Thermostability and Freeze Denaturation of Grass Prawn (*Penaeus monodon*) Muscle Proteins

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The thermostability of actomyosin (AM) and freeze denaturation of muscle proteins from grass prawn were investigated. A comparison of the inactivation rate constant (K_D) of AM Ca-ATPase at 0-45 °C showed that thermostability of AM decreased with an increase of incubation temperature. From the Arrhenius plot of K_D, the AM at zone I (0-25 °C) was more stable than at zone II (25-45 °C). The AM was stable around pH 7.6, which was almost the same as for other shellfishes. The stability of K_D at 35 °C of this species was lower than that of milkfish, tilapia hybrid, tilapia, and carp. Except for the extractable AM, the total SH and Ca-ATPase activity of AM decreased significantly immediately after freezing and further decreased during 6 months of frozen storage at -10, -20, -30, and -40 °C ($p < 0.01$). The K_D lowered as frozen storage temperature decreased; however, there was no significant difference between -30 and -40 °C ($p > 0.01$).

INTRODUCTION

Grass prawn, also known as black tiger, is an important aquatic shellfish in southeast Asia and also an important exportation commodity in Mainland China, Taiwan, and other Asian countries. In addition to the 5% of the total grass prawn which is exported in the live state, about 95% is exported frozen to Japan, the United States, and other countries (Liau, 1987). However, frequent complaints of decomposition, discoloration, and drip loss (resulting in short weight) from importing countries are made. The discoloration is due to the enzymatic oxidation of tyrosine and tyrosine-like components in the presence of oxygen and tyrosinase to produce a pigment, melanin. The drip loss may be due to the aggregation of myofibrillar proteins during frozen storage (Jiang, 1977; Jiang and Lee, 1985; Jiang et al., 1987a-c; Kurokawa, 1979; Matsumoto, 1979, 1980; Noguchi, 1982).

The myosin ATPase activity is a widely used index to evaluate the stability of muscle proteins during icing, freezing, and subsequent storage (Arai, 1974; Arai and Takashi, 1977). By measurement of the changes in Ca-ATPase activity, rabbit and chicken were found to be more stable than fish (Arai and Takashi, 1977). In addition, the thermostability of fish proteins increases with the increase of habitat temperatures and varies with species and storage conditions (Arai, 1974; Arai and Takashi, 1977; Connell, 1961; Seki, 1977; Suyama and Konosu, 1987; Suzuki, 1981). Although the stability of muscle proteins from subtropical and tropical fishes is well studied (Jiang, 1977, 1988; Seki, 1977; Tsai et al., 1989), that of grass prawn proteins is still lacking. Accordingly, the aim of this study was to investigate the thermostability and freeze denaturation of grass prawn muscle proteins. The results will consequently provide basic functionality information for studies on the storage and processing of this species.

MATERIALS AND METHODS

Grass prawns (*Penaeus monodon*) (50-60 shrimp/kg; reared for 4 months) were caught from an aquatic farm and transported immediately to the laboratory by oxygen-pumping tank. After the head and shell were removed, the peeled prawns were used to extract the actomyosin (AM).

Preparation of AM. AM was extracted according to the method of Noguchi and Matsumoto (1970) and suspended in 0.05 M KCl solution (pH 7.2).

Determination of the Inactivation Rate Constant (K_D) of AM at Various Temperatures. AM (1.0-5.0 mg/mL, pH 7.2) was incubated at various temperatures (0, 10, 20, 30, 35, 40, and 45 °C). At definite time intervals, all AMs were iced for 5 min and then placed in a 25 °C water bath for 5 min to elevate the temperature. The Ca-ATPase activity was then determined according to the method of Arai (1974).

Determination of the Inactivation Rate Constant (K_D) of AM at Various pHs. AMs with different pHs (5.4-9.4) were prepared by using 0.1 M Tris-maleate buffer (pH 5.4-8.4, adjusted using 0.1 M NaOH) and 0.1 M glycine-NaOH buffer (pH 8.8 and 9.4) and then incubated at 25 °C. At definite time intervals, the Ca-ATPase activity was measured; the inactivation rate constant (K_D) of AM Ca-ATPase was then calculated.

