid is 0.49%, these values were adjusted by the ratio of 0.49/0.45. The minimum requirement of lysine can be calculated from proportional expression as follows.

CDO activity : $0.603 \times 0.49 / 0.45 = 0.657$ (0.66)

Table 2. Food intake, body weight gain and liver weight of rats fed on different level of sulfur amino acids diets

SAA level (%)	Food intake (g/18days)	Body weight gain (g618days)	liver weight (g)
0.20	173.5 ± 9.32[a)]	19.0 ± 4.95[a)]	7.27 ± 0.10[a)]
0.30	231.8 ± 14.67[a)]	59.3 ± 6.24[a)]	8.26 ± 0.26[a)]
0.45	267.4 ± 9.62	82.5 ± 5.73	9.79 ± 0.83
0.50	303.0 ± 11.02	83.5 ± 6.80	10.05 ± 0.73
0.55	303.8 ± 11.10	90.5 ± 4.55	11.00 ± 0.64
0.60	322.0 ± 9.27	97.5 ± 6.49	12.12 ± 0.44

Each value represents mean ± SE (n=6)

a) Significantly different from 0.60% SAA group, $p < 0.01$

Table 3. Hepatic total glutathione, cysteine and taurine contents of rats fed on different level of sulfur amino acids diets

SAA level (%)	Total glutathione (μmol/B.W.)	Cysteine (nmol/B.W.)	Taurine (nmol/B.W.)
0.20	84.8 ± 2.79	0.50 ± 0.06	53.4 ± 2.46
0.30	126.8 ± 9.50[a)]	1.17 ± 0.13[a)]	69.5 ± 3.33[a)]
0.45	225.6 ± 23.30	2.13 ± 0.18	107.1 ± 6.31
0.50	287.9 ± 51.70	1.91 ± 0.20	97.8 ± 19.80
0.55	299.3 ± 38.00	2.55 ± 0.17	126.2 ± 22.60
0.60	279.0 ± 28.50	2.42 ± 0.33	111.0 ± 4.24

Each value represents mean ± SE (n=6)

a) Significantly different from 0.45% SAA group, $p < 0.01$

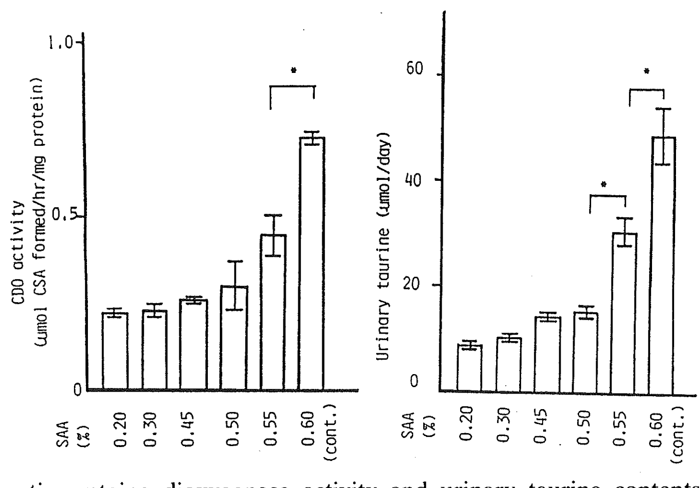

Fig. 1. Hepatic cysteine dioxygenase activity and urinary taurine contents of rats fed on different level of sulfur amino acids diets Mean±SE (n=6) $^*p<0.01$.

