Effects of Chloramphenicol on Post-mortem Biochemical Changes in Scallop Adductor Muscle[†]

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To clarify the role of endogenous enzymes and bacterial enzymes on post-mortem biochemical changes in scallop adductor muscle, the effects of chloramphenicol (CP) on these changes during storage at 5 and 10°C were investigated.

Both muscle with and without CP added showed the same ATP breakdown pattern; degradation to inosine (HxR) and hypoxanthine (Hx) was not inhibited by CP, therefore, degradation to HxR and Hx was mainly caused by endogenous enzymes. D-Lactic acid, an end product of glycolysis, increased to a certain level in both muscle with CP and muscle without CP in the early stage of storage. The acid level then remained constant in the muscle with CP, but increased markedly as decomposition progressed in the muscle without CP. D-Lactic acid thus forms due to both endogenous and exogenous enzymes. The final content of octopine, another end product of glycolysis, was higher in the muscle with CP than in the muscle without CP, and this difference is thought to be caused by D-lactic acid formation in the muscle without CP.

Key words: scallop, chloramphenicol, octopine, ATP-related compounds, D-lactic acid, post-mortem changes

Post-mortem biochemical changes in invertebrates are known to be different from those in vertebrates,^{1–3)} but no detailed information is available. In the previous report,⁴⁾ the authors showed that the post-mortem biochemical changes in scallop adductor muscle differed markedly from those in fishes^{5,6)} and prawn⁷⁾; decomposition progressed faster at higher storage temperatures, though the decrease in ATP and the increases in inosine (HxR) and hypoxanthine (Hx) were faster at lower storage temperatures, consequently the K value increased faster at lower storage temperatures. D-Lactic acid, not L-lactic acid, was detected, and while decomposition progressed it accumulated as a possible end product of the post-mortem biochemical changes in scallop adductor muscle together with octopine.

In this report, to clarify the role of endogenous enzymes and bacterial enzymes in the post-mortem biochemical changes of scallop adductor muscle, the effects of antibiotic chloramphenicol on these changes were investigated.

Materials and Methods

Materials

Live scallop *Patinopecten yessoensis* was obtained from Tokyo Central Wholesale Market in May 1993. The striated part of the adductor muscle was chopped into small pieces and made into composite samples. Composite samples were divided into 4 portions, and 0.1% chloramphenicol (CP) was added to 2 portions. Samples with and without CP were then stored at 5°C and 10°C. At regular intervals, muscle was withdrawn from each portion and subjected to the following tests.

Sensory Evaluation

The degree of freshness of scallop adductor muscle was evaluated by

trained sensory panels and classified into three stages mainly based on its odor: acceptable (no smell), initial decomposition (faintly putrid smell), and advanced decomposition (putrid smell).

Preparation of Extract

About 5 g of muscle was homogenized in 20 ml ice-cold 6% perchloric acid and the suspension was centrifuged at 10,000 rpm for 10 min. The procedure was repeated and the supernatants were combined. After neutralization with KOH aqueous solution, the supernatant was made up to 50 ml with distilled water. This solution was analyzed for ATP, ATP-related compounds, octopine, arginine, and lactic acid.

Determination of ATP and Its Related Compounds

The contents of ATP and its related compounds were analyzed by HPLC using the method previously described.⁴⁾

Determination of Octopine and Arginine

Octopine and arginine were converted to fluorescent derivatives with benzoin, then analyzed by HPLC using the method previously described.⁴⁾

Determination of Lactic Acid

L- and D-Lactic acids were analyzed using L-lactate dehydrogenase (EC 1.1.1.27, Boehringer Mannheim Co.) and D-lactate dehydrogenase (EC 1.1.1.28, Boehringer Mannheim Co.), respectively, according to the method of Noll.⁸⁾

Measurement of pH Value

Two grams of chopped muscle were homogenized in 20 ml deionized water, then the pH value of homogenate was measured with a Horiba F-12 pH meter.

Results

Sensory Evaluation

Portions of chopped scallop adductor muscle without CP were judged to be at the stage of initial decomposition on

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Fig. 1. Changes in ATP and its related compounds in chopped scallop adductor muscle during storage at 5°C.

Fig. 3. Changes in ATP and its related compounds in chopped scallop adductor muscle during storage at 10°C.

Fig. 2. Changes in ATP and its related compounds in chopped scallop adductor muscle with added chloramphenicol during storage at 5°C.

