Freeze Denaturation of Squid Actomyosin

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Denaturation of actomyosin from the obliquely striated mantle muscle of squids (Todarodes pacificus) was studied by measuring the changes in Ca⁺⁺-ATPase activity, relative viscosity, and solubility during frozen storage at three different temperature zones of maximum ice crystal formation (−3°C, −5°C), the eutectic point (−11°C), and −20°C. The logarithms of Ca⁺⁺-ATPase activity, relative viscosity and solubility of the actomyosin solutions (0.6 M KCl) and suspensions (0.05 M KCl) tended to decrease during frozen storage. The denaturation of squid actomyosin at the zone of maximum ice crystal formation significantly differed by only two degree of temperature difference between −3°C and −5°C, and it (0.05 M KCl) at −5°C was less than those of other temperature. The denaturation at −11°C was more rapid than at −5°C. The logarithms of Ca⁺⁺-ATPase activity, relative viscosity, and solubility were changed slower in the suspensions (0.05 M KCl) than the solutions (0.6 M KCl) at all experimental temperatures.

Key words: Squid, Actomyosin, Freeze denaturation of protein

Introduction

Freezing storage is generally known to be an effective method for food preservation. However, food proteins are denatured by freezing and, in particular, fish muscle proteins are more susceptible to freezing than other proteins.

Fukuda et al. (1984) reported that the myofibrillar protein of Chub mackerel was much more denaturated when frozen during post-rigor than during pre-rigor. Okada et al. (1985) and Oguni et al. (1987) reported that the freeze denaturation of carp myosin B in 0.6 M KCl was caused by a concentrated KCl solution in the temperature range from the freezing point to the eutectic point. The apparent rate constants for the inactivation of fish myosin B Ca⁺⁺-ATPase increased as the temperature decreased from −4°C to −10°C, and reached a maximum in the range of −10°C to −1°C (Inoue et al., 1992). Since this temperature range was very close to the eutectic point of KCl (11.1°C), the denaturation of myosin B during frozen storage might be affected by the concentrated KCl solution yielded in the range above the eutectic point. Takatori et al. (1992) reported that the rate constant for inactivation of myosin B Ca⁺⁺-ATPase (KF) was affected by KCl concentration rather than temperature in the range above the eutectic point of the KCl solution. The effect of storage temperature on the freeze denaturation of carp myosfibrils with KCl was studied in the range of −3°C to −30°C and the denaturation increased with a decrease in temperature the −3°C to −13°C, but it decreased with a decrease in the range of −13°C to −30°C (Takahashi et al., 1993). As mentioned above, the freeze denaturation of fish proteins is generally caused by concentrated salts during frozen storage of the same temperature.

In the case of study on squid proteins, Horie et al. (1975) and Takahide et al. (1977, 1978, 1980) prepared actomyosin, actin, myosin, tropomyosin, and paramyosin from squids and reported their physicochemical properties. Ikuo et al. (1980) isolated the highly purified myosin from mantle muscle of squid and investigated its properties.

In the present studies, to characterize the freeze denaturation of squid actomyosin, 0.05 M- and

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0.6 M KCl-squid actomyosins were prepared, and Ca\(^{2+}\)-ATPase activity, relative viscosity, and solubility were measured during frozen storage (−3, −5, −11, and −20°C).

**Materials and Methods**

1. **Materials**
   Mantle muscle of live squid, Todarodes pacificus (weight, 400~450 g; length, 45~50 cm) was used for this experiment. All experiments were carried out 0~4°C unless otherwise indicated.

2. **Preparation of Actomyosin**
   Squid mantle muscles were skinned off both sides according to the actomyosin preparation procedure in Fig.1 (Iguchi et al., 1981). Minced muscles were homogenized with 10 volumes of cold 0.05 M KCl (Tris-maleate buffer, pH 7.0) solution and then washed three times with the previous buffer. After centrifugation (6,500×g), the actomyosin was mixed with 1.5 volumes of cold 1.2 M KCl (tris-maleate buffer, pH 7.0) solution for 60 min with stirring and centrifuged at 6,500×g for 30 min. The supernatant was mixed with 3 volumes of deionized water and centrifuged at 6,500×g for 30 min. The residue was homogenized with 1.4 volume of 1.2 M KCl (tris-maleate buffer, pH 7.0) solution and the actomyosin solution was prepared. The actomyosin suspension was prepared with 0.1M KCl (tris-maleate buffer, pH 7.0) solution. 10 ml portion of the actomyosin solution and suspension (about 5 mg/ml) were frozen to the prescribed temperatures (−3, −5, −11, and −20°C) in a bath of ethanol/dry ice mixture, and were placed in each freezer at the above temperatures. The samples were removed from the freezer at intervals of appropriate days and thawed in room temperature. The thawed actomyosin solution and suspension were homogenized and adjusted to the concentration of protein (3.0~3.5 mg/ml) and subjected to the following analyses.