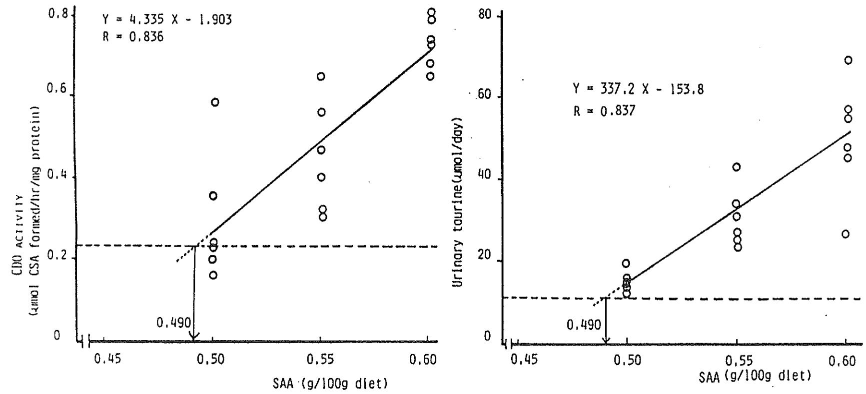

Fig. 2. Correlation between cysteine dioxygenase activity and urinary taurine contents and sulfur amino acids level of rats fed on different level of sulfur amino acids diets.

Table 4. Food intake, body weight gain and liver weight of rats fed on 0.45% limitation of sulfur amino acids and different level of lysine diets

Lys. level (%)	Food intake (g/18days)	Body weight gain (g/18days)	Liver weight (g)
0.175	221.7 ± 8.48[a)]	16.8 ± 1.20[a)]	8.15 ± 0.39[a)]
0.350	307.6 ± 6.64	68.7 ± 2.70[a)]	10.36 ± 0.48
0.450	346.5 ± 8.18	100.8 ± 3.06	11.89 ± 0.39
0.500	341.9 ± 13.00	101.2 ± 4.03	12.35 ± 0.41
0.550	350.5 ± 11.20	109.0 ± 1.21	12.32 ± 0.44
0.650	323.3 ± 8.07	107.2 ± 2.93	11.35 ± 0.27
0.700	312.3 ± 8.78	110.0 ± 3.27	11.83 ± 0.56

Each value represents mean ± SE (n=6)

a) Significantly different from 0.700% lysine group, $p < 0.01$

Table 5. Hepatic total glutathione, cysteine and taurine contents of rats fed on 0.45% limitation of sulfur amino acids and different level of lysine diets

Lys. level (%)	Total glutathione (μmol/B.W.)	Cysteine (nmol/B.W.)	Taurine (nmol/B.W.)
0.175	295.3 ± 19.93	0.87 ± 0.18[a)]	92.0 ± 4.74
0.350	318.6 ± 33.40	1.90 ± 0.87	130.7 ± 21.10
0.450	371.0 ± 17.26	1.62 ± 0.06	133.8 ± 21.45
0.500	312.4 ± 25.03	1.68 ± 0.13	91.2 ± 20.06
0.550	341.2 ± 28.05	1.94 ± 0.22	63.3 ± 2.10
0.650	284.6 ± 17.44	2.45 ± 0.09	68.2 ± 5.28
0.700	305.7 ± 13.13	2.26 ± 0.17	82.3 ± 2.91

Each value represents mean ± SE (n=6)

a) Significantly different from 0.350% lysine group, $p < 0.01$

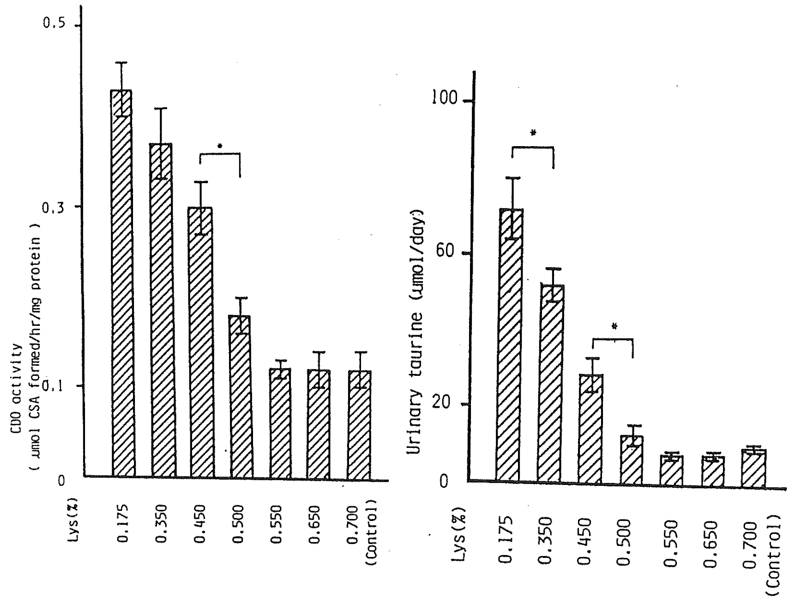

Fig. 3. Hepatic cysteine dioxygenase activity and urinary taurine contents of rats fed on 0.45% limitation of sulfur amino acids and different level of lysine diets. Mean±SE (n=6) *p< 0.01.