Fig. 4. Changes in ATP and its related compounds in chopped scallop adductor muscle with added chloramphenicol during storage at 10°C.

the 2nd day of storage at 10°C, or on the 3rd day of storage at 5°C, and to be at the stage of advanced decomposition on the 3rd day of storage at 10°C or on the 4th day of storage at 5°C. On the contrary, for portions with CP, no evidence of decomposition was detected during storage either at 10°C or 5°C for 10 days.

ATP and Its Related Compounds

Figures 1 to 4 show the changes in ATP and its related compounds during storage. Adenosine (AdR) was detected throughout the storage period tested, though in very small quantities (less than 1% of the total amount of ATP and its related compounds) and showed practically no change. Therefore, AdR does not appear in Figs. 1 to 4. The total amount of ATP and its related compounds was about 5.4 $\mu\text{mol/g}$ at the start, and remained constant in the portions with CP during the storage period of 10 days regardless of the storage temperature. However, in the portions without CP, the amount began to decrease as decomposition progressed (after the 6th day at 5°C or after the 5th day at 10°C), falling to about 70% of the initial value. Even at this highly decomposed stage, xanthine

production was not observed.

At the beginning, ATP accounted for 45% of the total amount of ATP-related compounds, ADP 33%, AMP 18%, IMP 1%, AdR less than 1%, inosine (HxR) 2%, and hypoxanthine (Hx) 1%. ATP gradually decreased during storage in both portions with and without CP, and on the 6th day of storage it nearly disappeared. With the progress of ATP degradation, HxR slowly accumulated and Hx accumulated constantly, then HxR began to decrease after 3 to 4 days of storage. Hx accumulation continued, and Hx accounted for approximately half of the total amount of ATP-related compounds on the 6th day of storage. During the early stage of storage, the rates of accumulation of HxR and Hx were higher at 5°C than at 10°C within both portions with and without CP, then these rates were reversed in the later stage of storage. For portions without CP, Hx accumulation accelerated as muscle decomposition fully progressed.

The changes in the K value during storage are summarized in Fig. 5. The K value was 6% immediately after death, and gradually increased during the first 3 days of storage to around 70%, then remained constant during the

Fig. 5. Effects of chloramphenicol (CP) on changes in the K value of chopped scallop adductor muscle during storage at 5 and 10°C.
○, muscle without CP stored at 5°C; ●, muscle without CP stored at 10°C; □, muscle with CP stored at 5°C; ■, muscle with CP stored at 10°C.

Fig. 7. Effects of chloramphenicol (CP) on changes in arginine in chopped scallop adductor muscle during storage at 5 and 10°C.
Symbols used are the same as Fig. 5.

Fig. 6. Effects of chloramphenicol (CP) on changes in octopine in chopped scallop adductor muscle during storage at 5 and 10°C.
Symbols used are the same as Fig. 5.

Fig. 8. Effects of chloramphenicol (CP) on changes in D-lactic acid in chopped scallop adductor muscle during storage at 5 and 10°C.
Symbols used are the same as Fig. 5.

rest of the storage period with the exception of the portion without CP stored at 10°C, in which the K value increased to nearly 80% at the end of the storage period.

Octopine and Arginine

Figure 6 shows the changes in octopine content during storage. Immediately after death, octopine content was 2 μmol/g and increased to 12–16 μmol/g at the end of the storage period. The rate of octopine increase during the early stage of storage was higher at 5°C than at 10°C. Also octopine increased faster in portions with CP than without, and the final content was larger in portions with CP.

The changes in arginine content during storage are shown in Fig. 7. Arginine content was 7 μmol/g at the start, and gradually decreased during storage to about 2 μmol/g. The differences in the changes in arginine content among the 4 portions were fairly small. The arginine content decreased as the octopine content increased.

Lactic Acid

Figure 8 shows the changes in D-lactic acid during storage. Both L-lactic acid and D-lactic acid were detected, but the L-lactic acid content was very small (less than 1 μmol/g). At the beginning, the D-lactic acid content was less than 1 μmol/g, then increased to around 6 μmol/g in the early stage of storage irrespective of the addition of CP. For portions with CP, the content remained constant throughout the rest of the storage period tested, but for portions without CP, it increased further as decomposition progressed, reaching 16 μmol/g for the portion stored at 5°C and 25 μmol/g for the portion stored at 10°C.

pH Value

The changes in pH value of scallop adductor muscle during storage are shown in Fig. 9. Muscle pH immediately after death was 6.89, gradually decreasing during storage to 6.05 in portions with CP. However, in portions without

Fig. 9. Effects of chloramphenicol (CP) on changes in pH value in chopped scallop adductor muscle during storage at 5 and 10°C. Symbols used are the same as Fig. 5.

