Short Paper

Determination of hepatic cysteinesulfinate decarboxylase activity in fish by means of OPA-prelabeling and reverse-phase high-performance liquid chromatographic separation

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Taurine is known as one of the final metabolites of sulfur amino acids in mammals.1 In mammals, this sulfur-containing substance is considered to be involved in physiological functions such as membrane protection, detoxification, and antioxidation.2 Although it is generally believed that fish contain large amounts of taurine in their tissues,3 there is little information available on the capacity of taurine biosynthesis in fish. Only Yokoyama et al.4,5 have reported that rainbow trout are able to synthesize a considerable amount of taurine in their liver via the same metabolic pathways as mammals.4,5

However, Park et al.6 have reported that the supplementation of taurine to the diet of juvenile flounder improves their growth performance.7 Takagi et al.8 have also demonstrated that the addition of taurine to the substituted protein diet of red sea bream reduces the incidence of green liver and elevates feed efficiency.7 These data suggest the necessity of extensive studies on the ability of taurine biosynthesis in fish. It seems that marine fish cannot synthesize sufficient amounts of taurine in its pure form.

In the present report, we developed an analytical method for determining the cysteinesulfinate decarboxylase [EC 4.1.1.29] activity in fish by means of the OPA-prelabeling and reverse-phase high performance liquid chromatography (HPLC) because this enzyme is known to play a regulatory role in the biosynthesis of taurine in mammals.8 After the oxidation of hypotaurine to taurine, which is formed by the enzyme reaction, we measured the taurine content and expressed this content as the enzyme activity.

Freshly isolated liver was minced and homogenized in 2.5-fold volumes of 0.25 M sucrose containing 10 mM sodium phosphate buffer (pH 7.4). The supernatant obtained after centrifugation at 1500 × g for 5 min was dialyzed against 10 mM phosphate buffer for 4 h to remove the endogenous taurine. This operation was carried out at a temperature of 4°C or below. The crude enzyme solution was used for the enzyme assay.

Total incubation volume was 1.0 mL and the incubation mixture consisted of 100 mM sodium phosphate buffer (pH 7.2), 1.0 mM cysteinesulfinate, 0.2 mM pyridoxal 5′-phosphate, 4 mM 2-mercaptoethanol, and 0.4–1.0 mg protein of the crude enzyme solution. Protein content was estimated by the colorimetric method described by Lowry et al.9 The reaction was started by adding the enzyme solution and incubation was continued at 35°C for 60 min. The reaction was terminated by heating the mixture at 70°C for 3 min, and β-Alanine (0.2 μmole) was then added as an internal standard. During incubation, hypotaurine formed by the enzymatic reaction is thought to be oxidized to taurine by either enzymatic and/or non-enzymatic reactions. Thus, the formed hypotaurine was converted into taurine by the addition of 200 μL of 31% H2O2 and 29% NH2OH (1:1, v/v) mixture. After standing overnight, an aliquot of the solution was dried under a stream of

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products in bluegill liver preparation is shown in Fig. 1. OPA-taurine was eluted at 10.5 min and \( \beta \)-alanine at 11.5 min. As shown in Fig. 2, the enzyme reaction was increased when both protein concentration (0–1.5 mg) and incubation time (0–60 min) were increased. Ninety percent of cysteinesulfinate added to the incubation mixture was recognized as taurine when 1.0 mg of protein was used for the reaction at 35°C for 60 min. An excess of protein (2.0 mg) did not affect the formation of taurine. No taurine was observed when the enzyme solution was heated earlier at 70°C for 3 min. In this case, both cysteic acid derived from cysteinesulfinate and a small amount of endogenous taurine were detected. When 0.2 mmole of \( \beta \)-alanine was used as an internal standard, a taurine content in the range of 0.014–1.40 mmole was calculated with a linear relationship.

Under the conditions described above, we analyzed the cysteinesulfinate decarboxylase activity...
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in various types of fish. These data are summarized in Table 1. Cysteinesulfinate was converted into taurine by bluegill liver preparation at a rate of 15.84 ± 1.27 nmol/min per mg of protein. In addition to bluegill, an inferior activity was observed in the liver of rainbow trout. However, negligible activity was found in the livers of other types of fish. Thus, there may be differences between species of fish for this enzyme activity as reported in mammals.11

Compared with another report, which studied the hepatic cysteinesulfinate decarboxylase activity in rats by means of OPA-prelabeling HPLC,12 the method described in the present report is more sensitive. This is because hypotaurine and the taurine formed during incubation are detected as taurine, and the use of an internal standard makes the experimental data more accurate. Other pathways for taurine biosynthesis in fish by way of cysteamine and/or sulfate may exist; therefore, we are currently trying to apply this method to other taurine biosynthesis assays in fish.

REFERENCES


Table 1  Cysteinesulfinate decarboxylase activities in fish

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Bodyweight</th>
<th>n</th>
<th>Activity (nmol/min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluegill</td>
<td>Lepomis macrochirus</td>
<td>25–40 g</td>
<td>4</td>
<td>15.82 ± 1.27</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Oncorhynchus mykiss</td>
<td>135–156 g</td>
<td>3</td>
<td>2.33 ± 0.35</td>
</tr>
<tr>
<td>Red sea bream</td>
<td>Pagoecus major</td>
<td>0.2–1.0 kg</td>
<td>3</td>
<td>0.24 ± 0.06</td>
</tr>
<tr>
<td>Japanese flounder</td>
<td>Paralichthys olivaceus</td>
<td>0.3–1.0 kg</td>
<td>3</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td>Carp</td>
<td>Cyprinus carpio</td>
<td>35 g</td>
<td>1</td>
<td>0.30</td>
</tr>
<tr>
<td>Yellowtail</td>
<td>Seriola quinqueradiata</td>
<td>2.5 kg</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>Finespotted flounder</td>
<td>Pleuronichthys cornutus</td>
<td>100 g</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>Hilgendorf saucard</td>
<td>Helicolenus hilgendorfi</td>
<td>200 g</td>
<td>1</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Activity was described as the content of taurine formed during incubation (mean ± SD).