Food Sci. Technol. Res., **8** (3), 200–206, 2002

Effect of Protein Hydrolysate from Antarctic Krill on the State of Water and Denaturation of Lizard Fish Myofibrils during Frozen Storage

Nong ZHANG[1], Yasumitsu YAMASHITA[1] and Yukinori NOZAKI[2]*

[1]*Graduate School of Marine Science and Engineering, and* [2]*Faculty of Fisheries, Nagasaki University, Bunkyo, Nagasaki 852-8521, Japan*

Received May 24, 2001; Accepted April 17, 2002

Protein hydrolysates were prepared from Antarctic krill and two types of shrimp by enzymatic treatment using protease. Hydrolysates prepared from the krill were added to lizard fish myofibrils, and changes in the amount of unfrozen water in myofibrils during freezing were analyzed by differential scanning calorimetry. Ca-ATPase activity of myofibrils was measured concurrently and the results were compared with those using hydrolysates from shrimp. The amount of unfrozen water increased after addition of the hydrolysates and decreased moderately during frozen storage. When hydrolysates were not added to myofibrils, the amount of water rapidly decreased during frozen storage. The decrease in ATPase activity during frozen storage followed that of unfrozen water, indicating a close correlation between ATPase activity and the amount of unfrozen water. These results suggest that the denaturation of myofibrils may be suppressed by the addition of hydrolysates, since the hydrolysates appeared to increase the amount of unfrozen water.

Keywords: krill, myofibrils, ATPase, hydrolysate, freeze-denaturation, unfrozen water, frozen storage

The use of Antarctic krill as a source of marine protein has been proposed due to its high harvest potential. Krill chemical constituents (Hansen & Meiklen, 1970; Sidhu *et al*., 1970), proteinase (Suzuki, 1981), protein concentrates (Yanase, 1979; Kuwano & Mitamura, 1977), nutritive value (Arai *et al*., 1976; Watanabe *et al*., 1976) and safety as a food (Watanabe *et al*., 1976) have been studied previously. However, the use of Antarctic krill is presently limited to meat extracts, dried products, fishing bait, aquaculture feed, and material for carotenoid pigments.

Fisheries products are generally easily putrefied by microbial proliferation and action at ambient temperatures due to their (products) high water and nutrient content. In addition, fish bodies are labile because the content of substrate proteins is relatively low; they are prone to physical, chemical, and enzymatic damage and hence, frozen storage is widely used for fish. However, because water in fish is closely related to protein structure maintenance and functions (Kauzman, 1959; Nemethy & Scheraga, 1962; Kavanau, 1965), the quality of fish is markedly affected by freezing, resulting in a drop in water-holding capacity, which renders some of the proteins insoluble (Migita *et al*., 1956, 1960; Suzuki, 1971; Niwa *et al*., 1987). To prevent such unwanted changes, addition of denaturation-inhibiting substances such as sugar, amino acids, organic acids, and phosphate is common (Hanafusa, 1973; Akiba, 1973; Matsuda, 1973; Matsumoto *et al*., 1985; Nozaki *et al*., 1991, 1993). Due to a growing interest in healthier and more natural lifestyles in recent years, studies are being conducted on improvements in the quality and function (e.g., biological defense, delay of senescence.) of food. Demand for natural substances with these qualities has also increased. Possible uses and functions of enzymatic hydrolysates from various foods have recently generated attention, and the use of fishery products (Suetsuna *et al*., 1988; Iwamoto *et al*., 1991; Sugiyama *et al*., 1991; Yokoyama *et al*., 1992), residues from seafood processing (Miyake, 1982), and fish testes (Murata *et al*., 1991) have been studied. In particular, enzymatic hydrolysates from seafood processing residues, which at present are not effectively utilized, have the potential to be used without waste, thereby increasing their value. Few studies however, have focused on the use of enzymatic hydrolysates for food, and more research is expected in this field.

The purpose of the present study was to determine the usefulness of Antarctic krill enzymatic hydrolysates as anti-freeze-denaturation substances for protein.

Materials and Methods

Materials Frozen Antarctic krill (*Euphausia superba)* reared by Maruha Inc. (mean body length, 6.15 cm; mean body weight, 0.75 g) was used in the experiments. Fresh Kuruma prawns (*Penaeus japonicus,* mean body length, 21.43 cm; mean body weight, 62.67 g) and fresh Tora velvet shrimps (*Metapenaeopsis acclivis,* mean body length, 7.22 cm; mean body weight, 2.03 g) were purchased at a Nagasaki fish market.