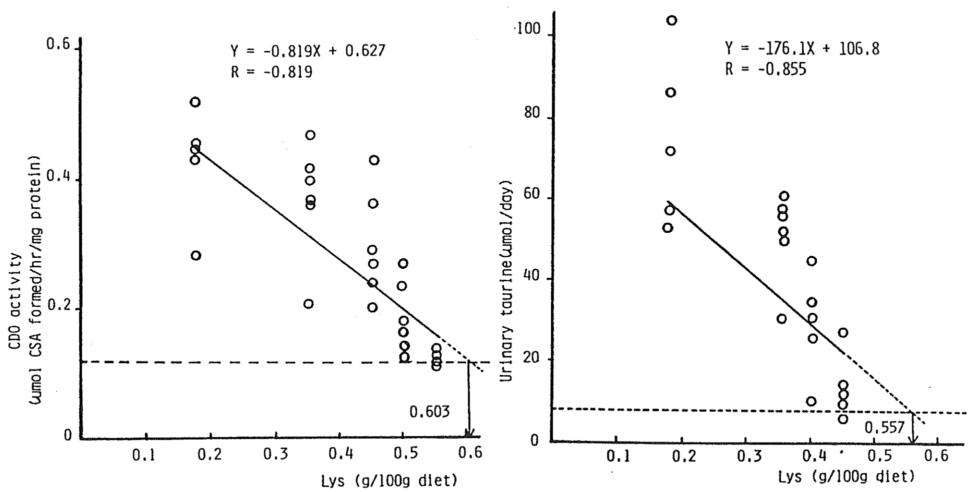

Fig. 4. Correlation between cysteine dioxygenase activity and urinary taurine contents and lysine level of rats fed on 0.45% limitation of sulfur amino acids and different level of lysine diets.

Table 6. Food intake, body weight gain and liver weight of rats fed on 0.45% limitation of sulfur amino acids and different level of threonine diets

Thr. level (%)	Food intake (g/18days)	Body weight gain (g/18days)	Liver weight (g)
0.125	162.9 ± 3.60[a)]	-3.7 ± 0.76[a)]	7.82 ± 0.37[a)]
0.250	321.9 ± 15.60[a)]	73.2 ± 5.44[a)]	11.28 ± 0.30
0.300	350.6 ± 5.05	95.0 ± 2.42[a)]	12.36 ± 0.29
0.350	354.3 ± 11.18	112.5 ± 4.94	13.83 ± 0.53
0.400	361.0 ± 4.91	113.0 ± 2.29	12.54 ± 0.30
0.500	374.7 ± 10.28	122.3 ± 4.69	12.55 ± 0.44

Each value represents mean ± SE (n=6)

a) Significantly different from 0.500% threonine group, $p < 0.01$

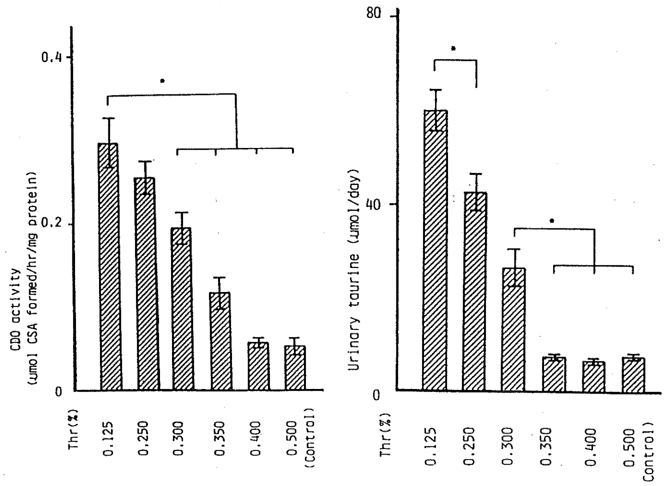

Fig. 5. Hepatic cysteine dioxygenase activity and urinary taurine contents of rats fed on 0.45% limitation of sulfur amino acids and different level of threonine diets. Mean ± SE (n=6), *$p < 0.01$.