Ca-ATPase Activity. To a 1-mL AM solution (1-5 mg/mL) were added 0.5 mL of 0.5 M Tris-maleate buffer (pH 7.0), 0.5 mL of 0.1 M $CaCl_2$, 7.5 mL of deionized water, and 0.5 mL of 20 mM adenosine 5'-triphosphate solution (ATP, Sigma grade, pH 7.0). After ATP was added, the rates of release of inorganic phosphate at 25 °C for 1, 2, and 3 min of reaction time were measured to determine the Ca-ATPase activity. Five milliliters of 15% trichloroacetic acid was added to stop the reaction; the quantity of releasing inorganic phosphate was determined according to the method of Arai (1974). The Ca-ATPase specific activity was defined as micromoles of inorganic phosphate liberated per milligram of protein within 1 min of reaction at 25 °C. The inactivation rate constant (K_D) of AM Ca-ATPase was calculated according to the method of Arai (1974), i.e., $K_D = (\ln c_0 - \ln c_t)/t$, where c_0 is the Ca-ATPase activity before incubation, c_t is the Ca-ATPase activity after t s of incubation, and t is the incubation time (s).

Denaturation of Muscle Protein of Frozen Grass Prawn at Various Temperatures. Headless grass prawns were frozen to a body temperature below -18 °C using an air blast freezer (wind velocity, 3.0 m/s; temperature, -40 °C). The resulting samples were then stored at -10, -20, -30, and -40 °C for 6 months. At 2-month intervals, samples were removed and thawed to 0 °C (measured using a microcomputer thermometer, Model 7001 CH, Jenco Electronic Co. Ltd., Taiwan) with running tap water (about 25 °C). AM was extracted. The Ca-ATPase activity was measured. The protein concentration was determined using Biuret method modified by Umemoto (1966). Extractability of

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Table I. Effect of the Incubation Temperatures on the Inactivation Rate Constant (K_D)[a] of Actomyosin Ca-ATPase of Grass Prawn, Compared with That of Milkfish, Hybrid Tilapia, Tilapia, and Carp

	$K_D \times 10^5$, s^{-1}, at temp of						
	0 °C	10 °C	20 °C	30 °C	35 °C	40 °C	45 °C
grass prawn	1.04	1.38	2.72	10.97	34.37	166.8	206.9
milkfish[b]	0.52	0.84	1.23	7.08	18.0	41.3	140
hybrid tilapia[b]	0.36	0.76	1.55	3.06	4.12	28.1	96.7
tilapia[b]	0.45	0.89	1.78	5.12	8.15	37.5	124
carp[b]	0.40	0.83	1.47	3.11	10.1	58.6	170

[a] $K_D = (\ln C_0 - \ln C_t/t$, where C_0 is the Ca-ATPase specific activity before incubation, C_t is the Ca-ATPase specific activity after t s of incubation, and t is the incubation time (s). Concentration of actomyosin, 1.0–5.0 mg/mL; pH 7.2. Means of three determinations from each sample were used to calculate the K_D values. [b] Data from Tsai et al. (1988).

AM was expressed as milligrams of extractable actomyosin per gram of fish meat.

Determination of the Total SH of AM. The total SH of AM was determined according to the method of Buttkus (1971). To 1.0 mL of AM solution (5–10 mg/mL) was added 9 mL of chilled solution (mixture of 50 mM KH_2PO_4–K_2HPO_4, 6 mM ethylenediaminetetraacetic acid, 0.6 M KCl, and 8 M urea, pH 8.0), and the mixture was stirred for 30 min at 25 °C; 0.02 mL of 0.01 M 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) was added to 3 mL of the mixture, and the mixture was incubated at 40 °C for 15 min. The absorbance at 412 nm was measured to calculate the total SH according to the method of Ellman (1959).

Statistical Analysis. All measurements from each sample were conducted in triplicate. The SAS statistical program adapted to the IBM-PC 386 computer was used to perform ANOVA using Duncan's multiple range test for significance (*SAS Statistics Guide for Personal Computers*, Release 6.03, 1988). Significance was noted at $p < 0.01$.

