The Effect of a High-Protein Diet on Cystathionine β-Synthase Activity and Its Transcript Levels in Rat Liver

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(Received May 22, 1996)

Summary  Hepatic cystathionine β-synthase activity was increased in rats fed a high-protein diet for three or five days as compared to rats given laboratory chow (day 0). In the low-protein diet group, the enzyme activity decreased after both feeding periods. Similar changes in the cystathionine β-synthase mRNA levels were observed in the rats fed these diets. The changes in cystathionine β-synthase activity caused by alteration of the dietary protein content were mainly due to changes in the mRNA levels.

Key Words  cystathionine β-synthase, transcript level, high protein diet, rat liver

The pathway for the methionine metabolism in mammalian liver is a cycle with a unidirectional outlet formed by cystathionine β-synthase reaction (1). This reaction is a rate-limiting step of cystein formation from methionine since cystathionine β-synthase activity is decreased by the addition of cysteine to the diet, and has been explained as a methionine-sparing effect of cysteine (1–4).

Previously, we suggested that the decrease in cystathionine β-synthase activity induced by cysteine was due to the change in the level of mRNA for the enzyme. On the other hand, Nakagawa (5) reported that cystathionine β-synthase activity was not changed after starvation for three days, but was altered by dietary protein content. However, the molecular mechanism of this regulation has not yet been clarified. In this study, we compared the cystathionine β-synthase mRNA level with the enzyme activity in the livers of rats fed diets high or low in protein (80% or 10% casein, respectively).

Methods

Animals and diets. We used 6-week-old Sprague-Dawley rats (Nippon SL AC, Shizuoka, Japan). After the rats were fed laboratory chow for three days,

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Table 1. Composition of experimental diets (%).

<table>
<thead>
<tr>
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<th>High-protein</th>
<th>Low-protein</th>
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<tbody>
<tr>
<td>Casein</td>
<td>80.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Dextrin</td>
<td>0.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Mineral mixture(^a)</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Vitamin mixture(^a)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Oil(^b)</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Mineral and vitamin mixtures (Harper’s mixture) were obtained from Oriental Yeast Co., Tokyo, Japan.
\(^b\) Mixture of liver oil and corn oil (1:4).

three rats were sacrificed (day 0 group) and the remaining rats were divided into two groups each consisting of 6–7 rats; high-protein group (80% casein, on the third and fifth day) and low-protein group (10% casein, on the third and fifth day). Table 1 shows the compositions of the experimental diets. The rats had free access to these diets, and were maintained in a room at 22±1°C and 50±10% humidity with 12 h of light daily from 0800–2000. After three or five days on the experimental diet, the rats were sacrificed and their livers immediately removed, weighed, frozen on dry ice and stored at −70°C until assay.

Cystathionine β-synthase activity. About 2 g of liver tissue was homogenized by a method described previously (6). Cystathionine β-synthase activity in the supernatant was determined as described by Kashiwamata and Greenberg (7). The protein concentration was determined by the biuret method (8). One unit of activity was defined as the amount of enzyme which catalyzed the formation of 1 nmol of cystathionine within a 45-min incubation period at 37°C, and cystathionine β-synthase activity was expressed as unit/mg protein.

Isolation of total RNA and Northern blot analysis. Total RNA was isolated from the rat livers by the acid guanidine thiocyanate-phenol-chloroform method as described by Chomczynski and Sacchi (9). The electrophoresis of total RNA (20 μg/lane), and transfer onto nylon filters and hybridization of the filters with labeled probes were carried out as described previously (10). The results were analyzed by densitometry of the bands on autoradiograms, and the level of cystathionine β-synthase mRNA was corrected for that of β-actin mRNA (6). The probes for β-actin and cystathionine β-synthase were described previously (11). The cDNAs were labeled with [α-32p]dATP by the method using the random oligonucleotide primer (12).

Statistical analysis. The significance of differences in mean values between two groups was evaluated by Student’s t-test. Differences with $p < 0.05$ were considered significant.
Results and discussion

The mean body weight of rats in the day 0 group was 199±4 g. The experimental diets containing 10% or 80% casein caused similar increases in body weight. The body weights after five days of feeding were 225±7 g in the 10% casein group and 214±9 g in the 80% casein group.

Figure 1 shows the cystathionine β-synthase activity in the livers of rats fed the two diets in comparison with that on day 0. The enzyme activity was significantly higher in the rats fed a high-protein diet as compared to that of day 0 rats given laboratory chow containing 25% protein (p<0.01 on day 5). On the other hand, the enzyme activity was significantly lower in the low-protein diet group as compared to that of the day 0 group (p<0.05 on day 3, p<0.01 on day 5). There was a significant difference (p<0.01) between the high-protein group and the low-protein group on days 3 and 5. These findings coincided with Nakagawa's findings that a high-protein diet enhanced cystathionine β-synthase activity (5).

The levels of cystathionine β-synthase mRNA in the livers were measured using Northern blot hybridization analysis. As shown in Fig. 2, the mRNA levels in the high-protein group were about 2.6-fold higher than those in the low-protein group on days 3 and 5; these differences being significant (p<0.05). Thus, changes in cystathionine β-synthase activity caused by dietary protein content are mainly due to changes in the mRNA level.

Glucocorticoid hormone and glucagon have been reported to increase cystathionine β-synthase activity in the rat liver (5). Since secretion of these hormones is simulated by protein intake (13-15), the effect of a high-protein diet on the gene expression of this enzyme appears to be mediated by these hormones. However, the possibility that amino acids are directly involved in the gene expression cannot be
Fig. 2. Cystathionine β-synthase mRNA levels in rat liver. Six-week-old rats were given a diet containing 80 (○) or 10% casein (■) for three or five days. The mRNA levels were determined as described in Methods. (A) Representative autoradiograms of Northern blot analysis are shown. CS, cystathionine β-synthase. (B) The cystathionine β-synthase mRNA levels were expressed as percentages of the values for rats given laboratory chow. Values are M±SD of 3–4 rats. a, p < 0.05 vs. casein 80% groups; b, p < 0.05 vs. day 0 rats.

We previously reported that the ratio of cysteine to methionine in the diet affected the levels of enzyme activity and mRNA of cystathionine β-synthase by an unknown mechanism. In this study, the diets contained only casein as a protein source. Both low-protein and high-protein diets contain sulfur amino acids at the same ratio. Thus, we consider that the gene expression of cystathionine β-synthase is regulated by at least two different mechanisms. However, further studies are required to address this problem.

REFERENCES

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