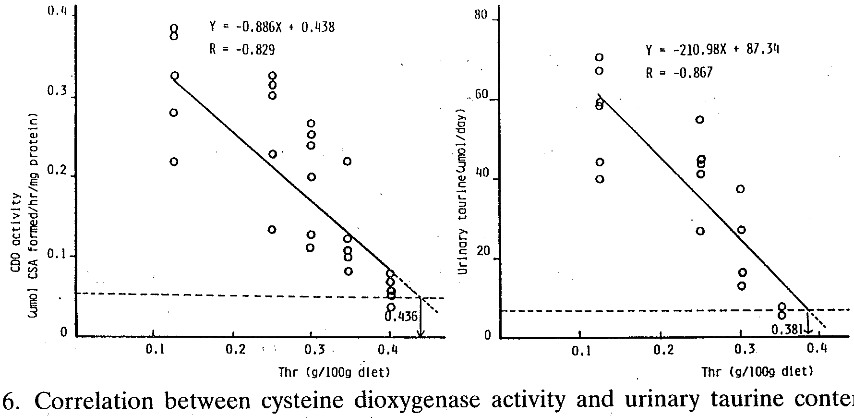

Fig. 6. Correlation between cysteine dioxygenase activity and urinary taurine contents and threonine level of rats fed on 0.45% limitation of sulfur amino acids and different level of threonine diets.

urinary taurine : 0.557×0.49 / 0.45=0.607 (0.61)

The level of 0.61~0.66% was estimated as minimum requirement of lysine.

Table 6 showed the food intake, body weight gain and liver weight of rats fed on 0.45% limitation of sulfur amino acids and different level of threonine diets. Body weight gain was remarkably low of rat fed on under level of 0.300% threonine. The values of hepatic total glutathione and cysteine were decreasing with increase of threonine level (Table 7). CDO activity was remarkably decreased to the level at 0.400% of threonine and was plateu over the level. The value of urinary taurine was plateau over 0.350% of threonine. The values were very low (Fig. 5). Fig. 6 showed that correlation between CDO activity and urinary taurine excretion and dietary threonine contents. The intersection of regression curve to minimum level (dotted line) was 0.436 in CDO activity and 0.381 in urinary taurine.

The value is amount of capture of 0.45% of sulfur amino acids. Since the requirement dose of sulfur amino acid is 0.49%, these values were adjusted by the ratio of 0.49/0.45. The minimum requirement of threonine can be calculated from proportional expression as follows.

Table 7. Hepatic total glutathione, cysteine and taurine contents of rats fed on 0.45% limitation of sulfur amino acids and different level of threonine diets

Thr. level (%)	Total glutathione (μmol/B.W.)	Cysteine (nmol/B.W.)	Taurine (nmol/B.W.)
0.125	488.5 ± 14.28	3.11 ± 0.25	80.7 ± 4.63
0.250	424.6 ± 9.67[a)]	2.78 ± 0.17	81.3 ± 4.82
0.300	372.2 ± 17.78	2.58 ± 0.20	82.0 ± 8.16
0.350	360.8 ± 7.97	2.51 ± 0.05	106.6 ± 12.48
0.400	295.2 ± 24.88	2.24 ± 0.13[a)]	79.7 ± 6.39
0.500	275.6 ± 17.42	1.66 ± 0.07	81.5 ± 11.55

Each value represents mean ± SE (n=6)

a) Significantly different from 0.500% threonine group, $p < 0.01$

CDO activity : 0.436×0.49 / 0.45=0.475 (0.48)

Urinary taurine : 0.381×0.49 / 0.45=0.415 (0.42)

The level of 0.42~0.48% was estimated as minimum requirement of threonine.

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