RESULTS AND DISCUSSION

Thermostability of the Grass Prawn AM. The thermostability of AM is related to the stability of the frozen fish protein. Thus, this property is frequently used to evaluate the tolerance of fish muscle to freezing and subsequent storage. K_D values of AM and myosin Ca-ATPase activities were usually used (Suzuki, 1981; Seki, 1977). The Ca-ATPase activity decreased linearly with incubation time, and the slopes increased as incubation temperature increased (data not shown). In this study, the K_D values of grass prawn AM increased with increased incubation temperatures. Grass prawn AM at 0–45 °C was less stable than that of milkfish, tilapia, tilapia hybrid, and carp (Table I). The K_D of grass prawn AM Ca-ATPase increased markedly when the incubation temperatures were higher than 30 °C (Table I) (Tsai et al., 1989). These results suggested that the thermostability of myofibrillar proteins varied with the species and might be related to the environmental living conditions.

From the Arrhenius plot (Figure 1), the stability of AM could be divided into two zones: zone I, 0–25 °C; zone II, 25–45 °C. The increasing rate of the K_D with the increase of incubation temperature was higher at zone II than at zone I. The activation energy (E_a) of zone I was 76.2 kcal/mol, while that of zone II was 37.8 kcal/mol. This phenomenon suggests that the fresh storage temperature during handling should always be, at least, lower than 25 °C.

Effect of pH on the Thermostability of AM. The AM of grass prawn at 25 °C was most stable at pH 7.6 (Table II), which was almost the same as that of other shellfish (Suyama and Konosu, 1987). The thermostability of AM is highly related to the stability of frozen fish proteins and frequently used to evaluate the freezing

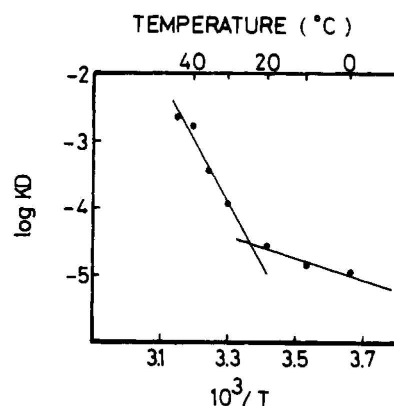

Figure 1. Arrhenius plot of K_D vs actomyosin Ca-ATPase.

Table II. Effect of pH on the Inactivation Rate Constant (K_D)[a] of Actomyosin Ca-ATPase of Grass Prawn

	pH					
	5.4	6.3	7.6	8.2	8.8	9.4
$K_D \times 10^5$, s^{-1}	20.3	7.78	3.06	3.21	15.0	20.6

[a] Refer to the footnote of Table I. Concentration of actomyosin, 1.0–5.0 mg/mL; incubation temperature; 25 °C. Means of three determinations from each sample were used to calculate the K_D values.

Table III. Effect of Storage Temperature on the Extractable Actomyosin of Grass Prawn

storage temp, °C	unfrozen	storage time, months			
		0	2	4	6
−10	93.8aA (100.0)	92.3aA (98.4)	50.2cB (53.5)	42.1cC (44.9)	38.6dC (41.2)
−20	93.8aA (100.0)	92.3aA (98.4)	72.3bB (77.1)	68.5bC (73.0)	67.0cC (71.4)
−30	93.8aA (100.0)	92.3aA (98.4)	84.5aB (90.1)	80.8aC (86.1)	78.5bC (83.4)
−40	93.8aA (100.0)	92.3aA (98.4)	83.8aB (89.3)	81.2aC (86.6)	81.0aC (86.4)

[a] The extractable AM is expressed as milligrams of extractable AM per gram of meat. Three determinations from each sample were used for Duncan's multiple range test. Values in the same column bearing different lower-case letters differ significantly ($p < 0.01$). Values in the same row bearing different upper-case letters differ significantly ($p < 0.01$). Values in parentheses are the percent ratio relative to the unfrozen sample.

tolerance of fish muscle (Suzuki, 1981; Seki, 1977). According to Tsai et al. (1989), the stability of milkfish, tilapia, and carp AM at pH 7.0 around 0–45 °C was highly related to that at frozen temperatures (−10 to −40 °C). Therefore, when prawn muscle is in the neutral or near alkaline pH region, it might be more stable against freezing and subsequent storage. The data obtained support the study by Jiang et al. (1990), who reported that a phosphate immersion treatment before freezing decreased the drip loss and improved the textural quality of frozen grass prawn. The phosphate immersion treatment not only increased the water holding capacity but elevated the pH of the muscle into the desired region.

