Decomposition of Carnosine and Its Methyl Derivatives in Aquatic Animal Meats during Storage

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The decomposition of carnosine, anserine, and balenine during storage of the big-eye tuna and the sei whale meats was studied with a view to ascertain the formation of methyl derivatives of histamine and β-alanyl-histamine. A chromatographic determination of these amines on a 15 cm column of a cation exchange resin (JEOL, LCR-2) was achieved using a borate buffer of pH 10.2 having sodium concentration of 0.32 N, at 52°C. When the big-eye tuna meats with and without addition of chopped sardine skin were stored at 5°C for 12 days or 20°C for 5 days, and when the sei whale meats with and without addition of mackerel slime at 25°C or 37°C for 3 days, the meats turned putrid in the late stages of the storage periods. The amounts of anserine in the big-eye tuna, and carnosine and balenine in the sei whale did not decrease. Although a lot of histidine was decarboxylated into histamine in the big-eye tuna meat at 20°C, the methyl derivatives of histamine and β-alanyl-histamine were not detected.

Migrating fishes such as tuna and mackerel contain a large amount of free histidine in their muscular tissues. During storage of these fishes, a lot of histidine changed into histamine by the action of decarboxylase in Proteus morganii1-3 and psychrophilic-halophilic histamine-forming bacteria (N-group bacteria).4-6 We have determined that the muscles of migrating fishes,7,8 some species of elasmobranchs7,9 and whales7,10 are also rich in carnosine (β-alanyl-histidine), anserine (β-alanyl-π-methylhistidine), and balenine (β-alanyl-τ-methylhistidine). However, little is known about the bacterial decomposition including the decarboxylation of such imidazole dipeptides.

This study was undertaken to determine the decomposition of the imidazole dipeptides during the storage of tuna and whale meats and whether β-alanyl-histamine [cancine,11,12 3-amino-N-{2-(imidazole-4-yl)ethyl}propanamide] and its methyl derivatives are formed from the dipeptides or not.

Materials and Methods

Materials

Dorsal meats of frozen big-eye tuna Thunnus obesus and sei whale Balaenoptera borealis were used as materials.

Storage of Meats

The big-eye tuna meat was minced and the aliquots were allowed to stand in glass bottles at 5°C, 20°C, and 35°C for 5 to 16 days. In the other experiment, the tuna meat obtained from a different individual was mixed with the chopped skin of fresh sardine in order to accelerate the spoilage, and stored at 5°C for 12 days and at 20°C for 5 days together with the specimens without the chopped skin. The sei whale meat was minced and allowed to stand in glass bottles at 25°C for 6 days. In the second experiment, the meat obtained from a different individual was stored at 25°C or 37°C for 3 days, and that mixed with the skin slime of mackerel left at 30°C for 24 h was stored at 25°C for the same time.

Measurement of Volatile Basic Nitrogen

The meats before and after storage were extracted with 5% trichloroacetic acid and the content of volatile basic nitrogen in the extracts was measured by a microdiffusion method.13 Determination of Carnosine and Its Methyl Derivatives

Carnosine, anserine, and balenine were quantitatively determined with an amino acid analyzer (JEOL, JLC-6AS) using a column (0.8 x 70 cm) of a cation exchange resin (JEOL, LCR-2) and lithium citrate buffer (pH 7.80, lithium concentration 0.5 N).14

For a qualitative analysis of the imidazole dipeptides, thin layer chromatography was done using silica gel G plates and solvents as follows:

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Fig. 1. Chromatography of β-alanyl-histamine (A), β-alanyl-α-methylhistamine (B), and β-alanyl-γ-methylhistamine (C) on a 30 cm column of Dowex 50W-X8, using pyridine-acetate buffer, pH 4.05.

Fig. 2. Chromatography of β-alanyl-histamine (A), β-alanyl-α-methylhistamine (B), β-alanyl-γ-methylhistamine (C), histamine (a), α-methylhistamine (b), γ-methylhistamine (c), and β-alanine (d) on a 15 cm column of a cation exchange resin (JEOL, LCR-2) using borate buffer, pH 10.2.

Determination of Histamine, β-Alanyl-histamine and Their Methyl Derivatives

The separation of β-alanyl-histamine, β-alanyl-α-methylhistamine and β-alanyl-γ-methylhistamine was made according to the determination method of β-alanyl-histamine by Arnould and Tankosic. The chromatogram obtained is shown in Fig. 1.

For the simultaneous determination of these three amines in addition to their hydrolyzed compounds such as β-alanine, histamine, α-methylhistamine and γ-methylhistamine, we modified an analytical method of histamine using a 0.8~15 cm column of a cation exchange resin (JEOL, LCR-2), and it was found that these compounds were separated rapidly and sufficiently with the borate buffer of pH 10.2 having sodium concentration of 0.32 N, at 52°C. A typical chromatogram is shown in Fig. 2.

Results and Discussion

Changes of Anserine and Histidine in Big-eye Tuna Meat during Storage

In the preliminary experiment, the big-eye tuna meat was stored at 5°C, 20°C, and 35°C for 5 to 16 days, and the change in the amount of anserine was determined. Although the meat turned putrid with the lapse of storage periods, there was no significant change in the level of imidazole dipeptides (data not shown).

In order to accelerate the decomposition of the constituents, the chopped sardine skin was mixed into the big-eye tuna meat, and both the meats with and without the chopped skin were stored at 5°C for 12 days and at 20°C for 5 days. The
Fig. 4. Changes in the amounts of anserine (1), histidine (2), β-alanyl-α-methylhistamine (3), and histamine (4) of big-eye tuna meats during storage at 5°C. (Broken lines indicate the changes when sardine skin was added to the meat.)

