Distribution of Hepatic Cystathionine $\gamma$-Lyase Activity in Fish

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Abstract: Distribution of cystathionine $\gamma$-lyase activity was studied in fish. Liver homogenates were incubated with L-homoserine at 25°C for 60 min, and 2-oxobutyrate formed by the enzymic reaction was determined and defined as the enzyme activity. Fish enzymes showed the maximum activities when the reactions were performed at pH 8.9 with Tris-HCl buffer. The highest enzyme activity was observed in smelt fish followed by that in yellowtail, and the lowest activity was found in carp. Addition of DL-propargylglycine or L-cysteine into the reaction mixtures inhibited 2-oxobutyrate productions, but the inhibition manners of cysteine were different among the examined species.

Key words: Cystathionine $\gamma$-lyase; Cysteine; Liver; Trans-sulfuration

Cysteine has been thought to be produced from methionine via trans-sulfuration pathway in fish because rainbow trout Oncorhynchus mykiss fed on cysteine-subtracted and methionine-supplemented diet did not show any abnormality during its growth (Walton et al. 1982; Cowey et al. 1992). However, our previous study dealing with existence and partial characterization of cystathionine $\gamma$-lyase (cystathionase, EC 4.4.1.1) in the liver of rainbow trout indicated that the enzyme activity in this fish was not only quite lower than that in mouse but also susceptible to inhibition by cysteine (Goto et al. 2005). The data lead us to a question whether fish is able to biosynthesize a considerable amount of cysteine from methionine for their growth. Hence, we studied the distribution and partial characterization of hepatic cystathionase in some kinds of fish.

Preparation of crude enzyme solution and enzyme assay procedure are described in our previous report (Goto et al. 2005). Briefly, liver collected from fish fed on commercial diets was homogenized, centrifuged (1500 × g), and dialyzed against 10 mM phosphate buffer (pH 7.4) to remove endogenous organic acids. A reaction mixture (1.0 ml) consisting of 20 mM L-homoserine, 0.04 mM pyridoxal 5'-phosphate and 100 mM Tris-HCl buffer (pH 8.9) was incubated with liver preparation (1–4 mg protein) for 60 min at 25°C. The enzyme reaction was terminated by adding 0.5 ml of 10% trichloroacetic acid, and the formed 2-oxobutyrate was converted into its hydrazone derivative by the continuous addition of 0.1% 2,4-dinitrophenylhydrazine in 2 N HCl (1.0 ml) and 3 N NaOH (1.0 ml). The hydrazone content was determined by measuring the absorption at 520 nm and defined as the enzyme activity. Blank test was performed by adding trichloroacetic acid into a vessel before the reaction. Protein content was determined by a colorimetric method (Lowry et al. 1951).

As demonstrated in our previous report (Goto et al. 2005), fish enzymes showed maximum activities at pH 8.9 in Tris-HCl buffer, and furthermore, the activities were elevated in proportion to L-homoserine concentration up to 10 mM (data not shown). Hence, we determined the distribution of cystathionase activities in fish. These data are summarized in Table 1. The highest activity was observed in smelt fish, followed by yellowtail, bluegill, rainbow trout, eel, Japanese flounder, and red seabream. In smelt fish, 2-oxobutyrate was produced at a rate of 10.75 ± 2.95 nmol/min/mg protein, which number was comparable to that of ICR mouse. The lowest was found in the liver of carp; the activity was one-fourth of that in smelt fish.

Effects of DL-propargylglycine and L-cysteine on fish cystathionase are shown in Fig. 1. Both compounds inhibited the enzyme activities. However, susceptibilities to cysteine were different among the species. Cysteine strongly inhibited 2-oxobutyrate productions in rainbow trout, eel, and red sea bream, but its inhibition were weaker in bluegill and smelt fish.

Our present data indicate that cystathionase activity apparently exists in the liver of fish but the susceptibilities of this enzyme to cysteine were different among the species. It seems that cysteine is a difficult amino acid to be biosynthesized from methionine when fish enzyme has a high susceptibility to cysteine. Thus, we think that the cysteine susceptibility is one of the possible causes for cystathionine accumulation.

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Table 1. Distribution of hepatic cystathionine γ-lyase activity in the liver of some fish species

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Body weight</th>
<th>n</th>
<th>Activity (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smelt fish</td>
<td>Plecoglossus altivelis</td>
<td>73 – 91 g</td>
<td>3</td>
<td>10.75 ± 2.95</td>
</tr>
<tr>
<td>Yellowtail</td>
<td>Seriola quinqueradiata</td>
<td>0.89 – 1.30 kg</td>
<td>5</td>
<td>7.26 ± 1.08</td>
</tr>
<tr>
<td>Bluegill</td>
<td>Lepomis macrochirus</td>
<td>18.6 – 32.3 g</td>
<td>8</td>
<td>4.94 ± 0.67</td>
</tr>
<tr>
<td>Rainbow trout *1</td>
<td>Oncorhynchus mykiss</td>
<td>136 – 235 g</td>
<td>5</td>
<td>4.78 ± 1.13</td>
</tr>
<tr>
<td>Eel *2</td>
<td>Anguilla japonica</td>
<td>200 – 300 g</td>
<td>5</td>
<td>2.98 ± 0.60</td>
</tr>
<tr>
<td>Japanese flounder</td>
<td>Paralichthys olivaceus</td>
<td>750 – 850 g</td>
<td>5</td>
<td>2.79 ± 0.26</td>
</tr>
<tr>
<td>Red seabream</td>
<td>Pogrus major</td>
<td>232 – 284 g</td>
<td>5</td>
<td>2.71 ± 0.18</td>
</tr>
<tr>
<td>Carp</td>
<td>Cyprinus carpio</td>
<td>26.5 – 39.5 g</td>
<td>5</td>
<td>2.37 ± 0.62</td>
</tr>
<tr>
<td>ICR mouse *1</td>
<td></td>
<td>17.3 – 21.9 g</td>
<td>3</td>
<td>11.47 ± 2.71</td>
</tr>
</tbody>
</table>

Activity was expressed as the amount of 2-oxobutyrate formed (mean ± SD).

*1 Goto et al. (2005), *2 Goto et al. (2004).

Fig. 1. Effects of DL-propargylglycine (■) and l-cysteine (○) on the activities of hepatic cystathionase in fish. Each inhibitor was added into the reaction mixtures and incubated at 25°C for 60 min. *1 Goto et al. (2005).

observed in rainbow trout and Japanese flounder Paralichthys olivaceus (Yokoyama et al. 1992, 1994; Park et al. 2001). In facts, both fish enzymes are susceptible to inhibition by cysteine as shown in Fig. 1. However, there is another possibility that other organs and/or pathways will be concerned with the cysteine biosynthesis in fish. Hence, studies to answer these questions are clearly needed and now in progress.

References