3. **Protein Determination**
   Protein concentration was determined by Biuret method (Gornall, 1949), standardized by micro-Kjeldahl.

4. **Ca\(^{2+}\)-ATPase activity**
   Ca\(^{2+}\)-ATPase activity was assayed as the method described by Uchiyama et al. (1978).

5. **Measurement of viscosity**
   Viscosity was measured at 20°C with an Ostwald type viscometer using 5.0 ml of sample solution.

<table>
<thead>
<tr>
<th>Minced muscle (500 g)</th>
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<tr>
<td>Homogenize with 10 vol. of 0.05 M KCl- Tris-maleate buffer, pH 7.0</td>
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<tr>
<td>Centrifuge at 6,500×g for 30 min.</td>
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<th>Residue</th>
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<tr>
<td>Homogenize with 1.5 vol. of 1.2 M KCl- Tris-maleate buffer, pH 7.0</td>
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<tr>
<td>by magnetic stirrer for 60 min.</td>
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<td>Centrifuge at 6,500×g for 30 min.</td>
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<th>Supernatant</th>
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<tr>
<td>Add 3 vol. of H2O.</td>
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<td>Centrifuge at 6,500×g for 30 min.</td>
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<th>Residue</th>
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<tr>
<td>Homogenize with 1.2 M KCl (10.7 vol.) by stirrer for 60 min.</td>
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<tr>
<td>Homogenize with 0.1M KCl for 60 min.</td>
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Actomyosin solution Actomyosin suspension

Fig. 1. Preparation of actomyosin from obliquely striated mantle muscle of squid.

6. **Measurement of solubility**
   Solubility was expressed as the ratio of the concentration of soluble protein to that of the original concentration of actomyosin (Jiang, 1987).

**Results and Discussion**

1. **Changes of Ca\(^{2+}\)-ATPase activity**
   The changes of the Ca\(^{2+}\)-ATPase activity of squid actomyosin in 0.6 M KCl solution during frozen storage at each temperature were shown in Fig. 2.

   In the case of −5°C and −11°C frozen storage, the Ca\(^{2+}\)-ATPase activity was markedly decreased and inactivated about 40% and 60%, after 10 days frozen storage, respectively. It suggests that the denaturation of squid actomyosin during frozen storage is affected by the concentrated KCl solution in the range above the eutectic point of KCl. The results in the present study were similar to the freeze denaturation of carp myosin B (Inoue et al., 1992; Takahashi et al., 1993). However, Inoue et al. (1992) reported that the remaining Ca\(^{2+}\)-ATPase activity of 0.6 M KCl-carp myosin B was 30% of the original activity for 24 hours in −11°C, and Okada et al. (1985) reported that the remaining Ca\(^{2+}\)-ATPase activity of 0.6 M KCl-carp myosin B was 40% of the original activity after 3 days at −5°C, which was different to findings in the present study. The facts suggest that the extent of freeze denaturation of squid actomyosin is less than carp myosin B in −5°C and −11°C. In the case of −3°C frozen storage, no significant loss of activity was observed during 7 days and then gradually decreased with the storage. The Ca\(^{2+}\)-ATPase
activity at $-3^\circ C$ frozen storage was much higher than that at $-20^\circ C$ and this result had an agreement with the result that Ca$^{2+}$-ATPase activity of fish myosin B at $-3^\circ C$ storage (partial freezing) was much higher than that at $-30^\circ C$ (Uchiyama, 1978). The Ca$^{2+}$-ATPase activity at the zone of maximum ice crystal formation ($-3^\circ$, $-5^\circ C$) significantly differed by only two degree of temperature difference and this finding suggests that the rate of ice crystal formation is much affected by a little difference in temperature at the zone of maximum ice crystal formation.

