Histidine Metabolism in Fish—V.
The Effect of Protein-deficiency and Fasting on the Activities of Histidine Deaminase and Urocanase in Carp Liver*

Morihiko Sakaguchi and Akira Kawai**
(Received April 6, 1970)

Free histidine, which is well-known to be contained in large amounts in the muscle of dark-fleshed fish and was recently found to occur in relatively high concentration in the carp muscle of young stage1), is supposed to be always kept at a constant level in their muscle under the normal physiological condition.

In the maintenance of the level of this compound, histidine deaminase and urocanase which are responsible for the first and second steps of the degradative process are thought to be regulated in various manners in tissues of fish. The levels of both the enzymes have been reported to vary with the administration of proteinous and non-proteinous compounds to rats2,3).

In the present paper, the effect of protein-deficiency and fasting for long periods on the levels of histidine deaminase and urocanase in carp liver was studied, as a preliminary trial for exploring the regulatory mechanism of histidine metabolism in fish.

Materials and Methods

Fish and diets. Carp, Cyprinus carpio, in young stage, weighting 30 ~ 50 g, were taken from Kyoto Prefectural Culture Pond in Kizu, Kyoto.

The fish were kept in aquaria (29 × 60 × 35 cm) at 15~25°C with continuous air-bubbling. After made to fast for two days, the fish were fed for one week on a commerical diet, followed by re-fasting for two days. Experimental diets, as shown in Table 1, were thereafter given twice a day, in the morning and in the evening, in total amounts of 5% body weight over the period of two weeks.

Preparation of tissue homogenates. Tissues were homogenized with 4 volumes of 1% KCl and centrifuged for 15 min. at 10,000×g, 0°C. The supernatant was used for the enzyme assays.

Enzyme assays. The methods mentioned by Tabor and Mehlerr4) for histidine

---

* A portion of this work was presented at the Annual Meeting of the Japanese Society of Scientific Fisheries, Tokyo, Japan, April, 1969.
** Research Institute for Food Science, Kyoto University, Kyoto, Japan (板目守彦・河合章：京都大学食糧科学研究所)
deaminase and urocanase assays were employed with the following modification. Histidine deaminase assay was carried out at pH 9.5, at which urocanase originally involved in the homogenate would be inactive. The assay system for histidine deaminase contained 3.0 ml of 0.2 M Tris-phosphate-HCl buffer (pH 9.5), 0.5 ml of enzyme solution, and 3.14 μmoles of L-histidine in a total volume of 4 ml. The assay system for urocanase contained 2.5 ml of 0.2 M Tris-HCl buffer (pH 7.5), 0.5 ml of enzyme solution, and 1.43 μmoles of urocanic acid in a total volume of 3.5 ml. Each mixture was incubated at 37°C and after the reaction was stopped by the addition of 0.5 ml of 30% perchloric acid, the precipitate was removed by filtration. The increase in UV absorption of the filtrate was measured at 277 mμ for histidine deaminase assay, and the decrease for urocanase assay.

Table 1. Composition of experimental diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Vitamin-salt mixture* (%)</th>
<th>Casein (%)</th>
<th>Potato starch (%)</th>
<th>Histidine content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. High casein</td>
<td>15</td>
<td>80</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>B. Low casein</td>
<td>15</td>
<td>5</td>
<td>80</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>C. Low casein + histidine</strong></td>
<td>15</td>
<td>5</td>
<td>80</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* Contains 5% vitamin mixture (purchased from Kohkin Chemical Co.), 5% salt mixture (4.7% NaCl, 7.2% MgSO₄, 9.4% NaH₂PO₄·2H₂O, 25.6% K₂HPO₄, 14.6% CaH₂(PO₄)₂·H₂O, 3.2% Fe-citrate, 35.0% Ca-lactate and 0.3% KI), 2% soybean oil, and 3% cellulose powder

** 2.7% histidine was supplemented to Diet B

Results and Discussion

Distribution of histidine deaminase and urocanase activities in various tissues. In the experiments to investigate the distribution of the enzyme activities, the fish were submitted to the experiments after fasting for a few days.