Preparation of enzymatic hydrolysates The enzymatic hydrolysates from the samples were prepared according to the method of Iwamoto *et al.* (1991). Distilled water was added twice to the sample (whole status), which was then heated at 90°C for 30 min to inactivate endogenous hydrolyzing enzymes. After homogenizing the sample, pH was adjusted to 8.0 with sodium hydrogen carbonate, and endo-type protease derived from *Bacillus subtilis* (Shin-Nihon Chemical Industries Inc.) was added at 0.1% (wet weight) to the samples. Enzymatic hydrolysis was performed at 60°C for 2 h and the reaction was terminated by heating at 90°C for 30 min. The pH was then adjusted

*To whom correspondence should be addressed.
E-mail: nozaki-y@net.nagasaki-u.ac.jp

to 6.0 by adding DL-malic acid, and exo-type protease derived from *Aspergillus* (Shin-Nihon Chemical Industries Inc.) was added at 0.1% (wet weight) to the samples. Enzymatic hydrolysis was performed at 60°C for 2 h and the reaction was terminated by heating at 90°C for 30 min. After filtering of the hydrolysate, the filtrate was heated to 80°C for 10 min and the lipid layer was removed from the surface. After cooling, the hydrolysate was subjected to ultrafiltration to remove products above a molecular weight of 30,000. After adjusting the pH to 1.0 with 10% HCl, the hydrolysate was desalted using a desalting apparatus (Model G3, Asahi Kasei Inc.). This step was repeated after adjusting the pH to 7.0 with 1 M NaOH, and the hydrolysate was subjected to spray drying (Model GA32, Yamato Scientific Inc.) to obtain a dried product.

Components, molecular weight distribution, and amino acid composition of hydrolysate Water content, total protein content, total lipids, ash content, and sugar content were determined using atmospheric pressure heating and drying (105°C), Kjeldahl, Soxhlet, heating (550°C), and phenol-sulfuric acid, respectively. The salt content was determined using a salt analyzer (Model SAT-2A, Toa Denpa Inc.).

Molecular weight distribution of the hydrolysate was determined by measuring absorbance at 220 nm for each fraction eluting from Sephadex G-25 column chromatography (inner diameter 2.2 cm, length 60 cm).

Amino acid analysis was performed using post-label (ninhydrine). Automated amino acid analysis grade (6 M) HCl was added to the sample and the hydrolysis reaction proceeded at 110°C for 20 h. The product was then analyzed by HPLC (Model JLC-300, Nihon Electronic Industries Inc.).

Preparation of myofibrils and frozen-storage Myofibrils were prepared according to the method of Katoh *et al*. (1977) with slight modification as described below. Fresh lizard fish muscles were cut into thin sections and washed with five volumes of 0.1 M KCl-20 mM Tris-maleate buffer (pH 7.0) three times with stirring. After addition of three volumes of the same buffer, the specimens were homogenized in a foam preventive type blender (Mitsubishi JM-H131) at 10,000 rpm for 90 s and passed through a nylon net (#16) to remove connective tissues. Subsequently, 20% Triton X-100 solution was added to obtain a final concentration of 1%, and the specimens were allowed to stand for 30 min followed by centrifugation at 750×*g* for 10 min. The sediment was mixed with five volumes of the same buffer, stirred, centrifuged again, and washed. This procedure was repeated four times. To remove as much buffer-derived KCl and other buffer compounds as possible from the sediment, the latter was mixed with five volumes of cold distilled water, washed by stirring, and centrifuged at 5000×*g* for 10 min. The supernatant was then removed. To remove excess water, the specimens were centrifuged again (27,000×*g*, 20 min) and the sediment obtained was retained as the myofibril samples. All procedures were performed at about 5°C. The myofibril samples were thus composed of water (87.1%), crude protein (12.4%), crude lipid (0.04%), and crude ash (0.43%).

Hydrolysate was added to 100 g of myofibrils (moisture content, 87.1%) at 2.5–12.5% of dry-weight and the pH was adjusted to 7.0 using 0.01 M NaOH or 0.01 M HCl. After mixing at 5°C for 20 min, about one gram of the sample was sealed in a microtube (inner diameter 8 mm, length 45 mm) and stored at –25°C. For the control, myofibrils without hydrolysate were identically processed and stored.