Effect of Frozen Storage Temperatures on the AM of Grass Prawn. *Change in the Extractable AM.* The freezing speed, duration, and temperature of storage are the most important factors affecting the protein quality of seafoods (Bito, 1976; Fukuda, 1986; Love, 1962a,b). Immediately after freezing, no significant change in the extractable AM was observed ($p > 0.01$) (Table III). During storage, extractable AM decreased with storage time; the decrease was highest at −10 °C and lowest at −40 °C. However, there was no significant difference between

Table IV. Effect of Storage Temperature on the Total SH[a] of Grass Prawn Actomyosin

storage temp, °C	unfrozen	storage time, months			
		0	2	4	6
−10	53.4aA (100.0)	46.8aB (87.6)	30.2cC (56.6)	28.4cC (53.2)	28.2cC (52.8)
−20	53.4aA (100.0)	46.8aB (87.6)	40.1bC (75.1)	36.7bD (68.7)	33.4bE (62.5)
−30	53.4aA (100.0)	46.8aB (87.6)	44.0aC (82.4)	41.8aD (78.2)	39.2aE (73.4)
−40	53.4aA (100.0)	46.8aB (87.6)	43.4aC (81.3)	40.6aD (76.0)	40.3aE (75.5)

[a] The total SHs are expressed as moles per 5×10^5 mg of protein. Three determinations from each sample were used for Duncan's multiple range test. Values in the same column bearing different lower-case letters differ significantly ($p < 0.01$). Values in the same row bearing different upper-case letters differ significantly ($p < 0.01$). Values in parentheses are the percent ratio relative to the unfrozen sample.

Table V. Effect of Storage Temperature on the Ca-ATPase Activity[a] of Grass Prawn Actomyosin

storage temp, °C	unfrozen	storage time, months				$K_D \times 10^3$, day^{-1}
		0	2	4	6	
−10	1.443aA (100.0)	1.082aB (75.0)	0.521cC (36.1)	0.503cC (34.9)	0.341cD (23.6)	5.28
−20	1.443aA (100.0)	1.082aB (75.0)	0.782bC (54.2)	0.705bD (48.9)	0.608bE (42.1)	2.91
−30	1.443aA (100.0)	1.082aB (75.0)	0.981aC (68.0)	0.890aD (61.7)	0.854aD (59.2)	1.30
−40	1.443aA (100.0)	1.082aB (75.0)	0.986aC (68.3)	0.890aD (61.7)	0.866aD (60.0)	1.28

[a] The Ca-ATPase activity is expressed as micromoles of inorganic phosphate released within 1 min of reaction at 25 °C per milligram of protein. $K_D = (\ln C_0 - \ln C_t)/t$, where C_0 is the Ca-ATPase specific activity at zero day, C_t is the Ca-ATPase specific activity after t days of storage, and t is the storage time (days). Values in the same column bearing different lower-case letters differ significantly ($p < 0.01$). Values in the same row bearing different upper-case letters differ significantly ($p < 0.01$). Values in parentheses are the percent ratio relative to the unfrozen sample.

samples stored at −30 and −40 °C during the first 4 months of storage ($p > 0.01$).

Change in the Total SH of AM. The total SH of all samples decreased significantly after freezing and further decreased during 6 months of storage ($p > 0.01$, Table IV). The decrease in extractable AM (Table III) reflected the aggregation of AM. The decrease in total SH might be because of the formation of disulfides on extractable AM molecules during freezing, subsequent storage, and/or extraction. This might also be because the aggregation of AM, even that which was still extractable, led to steric hindrance of the DTNB binding to SH groups and interfered with the measurement of total SH. No significant difference was obtained between the samples stored at −30 and −40 °C ($p > 0.01$).