CP, muscle pH rapidly decreased after the onset of decomposition in the muscle, falling to 5.90.

Discussion

It is well-known that IMP accumulates as ATP degradation progresses in fish muscle, and AMP in molluscs. In the muscle of kuruma prawn,⁷⁾ ATP degradation resulted in IMP accumulation, and further degradation to HxR or to Hx was very slow, but after muscle reached the stage of initial decomposition, a marked increase in Hx occurred. But when antibiotic CP was added to muscle,⁹⁾ degradation to HxR or to Hx was completely inhibited, therefore degradation to HxR or to Hx is thought to occur by microbial growth.

The same phenomenon was observed for the adductor muscle of Japanese common oyster, in which AMP accumulated instead of IMP. On the contrary, IMP or AMP did not accumulate in scallop adductor muscle, and further degradation to HxR or to Hx was rapid. Even when antibiotic CP was added, HxR or Hx formation in scallop adductor muscle was not inhibited. Consequently, in scallop adductor muscle, unlike kuruma prawn muscle or adductor muscle of Japanese common oyster, the accumulation of HxR and Hx is thought to be mainly due to endogenous enzymes. Since decomposition to HxR and Hx started even in the early stage of storage, and AMP, IMP, and AdR accumulations were very low, this enzymatic activity to form HxR and Hx was fairly strong. ADP degradation was slower than ATP degradation, and a fairly large proportion of ADP (around 20% of the total amount of ATP related compounds) remained even at the end of the storage period, together with large accumulations of degradation products, HxR and Hx. It therefore appears that there is a mechanism which inhibits the conversion of ADP to AMP. Hiltz and Dyer^{10,11)} reported that the increase in Hx content was linearly related to the decrease in taste of unfrozen or thawed sea scallop *Placopecton magellanicus* meat. Our results showed that in scallop adductor muscle, the accumulation of Hx was not affected by bacterial enzymes, but rather by storage temperature. In

order to maintain the quality of scallop adductor muscle, the storage temperature needs to be chosen carefully.

The K value, a freshness index defined as the molar ratio of HxR plus Hx to the total of ATP and its related compounds, increased faster at 5°C than at 10°C during the early stage of storage. The same temperature dependency was observed when scallop adductor muscle was stored at -3, 0, and 5°C.⁴⁾ Similar changes in the K value were reported for itayagai scallop *Pecten albicans* adductor muscle¹²⁾; the K value increased faster at -3 or 0°C than at 5 or 10°C. Sensory evaluation proved that the higher the storage temperature, the faster the decomposition progressed when stored at 5 and 10°C, which is the same result when stored at -3 to 5°C. Thus the K value cannot be used as a freshness index of scallop adductor muscle within the temperature range of -3 to 10°C.

Octopine accumulation was higher in portions with CP than without, while D-lactic acid accumulation in portions without CP was higher than in portions with CP. Since both octopine and D-lactic acid are end products of glycolysis in scallop adductor muscle, the difference in octopine accumulation is partly due to D-lactic acid formation. The rate of octopine accumulation in the early stage of storage was higher in portions with CP than without. Similarly, ATP degradation in the early stage of storage in portions with CP was slightly faster than in portions without CP. The causes of these phenomena are of great interest.

At the beginning of storage, D-lactic acid accumulated regardless of the addition of CP, indicating that D-lactic acid is formed by enzymes in muscle. Then in later stage of storage, D-lactic acid increased rapidly as decomposition progressed in portions without CP, suggesting that D-lactic acid is formed by microbial enzymes at this stage. Therefore, D-lactic acid forms in scallop adductor muscle due to both endogenous muscle enzymes and exogenous bacterial enzymes.

Previously, it was reported that lactate dehydrogenase activity in molluscs was absent or very weak.¹³⁻¹⁷⁾ Our results showed that D-lactic acid was produced both endogenously and exogenously. Previous reports referred to only L-lactate dehydrogenase. It may be possible that scallop adductor muscle contains D-lactate dehydrogenase.

There was little difference in the changes in muscle pH among the 4 portions in the early stage of storage. Hiltz *et al.*¹³⁾ reported that octopine formation was linearly related to the decline in muscle pH in scallop *Placopecton magellanicus* muscle, but no evidence of this linear relation was found in our study. The pH fell rapidly in portions without CP after the onset of decomposition. This rapid decrease is believed to be caused by the increase in D-lactic acid.

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