The changes of the Ca$^{2+}$-ATPase activity of squid actomyosin in 0.05 M KCl solution during frozen storage at each temperature were shown in Fig. 3. In the case of $-5$ and $-11^\circ C$ frozen storage, Ca$^{2+}$-ATPase activities were markedly decreased and showed about 40% after 10 days.

2. Changes of relative viscosity

The changes of relative viscosity in squid actomyosin in 0.6 M KCl were shown in Fig. 4. All samples were markedly decreased during 7 days and then were not changed. The significant difference between $-5^\circ C$ and $-11^\circ C$ frozen sample was not detected. Decrease of relative viscosity of samples at $-3^\circ C$ and $-20^\circ C$ was less than samples at $-5^\circ C$ and $-11^\circ C$. Oguni et al. (1975) reported that the relative viscosity of carp actomyosin in 0.6 M KCl stored at $-20^\circ C$, decreased to 50% of the original activity after 15 days. In the case of squid actomyosin in this study, relative viscosity was 60% of the original activity in $-20^\circ C$ after 15 days, and it suggests that the decrease of the actomyosin viscosity is less than the carp actomyosin during frozen storage.

The change of the actomyosin suspension in 0.05 M KCl was shown in Fig. 5. When the actomyosin was stored in $-5$ and $-11^\circ C$, its relative viscosities in 0.6 M KCl and 0.05 M KCl were decreased to 1.3 and 1.6 after 4 days, respectively. In the case of $-3^\circ C$ and $-20^\circ C$ storage, the significant difference between 0.6 M KCl and 0.05 M KCl-actomyosin was observed. The relative viscosity depended on the KCl concentration of the actomyosin solution before freezing and the decrease of 0.6 M KCl-actomyosin solution was more rapid than 0.05 M KCl-actomyosin suspension.

3. Changes of solubility.

The changes of solubility of 0.6 M KCl-actomyosin solution were shown in Fig. 6. In the case of samples in $-3^\circ C$ and $-20^\circ C$, the solubility was about 70% after 24 days. Oguni et al. (1975) and Ohnishi et al. (1978) reported that the solubility was about 50% after 25 days when 0.6 M KCl-carp actomyosin was stored at $-20^\circ C$. It is different from the present study. In $-5^\circ C$ and $-11^\circ C$, the solubility was markedly decreased until 10 days,
Freeze Denaturation of Squid Actomyosin

Fig. 4. Changes in the relative viscosity of squid actomyosin stored at 0.6 M KCl.

Fig. 5. Changes in the relative viscosity of squid actomyosin stored at 0.05 M KCl.

and then, any difference of solubility between samples was not detected.

The change of solubility in 0.05 M KCl-actomyosin suspension was presented in Fig. 7. The solubility of 0.05 M KCl-actomyosin suspension stored at –5°C and –11°C was similar to 0.6 M KCl-

actomyosin solution. In the case of stored at –3°C, the decrease of solubility was much slowed until 10 days, the decrease of solubility stored at –20°C was shown to 7 days and then much slowed. In the solubility, the decrease of 0.6 M KCl-actomyosin
solution was more rapid than 0.05 M KCl-actomyosin suspension as Ca^{2+}-ATPase activity and relative viscosity.

Conclusion

Denaturation of actomyosin solution (0.6 M KCl) and suspension (0.05 M KCl) from obliquely striated mantle muscle of squid was investigated by measuring the changes in Ca^{2+}-ATPase activity, relative viscosity, and solubility during the frozen storage at -3, -5, -11, and -20°C.

In the case of 0.6 M KCl-squid actomyosin solution, the Ca^{2+}-ATPase activity in -3 and -20°C was slowly decreased and about 10% was decreased in the sample of storage in -3°C after 10 days. The activities of samples in -5°C and -11°C were remarkably decreased during the initial storage period, so 60% of the initial activity was inactivated at -11°C after 10 days. The Ca^{2+}-ATPase activity of 0.05 M KCl-actomyosin suspension stored at -5°C and -11°C was inactivated about 30-40% after 10 days, and the activity of the samples at -3°C and -20°C was inactivated about 5%.

The relative viscosity of 0.6 M KCl-actomyosin solution was prominently decreased in the initial stage of storage, regardless of storage temperature. The samples stored at -5°C and -11°C were markedly dropped to 45% and then regularly maintained.

References