As shown in Table 2, high histidine deaminase activity was found in the liver, and low activity in the kidney and spleen, while no activity in the heart or muscle. Urocanase activity was detected only in the liver and kidney, and the enzyme activity of the liver was higher than that of the kidney.

Concerning the process of histidine degradation in the liver of carp, the authors have already demonstrated, using the liver homogenate, that histidine is easily metabolized to urocanic, formiminoglutaric, and glutamic acid in order. Furthermore, the fact that the activities of these two enzymes were highest in the liver supports the idea that histidine degradation in carp may mainly occur in the liver tissue.

The effect of protein-deficiency on histidine deaminase and urocanase activities. In order to examine the decrease in the enzyme activities caused by protein-deficiency, and restoration of the activities by the supplement of histidine to the protein-deficient diet,
carps were fed on the diets: 80% casein, 5% casein, and 5% casein plus 2.7% histidine, being compared with each other with respect to their activities of liver histidine deaminase and urocanase.

Feeding on 80% casein diet made approximately 5% gain in body weight of the fish, while feeding on 5% casein as well as on the diets of 5% casein plus histidine had hardly any increase in their body weights. The ratio of liver weight to whole body weight showed the value of 4.5% on 80% casein diet, on the other hand, 10% and 7.8% on the low protein diet and the histidine-supplemented diet, respectively. Namely, feeding on the latter diets caused abnormal enlargement of the livers of the fish.

As we can see in Table 3, carp fed on 5% casein diet had significantly low activities of histidine deaminase and urocanase in the liver, compared with the fish on the high casein diet. It is reasonably suggested that the substitution of casein for starch resulted in the depressed levels of both the enzymes. This effect may be considered to be a reveal of a kind of regulatory action for sparing histidine which is required as a constituent of various proteins in the fish body.

### Table 2. Distribution of histidine deaminase and urocanase activities in various tissues of carp. The values are represented as the averages for two fish*.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Weight of tissue (g)</th>
<th>Histidine deaminase**</th>
<th>Urocanase***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.6</td>
<td>4.2</td>
<td>13.2</td>
</tr>
<tr>
<td>Liver</td>
<td>3.0</td>
<td>25.7</td>
<td>19.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.4</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

* body weight: 94.2 and 73.6 g
** urocanic acid formed (µmoles/hr/g tissue
*** urocanic acid destroyed (µmoles/hr/g tissue

### Table 3. Effect of protein-deficiency on liver histidine deaminase and urocanase activities in carp. Values are given as means ± standard deviation.

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of fish</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Histidine deaminase (µmoles/hr/g tissue)</th>
<th>Urocanase (µmoles/hr/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. High casein</td>
<td>7</td>
<td>34.9±5.1</td>
<td>1.7±0.1</td>
<td>19.2±1.9</td>
<td>21.8±6.4</td>
</tr>
<tr>
<td>B. Low casein</td>
<td>6</td>
<td>35.2±4.2</td>
<td>3.5±0.4</td>
<td>2.0±0.2</td>
<td>4.8±2.1</td>
</tr>
<tr>
<td>C. Low casein+histidine</td>
<td>6</td>
<td>34.5±3.8</td>
<td>2.7±0.3</td>
<td>4.6±0.6</td>
<td>12.9±4.9</td>
</tr>
</tbody>
</table>

Significance by t test:

- Histidine deaminase, A vs B: P<0.01
- Urocanase, A vs B: P<0.01
- A vs C: P<0.01
- B vs C: P<0.01
- B vs C: P<0.05
- A vs B: P<0.01
- A vs C: P<0.05
- B vs C: P<0.05
Histidine deaminase and urocanase activities were elevated to an appreciable extent by the supplementation of almost the same amount of histidine (2.7%) to the low casein diet as contained in the high casein diet (Table 1). Both the enzymes, however, could not reach up to the levels obtained by feeding the fish on the high casein diet. Relatively high concentration of dietary protein may be required to bring about the same levels of the enzymes as observed in feeding on the high protein diet. Sahib and Krishna Murti reported that administration of histidine alone did not show any significant elevation of histidine deaminase level but histidine plus a little amount of casein or its hydrolysate produced obvious elevation of the enzyme level in the rat liver. The role of casein or its hydrolysate in elevating the enzyme levels in the fish liver is under our investigation.