Change in Ca-ATPase Activity. The Ca-ATPase activity of extracted AM decreased significantly after freezing and further decreased during storage ($p < 0.01$, Table V). However, no significant difference in Ca-ATPase activity of samples stored at −30 and −40 °C was observed ($p > 0.01$). The change of Ca-ATPase activity of all samples was almost concordant with the change of total SH. Using 48 sets of total SH and Ca-ATPase activity data (Tables IV and V) to perform linear regression analysis, the correlation coefficient (r) was 0.98; the linear regression equation was $y = -0.5742 + 0.0354x$, where x and y represent the total SH and Ca-ATPase activity, respectively. The decrease in Ca-ATPase was also reported to be correlated to the oxidation of SH (Buttkus, 1971; Hamada et al., 1977). Accordingly, the decrease in total SH during storage (Table IV) indicated that the loss of Ca-ATPase activity might be due to the oxidation of SH on the active site of AM.

Recently, Matsumoto et al. (1985) studied the change in myofibrillar protein Ca-ATPase of mackerel during storage at −15, −20, −25, −30, and −40 °C and found a linear relation between Ca-ATPase activity and storage time. Moreover, they compared the K_D of AM Ca-ATPase at different storage temperatures and concluded that the K_D of AM Ca-ATPase was a good index for evaluating the protein stability of frozen fish (Fukuda, 1986; Matsumoto et al., 1985). The K_D values of grass prawn AM increased with increased storage temperature (Table V). Compared with other species stored at −20 °C, the K_D value of this species was higher than that of tilapia hybrid (1.16×10^{-3} day^{-1}), tilapia (1.08×10^{-3} day^{-1}), and carp (1.53×10^{-3} day^{-1}) and almost the same as that of milkfish (2.66×10^{-3} day^{-1}) (Tsai et al., 1989).

In summary, during frozen storage, a decrease in the total SH of grass prawn AM suggested the formation of disulfides occurred. The decreases in extractable AM, total SH, and Ca-ATPase activity lowered as frozen storage temperature decreased.

ACKNOWLEDGMENT

This research was supported by the American Institute in Taiwan (AIT) under Grant FG-Ta-120 (TW-AES-27).

LITERATURE CITED

Arai, K. Evaluation of fish quality from the muscle protein studies. In *Sakana no Hinshitsu* (*Quality of Fish*); Koseisha, Ed.; Japanese Society of Scientific Fisheries: Tokyo, 1974; pp 55–80.

Arai, K.; Takashi, R. Studies on muscular proteins of fish. XI. Effect of freezing on denaturation of actomyosin ATPase from carp muscle. *Bull. Jpn. Soc. Sci. Fish.* **1977**, *43*, 533–541.

Bito, M. Studies on the retention of meat color of frozen tuna. *Bull. Tokai Reg. Fish. Res. Lab.* **1976**, *84*, 51–113.

Buttkus, H. The sulfhydryl content of rabbit and trout myosins in relation to protein stability. *Can. J. Biochem.* **1971**, *49*, 97–102.

Connell, J. J. The relative stabilities of the skeletal muscle myosins of some animals. *Biochem. J.* **1961**, *80*, 503–509.

Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70–75.

Fukuda, Y. Effect of freezing conditions on quality of frozen fish meat. *Refrigeration* (*Japanese*) **1986**, *61*, 18–29.

Hamada, I.; Tsuji, K.; Nakayama, T.; Niwa, E. Oxidative denaturation of actomyosin. *Bull. Jpn. Soc. Sci. Fish.* **1977**, *43*, 1105–1111.

Jiang, S. T. Studies on the denaturation of mullet muscle proteins during frozen storage. *Refrigeration* (*Japanese*) **1977**, *52*, 621–629.

Jiang, S. T.; Lee, T. C. Changes in free amino acids and protein denaturation of fish muscle during frozen storage. *J. Agric. Food Chem.* **1985**, *33*, 839–844.

Jiang, S. T.; Tsao, C. Y.; Lee, T. C. Effect of free amino acids on the denaturation of mackerel myofibrillar proteins in vitro during frozen storage. *J. Agric. Food Chem.* **1987a**, *35*, 28–33.

