Influence on Post-mortem Rigor of Fish Body and Muscular ATP Consumption by the Destruction of Spinal Cord in Several Fishes

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Influence of destruction of spinal cord on the post-mortem rigor and muscular ATP consumption was examined in yellowtail, red sea bream, and plaice. In case of yellowtail and red sea bream, the sample whose spinal cord was destroyed (tested sample) showed 6-12 hours delay in reaching full-rigor compared with the control sample. On the other hand, in case of plaice, the tested sample attained full-rigor faster than the control, and the control sample did not reach to full-rigor during 24 h storage. Rate of ATP consumption was slower in the tested than in the control in yellowtail and red sea bream. In case of plaice, the ATP content was almost the same in both of the tested and the control, and was much in the control for 15-24 h storage. Then, it was concluded that destruction of the spinal cord delayed ATP consumption and process of post-mortem rigor except for the case of plaice, and such a specialty of plaice would be caused by the inferiority of autonomic nerve development.

Key words: post-mortem rigor, ATP, spinal cord, nerve, fish

In the killing of a live cultured fish, punch at the brain or a cut of the spinal cord is done generally. In some cases, in addition to the cut of the spinal cord, a wire is pushed in to destroy the spinal cord completely. It is thought that the freshness of fish is maintained for longer period by this method, but there are few studies which are concerned the influence on the freshness by such a method. Nakayama et al. *1 has reported the delay effect on the post-mortem rigor and ATP consumption which was evaluated by ATP/IMP ratio by the destruction of spinal cord about red sea bream. ATP consumption has been reported as the cause of post-mortem rigor in frigate mackerel,19) pacific mackerel,2-4) red sea bream,3-5,6) carp,5-7,10) yellowtail,11-14) flathead,12) Japanese striped knifejaw,12,14) sardine,4,15) plaice16,17) and horse mackerel.18) In comparison of slaughter methods, death by struggling is the fastest in ATP exhaustion and onset of post-mortem rigor in yellowtail19) and horse mackerel.18) Iwamoto et al.5) showed that process of post-mortem rigor was influenced by the storage temperature, and the rigor proceeded slower at 10°C than 0°C storage because ATP consumption rate was slower at 10°C. According to these reports, the decrease of ATP content is the major factor of post-mortem rigor progress.

The purpose of the present study is to examine the influence of destruction of the spinal cord on the rigor mortis process and ATP consumption rate in several fishes.

Materials and Methods

Samples
Yellowtail Seriola quinqueradiata (50-59 cm, 2.3-3.3 kg), red sea bream Pagrus major (27-39 cm, 714-1240 g), and plaice Paralichthys olivaceus (33-38 cm, 386-560 g) were obtained alive from a fish store (Uoshin suisan Co., Nara, Japan). They were punched at the brain and their spinal cord cut (control sample). Tested samples were prepared as follows. In the fish whose spinal cord was cut, a wire (2 mm in diameter, 30 cm in length) was pushed in to destroy the spinal cord (tested sample). The samples were stored at 5°C for 24 h. Three individuals were examined in each fish.

Measurement of the Rigor Index
Post-mortem rigor was evaluated according to the method of Bito et al.19) Briefly, fish were put on a horizontal table protruding the half of the body length from the edge of the table. The distance from the horizontal line to the base of the tail (L) was measured at selected time intervals after death, and the rigor index was calculated by applying these values to the following equation.

\[ \text{Rigor index} = \frac{L_0 - L}{L_0} \times 100 \]

(L_0: the value immediately after death)

Determination of ATP Content
Amount of ATP content was determined according to the method of Yokoyama et al.20) One gram of muscle was cut from the dorsal muscle of the fish body which was used for measurement of rigor index. The muscle was homogenized with 5 ml of 10% perchloric acid (PCA). The homogenate was centrifuged at 3,000 × g for 15 min, and the residue was washed twice with 5% PCA. The supernatants were combined and neutralized with 10 and 1 N KOH solution. The neutralized solution was centrifuged at 3,000 × g for 15 min, and the precipitate was washed with 10 ml of neutralized 5% PCA-KOH solution and centrifuged. The supernatants were combined and diluted to

25 ml with distilled water. The PCA extract was filtered through a 0.45 μm membrane, and 10 μl of the filtrate was analyzed by a CAPCELLPAK 5C18-AR (4.6 x 150 mm, Shiseido, Japan) which was equilibrated with a mixture of 20 mm citric acid, 20 mm acetic acid, and 40 mm triethylamine (pH 4.8). Flow rate was 1 ml/min and the column temperature was held at 40°C. The eluate was monitored by UV absorption at 260 nm.