The elevation and diminution of the enzyme levels thus observed may be in accordance with the induction and repression of these enzymes, although no data concerning the synthesis of enzyme proteins is available in this experiment. Substrate-induction and cata bolite-repression of histidine degrading enzymes are usually recognized in microorganisms. Similar types of regulatory mechanisms are supposed to be present also in the liver tissue of carp.

Effect of fasting on the enzyme levels. Liver histidine deaminase and urocanase activities of carp which was made to fast for 2 weeks were compared with those of the fish fed on 80% casein diet for the same period.

As presented in Table 4, histidine deaminase and urocanase activities based on the unit weight of the tissue of fasting fish were somewhat high, compared with those of the fish on the high protein diet, but the activities found in whole tissue of the liver were hardly affected by fasting. This phenomenon may be attributed to the condensation of the enzymes in the liver, since average loss of 33.3% in the liver weight were observed during the period of 2 weeks' fasting, whereas 20.6% loss in body weight for the same period. In regard to the effect of fasting on histidine deaminase level, Noda and Yoshida have observed a resembled phenomenon in rat liver. On the basis of these facts, it seems

Table 4. Effect of fasting on liver histidine deaminase and urocanase activities in carp.
Values are given as means ± standard deviation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of fish</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Histidine deaminase</th>
<th>Urocanase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(μmoles/hr./g)</td>
<td>(μmoles/hr./g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(μmoles/hr/whole tissue)</td>
<td>(μmoles/hr/whole tissue)</td>
</tr>
<tr>
<td>D. Feeding</td>
<td>6</td>
<td>37.2±0.6</td>
<td>1.8±0.1</td>
<td>20.1±2.1</td>
<td>21.5±1.7</td>
</tr>
<tr>
<td>E. Fasting</td>
<td>6</td>
<td>36.9±4.7</td>
<td>1.3±0.1</td>
<td>27.8±4.1</td>
<td>24.4±2.4</td>
</tr>
</tbody>
</table>

Significance by t test:
Histidine deaminase (μmoles/hr./g tissue), D vs E: P<0.01
(μmoles/hr./whole tissue), D vs E: P>0.05
Urocanase (μmoles/hr./g tissue), D vs E: P<0.05
(μmoles/hr./whole tissue), D vs E: P>0.05
reasonable to assume that histidine deaminase and urocanase are reserved during the long-term fasting with no diminished levels in order to catabolize histidine, which is released by the breakdown of body proteins for energy supply.

During the fasting for a few days, rat liver threonine dehydratase and arginase were reported to be raised in their levels, nevertheless glucose-6-phosphate dehydrogenase and glucokinase diminished. These reports suggest that the amino acid-catabolizing enzymes may be controled not to be readily diminished by fasting in contrast to the enzymes involved in carbohydrate metabolisms in the liver tissue.

In this paper, the elevation and diminution of the enzyme levels were discussed in relation to feeding the different diets and fasting, however, hormonal and other complicated control mechanisms should be put into consideration in future studies on histidine metabolism in fish.

Summary

1. Distribution of histidine deaminase and urocanase in various tissues of carp was examined. High histidine deaminase activity was found in the liver, and low activity in the kidney and spleen but not in the heart and muscle. Urocanase activity in the liver tissue was higher than that in the kidney.

2. Carp fed on 5% casein diet for 2 weeks gave the low levels of liver histidine deaminase and urocanase, compared with the fish on 80% casein diet. Supplementation of 2.7% of histidine to the low protein diet caused the appreciable elevation of both the enzyme levels.

3. Liver histidine deaminase and urocanase of carp fasting for 2 weeks showed the higher activities per unit weight than those of the fish on the high protein diet but there were no differences in the activities of whole liver tissue between these fish.

References