Jiang, S. T.; Hwang, B. S.; Tsao, C. Y. Protein denaturation and changes in nucleotides of fish muscle during frozen storage. *J. Agric. Food Chem.* **1987b**, *35*, 22–27.

Jiang, S. T.; Hwang, B. S.; Tsao, C. Y. Effect of adenosine-nucleotides and their derivatives on the denaturation of myofibrillar proteins in vitro during frozen storage at −20 °C. *J. Food Sci.* **1987c**, *52*, 117–123.

Jiang, S. T.; Hwang, D. C.; Chen, C. S. Denaturation and change in SH group of actomyosin from milkfish (*Chanos chanos*) during storage at -20 °C. *J. Agric. Food Chem.* **1988**, *36*, 433–437.

Jiang, S. T.; Moody, M. W.; Chen, H. C. Effect of pretreatments and freezing conditions on the quality of grass shrimp (*Penaeus monodon*). Presented at the Meeting of the International Institute of Refrigeration Commission C2 on "Chilling and Freezing of New Fish Products", Sept 18–20, 1990, Aberdeen, U.K.

Kurokawa, T. Kamaboko-forming ability of frozen and ice stored lizard fish. *Bull. Jpn. Soc. Sci. Fish.* **1979**, *45*, 1551–1556.

Liau, C. S. The trading of fishery products of Taiwan. *China Fish. Mon.* **1987**, *412*, 11–19.

Love, R. M. Protein denaturation in frozen fish VI. Cold storage studies on cod using the cell fragility method. *J. Sci. Food Agric.* **1962a**, *13*, 269–273.

Love, R. M. Protein denaturation in frozen fish VII. Effect of the onset and resolution of rigor mortis on denaturation. *J. Sci. Food Agric.* **1962b**, *13*, 534–539.

Matsumoto, J. J. Denaturation of fish muscle proteins during frozen storage. In *Proteins at Low Temperature*; Fennema, O., Ed.; ACS Symposium Series 180; American Chemical Society: Washington, DC, 1979; pp 205–312.

Matsumoto, J. J. Chemical deterioration of muscle proteins during frozen storage. In *Chemical Deterioration of Proteins*; Whitaker, J., Fujimaki, M., Eds.; ACS Symposium Series 123; American Chemical Society: Washington, DC, 1980; pp 95–109.

Matsumoto, I.; Ooizumi, T.; Arai, K. Protective effect of sugar on freeze-denaturation of carp myofybrillar proteins. *Bull. Jpn. Soc. Sci. Fish.* **1985**, *51*, 833–840.

Noguchi, S. Characteristics of fish proteins from processing point of view II. Protein denaturation of frozen fish muscle. *New Food Ind. (Japanese)* **1982**, *21*, 34–42.

Noguchi, S.; Matsumoto, J. J. Studies on the control of the denaturation of fish muscle proteins during frozen storage. *Bull. Jpn. Soc. Sci. Fish.* **1970**, *36*, 1078–1081.

Seki, N. Extraction of fish muscle proteins. In *Fish Proteins (Gyoniku Tanpakushitsu)*; Koseisha, Ed.; Japanese Society of Scientific Fisheries: Tokyo, 1977; pp 7–23.

Suyama, M.; Konosu, M. Functionality changes of meat during storage and processing. In *Marine Food (Suisan Shokuhin Gaku (Japanese)*; Koseisha, Ed.; Japanese Society of Scientific Fisheries: Tokyo, 1987; pp 168–202.

Suzuki, T. Characteristics of fish meat and fish protein. In *Fish and Krill Proteins, Processing Technology*; Applied Science Publishers: London, 1981; pp 1–45.

Tsai, S. F.; Wang, J. H.; Jiang, S. T. The stability of muscle proteins from some subtropic fish. *J. Agric. Food Chem.* **1989**, *37*, 749–752.

Umemoto, S. A modified method for estimation of fish muscle protein by Biuret method. *Bull. Jpn. Soc. Sci. Fish.* **1966**, *32*, 427–435.

Received for review September 11, 1990. Revised manuscript received April 15, 1991. Accepted August 13, 1991.

Registry No. ATPase, 9000-83-3.