Results and Discussion

Process of Post-mortem Rigor

The results of rigor index are shown in Figs. 1-3. In case of yellowtail and red sea bream, the control sample body started to be stiff within 3 h storage, and was in maximum rigor in 6-9 h after death. On the other hand, a tested sample showed slower process of rigor than the control. In the tested sample of yellowtail, post-mortem rigor started at 6 h and progressed rapidly till 9 h storage. Maximum rigor was observed at 15 h after death. In the tested sample of red sea bream, post-mortem rigor progressed rapidly in 3-6 h, but gradually in 6-21 h storage. After that, maximum rigor was attained at 24 h after death. According to these results, process of rigor mortis was delayed significantly by the destruction of the spinal cord in yellowtail and red sea bream. By the destruction of the spinal cord, the fish body stopped struggling promptly. This effect would be due to the destruction of the autonomic nerve. In case of the control sample, autonomic nerve would function even after the death of fish for a short time, so the muscle moves at random till autonomic nerve stops their function. Then, ATP consumption rate of the control in the early period of storage would be faster than the tested, and it might be the cause of the earlier onset of post-mortem rigor.

In case of plaice, however, the opposite result was obtained (Fig. 3). The onset of rigor mortis was earlier for 3 hours in the tested than the control. And the tested sample maintained the higher level of rigor than the control especially in 18-24 h storage. In case of plaice, body struggling was not observed with or without destruction of the spinal cord. Plaice lives on the bottom of sea and it swims less than other fishes. So, it would be speculated that autonomic nervous system which controls muscle exercise in plaice doesn't develop so well as pelagic fishes which swim constantly. Then, instead of stopping the function of autonomic nervous system, some stimulation would be given to plaice muscle by the destruction of the spinal cord, and the post-mortem rigor progressed earlier.

Change of ATP Contents

The results of ATP content determination are shown in Figs. 4-6. In case of yellowtail (Fig. 4), ATP content was almost the same between the tested and the control during 3 h storage. In 6-15 h storage, ATP content was more in the tested. In case of red sea bream (Fig. 5), ATP content was decreased rapidly till 6 h storage in the control. In the tested, ATP content was decreased slower than the control during storage for 15 h. As the case of plaice (Fig. 6), ATP content was almost the same between the control and the tested during 15 h storage, and the control sample maintained a higher level of ATP content than the tested in 18-24 h storage. These difference in ATP contents between the tested and the control would have a relationship to the destruction of autonomic nervous system as described.
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Fig. 4. Change of muscular ATP contents of yellowtail during storage at 5°C.
- the sample whose spinal cord was cut and destroyed by pushing the wire.
- the sample which was killed by cutting the spinal cord.

Fig. 5. Change of muscular ATP contents of red sea bream during storage at 5°C.
The symbols are the same of Fig. 4.

It was shown in some cases that the change of ATP contents did not correspond to the progress of rigor mortis. In the yellowtail, rigor mortis progressed rapidly in 3–9 h storage; but ATP content decreased slightly during the same period. In the tested red sea bream, the rigor was progressed gradually in 6–21 h, but the content of ATP decreased rapidly in 9–15 h storage. These cases show that ATP content does not always correspond with post mortem rigor.

Influence of Muscle Cutting on Post-mortem Rigor

At the beginning of this study, we measured the rigor index in the sample whose body was not accepted for any damage for excision of a piece of muscle to determine the ATP content. Because we thought that the cutting of muscle gave damage to the fish body, and rigor index might be lower than the actual. However, damaged sample showed higher index and faster process of rigor than the intact sample (Fig. 7). This result suggests that cutting of muscle might cause ATP exhaustion, and promote post-mortem rigor. Then, to discuss about the exact relationship between the rigor index and ATP contents, an experiment would be needed as follows; several samples which are killed at the same time are provided, and ATP contents are determined when each sample shows a specific rigor index. According to this method, the relationship between the ATP contents and rigor index of intact fish body would be clarified.

Muscle cell is connected with a neural end and it accepts a neural pulse at the cell membrane. Ca²⁺ is released from sarcoplasmic reticulum by the pulse, and muscle contracts by the elevation of Ca²⁺ concentration. The central
nervous system exists in the spinal cord and it links with peripheral nerves. By nature, the pulse from the central nervous system causes the muscle contraction. As the case of the present study, destruction of the central nervous system might stop the pulse to the muscle fiber, and stop the release of Ca\(^{2+}\) from sarcoplasmic reticulum, and resultantly, muscle contraction was also stopped completely. To study about the detail mechanism, histological observations on the connecting site of neural end and distribution of Ca\(^{2+}\) would be needed.

References