Measurement of the Ca-ATPase activity of myofibrils Myofibrillar Ca-ATPase activity was measured according to the method of Arai *et al*. (1970) to evaluate the denaturation of myofibrils during frozen storage. The myofibril samples were taken out of the freezer after various days of storage, thawed in a cold room at about 5°C, homogenized in 30 parts of 0.1 M KCl-20 mM Tris-maleate buffer (pH 7.0), and centrifuged at 750×*g* for 10 min. The sediment was mixed with the same buffer, washed by stirring, and centrifuged at 750×*g* for 10 min. This procedure was repeated twice. The sediment obtained was suspended in the same buffer, and myofibrillar Ca-ATPase activity was measured using the method described below. After reacting at 25°C with a final concentration of 100 mM KCl, 5 mM $CaCl_2$, 25 mM Tris-maleate (pH 7.0), 1 mM ATP, and 0.2–0.4 mg/ml of suspended myofibrils, the reaction was terminated by adding 30% trichloroacetic acid solution to a final concentration of 5%, and free inorganic phosphate was measured by colorimetry (Katoh *et al*., 1977). Protein concentration was measured by the biuret method (Gornall *et al*., 1949) using bovine serum albumin (fraction V) as the standard. The weight of the bovine serum albumin was corrected using the Kjeldahl method. The Ca-ATPase activity of the frozen myofibril samples was expressed as the ratio of the specific activity before freeze-storage (relative (%)). The rate constant of freeze-denaturation of myofibrillar Ca-ATPase (k_D) was obtained using the following formula (Matsumoto *et al*., 1985), $k_D=(\ln C_o-\ln C_t)/t$, where C_o and C_t denote the relative activity of myofibrillar Ca-ATPase before and t days after the storage, respectively.

Measurement of unfrozen water by differential scanning thermal analysis The amount of unfrozen water was determined using the method of Wakamatsu and Sato (1979) by a differential scanning calorimeter (DSC-100, Seiko Electronics Inc.). The hydrolysate-added myofibril samples were taken out of the freezer daily, and after thawing at 5°C for 30 min about 20 mg of the sample was placed in a tightly sealed aluminum container. After accurate determination of weight, heat of fusion was measured using a starting temperature of –40°C, a final temperature of 25°C, and a temperature increase rate of 1.0°C/min. The amount of water contained in the samples was determined by the heat-drying method (105°C) at atmospheric pressure. Assuming that the heat of fusion was due to the free water contained in the sample, the amount of free water was subtracted from the amount of water in the sample to obtain the amount of unfrozen water, which was expressed as the per-unit-dry-weight of the sample.

Results

Properties of hydrolysate The major component of the hydrolysate was protein, which accounted for 93% in Kuruma prawns, and 84–86% in the other two species. The amino acid composition of the hydrolysates prepared from the specimens is summarized in Table 1. Glutamic acid + glutamine (acidic amino acid) was the most abundant in all specimens (8.56, 12.35, 13.26 g/100 g dried matter in Antarctic krill, Kuruma prawns, and Tora velvet shrimps, respectively), and was followed by the acidic amino acid aspartic acid + asparagine (about 7 g/100 g dried matter). Arginine and lysine (basic amino acids), glycine, ala-

Table 1. Amino acid composition and crude protein of the various hydrolysates (g/100 g of dried matter)

Amino acid	Antarctic krill	Kuruma prawn	Tora velvet shrimp
Acidic amino acids			
Aspartic acid+Asparagine	6.12	7.36	7.67
Glutamic acid+Glutamine	8.56	12.35	13.26
Basic amino acids			
Arginine	4.85	6.31	6.90
Lysine	3.44	8.47	8.41
Histidine	2.40	2.59	1.40
Neutral amino acids			
Glycine	3.66	5.36	10.54
Alanine	3.72	4.99	4.22
Serine	2.30	3.25	2.68
Threonine	1.67	2.34	2.52
Valine	1.16	1.87	2.25
Leucine	3.37	4.90	4.81
Isoleucine	0.87	1.28	1.80
Phenylalanine	2.26	2.97	2.34
Tyrosine	1.97	2.23	1.71
Proline	2.79	3.42	1.99
Cystine	0.14	0.34	0.21
Methionine	1.21	1.70	1.61
Crude protein	86.16	92.67	84.93

nine, and leucine (neutral amino acids) were also abundant.

