Further Studies of Inosinic Acid Formation in Carp Muscle

Tsuneyuki Saito and Kenichi Arai
(Faculty of Fisheries, Hokkaido University)

A previous communication from this laboratory reported the formation of IMP* by slow freezing of carp muscle. The occurrence of AMP may occur as soon as ATP and ADP are converted to AMP. The purpose of the present paper is to establish the occurrence of the AMP deamination as a reaction mechanism in the formation of IMP of carp muscle.

One gram of fresh carp muscle was homogenized with 20 ml. of 1/7.5 M sodium chloride solution by hand in a mortar. Adenine and hypoxanthine nucleotides and nucleoside from the homogenized extracts were studied by the hydrochloric acid gradient-elution system on Amberlite IRA-400 which has been described previously. The results are summarized in Table 1.

### Table 1. Changes in the concentration of nucleotides in muscle homogenate

<table>
<thead>
<tr>
<th>Compound</th>
<th>Treatment</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inosine, hypoxanthine</td>
<td>0.00</td>
<td>0.08</td>
<td>0.43</td>
<td>0.67</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>0.04</td>
<td></td>
<td>0.08</td>
<td>0.10</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>IMP</td>
<td>0.00</td>
<td></td>
<td>5.66</td>
<td>8.51</td>
<td>7.26</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>0.94</td>
<td></td>
<td>0.44</td>
<td>0.48</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>5.76</td>
<td>2.86</td>
<td>0.06</td>
<td>0.08</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.74</td>
<td>6.76</td>
<td>6.67</td>
<td>9.84</td>
<td>9.85</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed in μ moles/g. of wet muscle. Amounts of inosine and of hypoxanthine are expressed as that of inosine.

- I: Extracted with 4% HClO₄ rapidly (standard)
- II: Homogenized with 1/7.5 M NaCl
- III: Same as II, Kept at −6°C. for 1.5 hrs.
- IV: Same as II, 3.54 μ moles of AMP premixed, kept at −6°C. for 1.5 hrs.
- V: Same as II, 3.54 μ moles of AMP premixed, kept at +6°C. for 1.5 hrs.

During the process of grinding muscle on the ice for about 10 minutes, 2.9 μ moles of ATP are decomposed while 2.04 μ moles of IMP are accumulated (treatment 11).

When muscle, homogenized with sodium chloride, is kept at −6°C. for 1.5 hours the greater parts of ATP and 0.5 μ mole of ADP are decomposed; on the other hand, 66 μ moles of IMP and small amounts of inosine and hypoxanthine are accumulated (treatment III).

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* The following abbreviations are used:
  IMP, Inosinemonophosphate; ATP, Adenosinetriphosphate;
  ADP, Adenosinediphosphate; AMP, Adenosinemonophosphate.
In each of the cases of treatments IV and V, in which 3.54 μ moles of AMP are added previously to muscle homogenate, the two, both the added material and that obtained from ATP and ADP are converted almost completely to IMP and furthermore to inosine and hypoxanthine at 6°C. or at −6°C. for 1.5 hours. But the rate of conversion to inosine and hypoxanthine is slow, especially at −6°C. In these cases it was noticed that there is almost a quantitative relationship between the amounts of ATP decomposed and the sum totals of base, nucleoside and nucleotides formed.

From these experiments it has been considered that the deamination process of AMP is so rapid that whenever the changes, accompanied by ATP splitting, occur in muscle tissue or in homogenate, considerable amounts of IMP are accumulated and then it converts to inosine and hypoxanthine slowly.

From these phenomena which were very remarkable at low temperature it may be considered that the action of AMP deaminase is not affected by such low temperature while that of 5'-nucleotide phosphatase is reduced as the temperature becomes lower.

References