Volatile basic nitrogen content of meats was measured as an index of the freshness. The results are shown in Fig. 3. At 5°C storage, the volatile basic nitrogen content of the meat almost unchanged during storage, whereas the meat containing the chopped skin reached to the level of 48 mg/100 g after 12 days. At 20°C storage, on the other hand, a considerable increase in the content was observed, and particularly, the meat with the chopped skin had 133 mg/100 g after 5 days.

The big-eye tuna meat used in this case contained 36.1 μmol/g (866 mg/100 g) of anserine and 51.0 μmol/g (790 mg/100 g) of histidine, and the changes in the amounts of these constituents together with β-alanyl-α-methylhistamine and histamine in the big-eye tuna meat at 5°C and 20°C storage are shown in Figs. 4 and 5, respectively.

Fig. 4 shows that the amount of anserine in the meats scarcely varied at 5°C, and β-alanyl-α-methylhistamine was not detected. Histidine contents decreased slightly, and reached to 43.6 μmol/g and 35.4 μmol/g in the meats with and without the chopped skin, respectively, during 12 days storage. The decreasing rate of anserine and histidine thus seemed not to be accelerated by the addition of sardine skin. Any histamine could not be detected throughout the storage as has already been reported by YAMANAKA et al. 19)

At 20°C, as shown in Fig. 5, the content of anserine decreased slowly with increasing the storage time, and reached to 28.7 μmol/g and 29.9 μmol/g in the meat with and without the chopped skin, respectively, after 5 days. One or 2 μmol/g of α-methylhistidine were detected in the meat and this may be the result of hydrolysis of peptides. Histidine content of tuna meat increased slowly during first 4 days and then decreased rapidly to 20.6 μmol/g on the 5th day, and in the case of meat containing the chopped skin, it decreased rapidly and then increased to 12.4 μmol/g after 5 days. The level of the histidine might be differed by the bacterial intake of free histidine and production of histidine by hydrolysis of meat proteins.

YAMANAKA et al. 19) reported that a large amount of histamine was detected during the storage of big-eye tuna meat at 20°C. In the present study, the formation of histamine in the tuna meats differed markedly from the addition of chopped skin; histamine was formed on the 5th day and reached to the level of 32.0 μmol/g in the meat without the chopped skin, whereas, it increased rapidly and reached maximum amount of 26.8 μmol/g on the 4th day and then decreased in the meat added chopped skin. The tendency of histamine production seemed to be correlative
Table 2. Changes in the amounts of carnosine, anserine, and balenine of sei whale meats during storage at 25°C and 37°C

<table>
<thead>
<tr>
<th>Storage (days)</th>
<th>Carnosine (µmol/g)</th>
<th>Anserine (µmol/g)</th>
<th>Balenine (µmol/g)</th>
<th>Lysine* (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29.3</td>
<td>0.8</td>
<td>52.1</td>
<td>0.3</td>
</tr>
<tr>
<td>3 at 25°C</td>
<td>25.8</td>
<td>0.5</td>
<td>51.3</td>
<td>2.6</td>
</tr>
<tr>
<td>at 37°C</td>
<td>13.9</td>
<td>0.6</td>
<td>46.3</td>
<td>16.8</td>
</tr>
<tr>
<td>3 at 25°C</td>
<td>13.0</td>
<td>0.3</td>
<td>45.4</td>
<td>7.3</td>
</tr>
</tbody>
</table>

* Free lysine content was determined in relation to the decomposition of the proteins of whale meat.

with that of histidine decomposition.

During the experiment, \( \pi \)-methylhistamine and \( \beta \)-alanyl-\( \pi \)-methylhistamine, corresponding to the decarboxylation products of \( \pi \)-methylhistidine and anserine, respectively, were not detected.

Changes of Carnosine and Its Methyl Derivatives in Sei Whale Meat during Storage

The sei whale meat is known to contain a large amount of balenine in addition to an intermediate amount of carnosine and a small amount of anserine.\(^7\) When stored at 25°C for 6 days, the meat was decomposed and the level of volatile basic nitrogen reached to 93.0 mg/100 g, whereas balenine was not broken down at all as shown in Table 1.

In the following experiment, the sei whale meat added with the mackerel slime was stored at 25°C for 3 days, and the decomposition of imidazole compounds was compared to that in the meat without addition of the slime when stored at 25°C or 37°C. The change in the amounts of carnosine, anserine, and balenine is shown in Table 2 in which the free lysine content is also tabulated as an indicator of the decomposition of proteins during the storage. It can be seen from the table that the fresh meat contained 29.3 µmol/g (662 mg/100 g) of carnosine, 0.8 µmol/g (19 mg/100 g) of anserine, and 52.1 µmol/g (1250 mg/100 g) of balenine, and the amounts of these dipeptides decreased somewhat rapidly at 37°C in the meat without the slime, and also at 25°C in the meat with the slime. However, the methyl derivatives of histamine and \( \beta \)-alanyl-histamine were not detected throughout the putrefaction of the sei whale meat. Although it could not be proved conclusively, there was evidence by the qualitative and quantitative determinations that a great part of the decomposition of these dipeptides is due to the hydrolysis of their peptide bonds.

\( \beta \)-Alanyl-histamine, decarboxylation product of carnosine, was first detected in the heart of crab *Carcinus maenas* and named as carcine by ARNOULD and FRENTZ.\(^1\) In the present study, we have prepared \( \beta \)-alanyl-\( \pi \)-methylhistamine and \( \beta \)-alanyl-\( \tau \)-methylhistamine, which have not been reported so far, and incidentally, propose to give the name of anseramine and balenamine because they correspond to the decarboxylated amines of anserine and balenine, respectively.

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References