Figure 1 shows the elution pattern of Sephadex G-25 gel filtration of the Antarctic kill hydrolysate. Many peaks were observed in the ranges corresponding to molecular weights below 12,000 in all samples. In particular, large peaks were noted in the regions around molecular weights of 1400 and 300, indicating that the hydrolysates of the crustaceans examined were mixtures of various peptides, dominated by those with molecular weights of about 1400 and 300. The hydrolysates from Kuruma prawn and Tora velvet shrimp had elution profiles similar to that of Antarctic krill hydrolysate (data not shown).

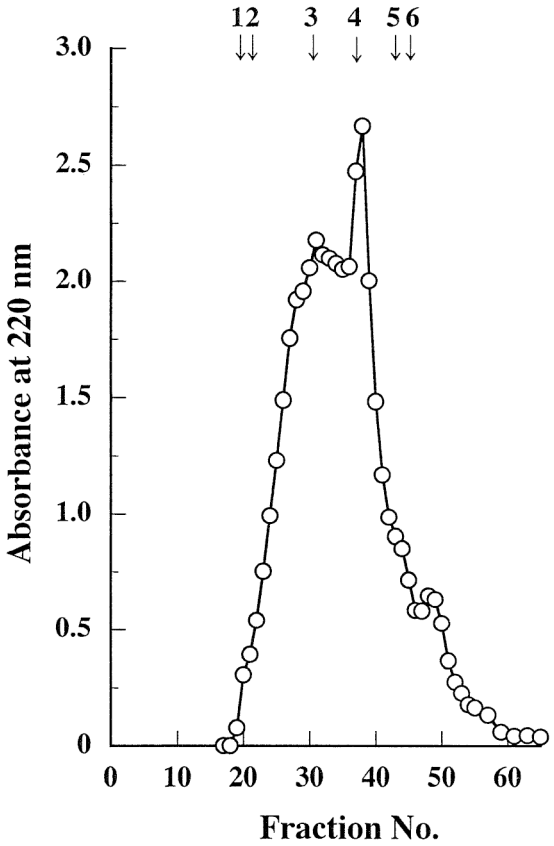

Fig. 1. Gel chromatograms of Antarctic krill hydrolysates on Sephadex G-25. Elution: 30 mM phosphate buffer (pH 7.0) containing 0.1 M NaCl. Flow rate: 30 ml/h. Fraction volume: 5 ml/tube. The numbers denote elution positions of the following compounds: (1) cytochrome C (M.W. 12,500); (2) aprotinin (M.W. 6511); (3) bacitracin (M.W. 1411); (4) glutathione (reduced) (M.W. 307); (5) glycyl-L-phenylalanine (M.W. 222); (6) L-phenylalanine (M.W. 165).

Denaturation of myofibrils during frozen storage Figure 2 shows the changes in specific activity of the myofibrillar Ca-ATPase during the storage period. In the control myofibrils (without hydrolysates), the residual activity dropped quickly to about 56% of the initial value in the first 15 days of storage and then continued to decrease more slowly until day 120, indicating that the denaturation process proceeded in two steps. When Antarctic krill hydrolysate was added at 2.5% or 5% (dry-weight) of myofibrils, the activity remained high at 84% and 95%, respectively, on day 10 of storage, then decreased gradually, showing a two-step change as in the control. For the Kuruma prawn hydrolysate, the activity on day 30 of storage was 67% and 84% at 2.5% and 5% of dry-weight, respectively, and then decreased gradually afterwards, showing a two-step denaturation. The Tora velvet shrimp hydrolysate exhibited 82% and 88% activity on day 30 of storage at 2.5% and 5% of dry-weight, respectively, and then decreased gradually, showing two-step denaturation similar to the control. When the hydrolysate levels were increased to over 7.5%, the denaturation process was slow, showing one-step denaturation until day 120 of storage.

The rate constant for freeze-denaturation (k_D) was calculated from the changes in myofibrillar Ca-ATPase activity during the storage period, and is shown in Table 2. For the control, the rate constant was 48.8×10^3/day in the first step (k_{D1}) and 19.7×10^3/day in the second step (k_{D2}). In contrast, the rate constant k_{D1} of the myofibrils with added hydrolysate was lower than that of the control. The hydrolysate from Tora velvet shrimp was particularly effective, and the hydrolysates from Antarctic krill and Kuruma prawns exhibited equal efficacy. These results indicated that freeze-denaturation of myofibrils was inhibited by addition of the hydrolysate and that Antarctic krill hydrolysate had comparable denaturation-inhibiting effects to that of Kuruma prawns.

Changes in unfrozen water levels We determined the heat of fusion of distilled water using the method that established a linear relationship between the amount of pure water and the heat of fusion up to about 25 mg of distilled water. The heat of fusion obtained from pure water was 333.3 J/g (n=4, p<0.001) which was in agreement with the known value of 339.0 J/g for pure water (Wakamatsu & Sato, 1979). We measured the amount of unfrozen water in the myofibrils with and without hydrolysate during frozen storage and plotted the data in the graph shown in Fig. 3. The amount of unfrozen water in myofibrils increased after addition of the hydrolysate compared to levels seen in the control (0.843 g H_2O/g dried matter). A marked effect was observed in up to 5% hydrolysate addition, whereas only a smaller response was observed above this level. Thus, the amount of unfrozen water in the myofibrils increased 1.3-fold upon addition of hydrolysate to 5% dry-weight, but an increase to 12.5% increased the amount of unfrozen water only 1.4-fold. These

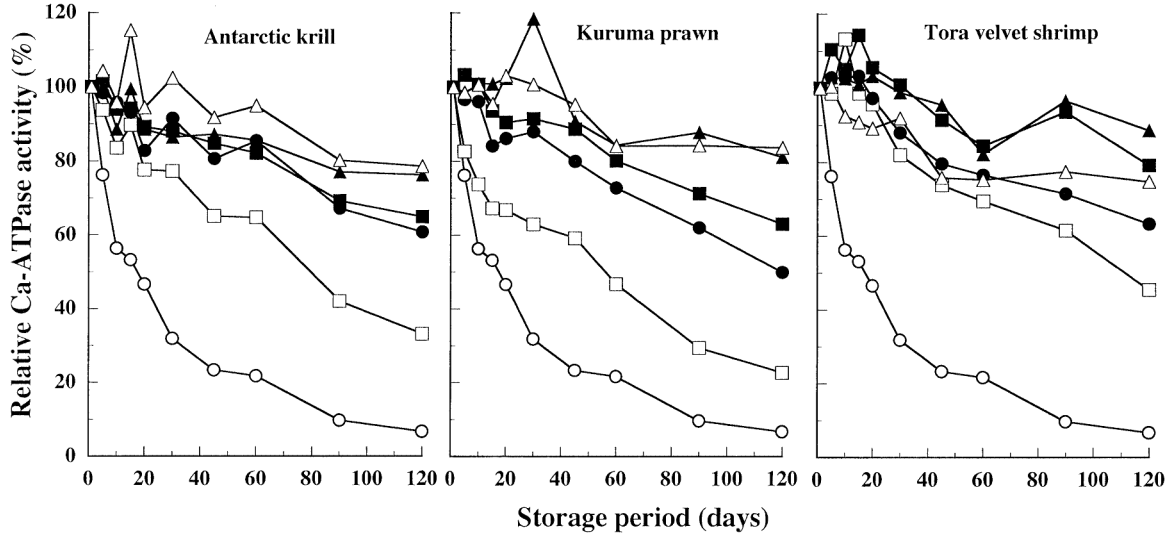

Fig. 2. Changes in Ca-ATPase activity of myofibrils during frozen storage at –25°C in the presence of varoius hydrolysates at varying concentrations. Symbols: (○), control; (□), 2.5%; (●), 5.0%; (■), 7.5%; (▲), 10.0%; (△), 12.5%.

Table 2. Rate constant (k_D) for inactivation of myofibrillar Ca-ATPase of myofibrils in the presence of various hydrolysates during frozen storage at –25°C ($\times 10^3$/day)

Amount of added hydrolysates (%)	Antarctic krill		Kuruma prawn		Tora velvet shrimp	
	k_{D1}	k_{D2}	k_{D1}	k_{D2}	k_{D1}	k_{D2}
Control	48.8 (15)[a)]	19.7	48.8 (15)	19.7	48.8 (15)	19.7
2.5	18.0 (10)	8.4	26.4 (30)	10.3	6.6 (30)	4.8
5.0	12.1 (10)	3.4	11.5 (30)	5.0	4.3 (30)	3.7
7.5	3.6	—	3.8	—	2.0	—
10.0	2.3	—	1.7	—	1.0	—
12.5	2.0	—	1.5	—	2.4	—

[a)]Numerals in parentheses indicate the turning point between k_{D1} and k_{D2}

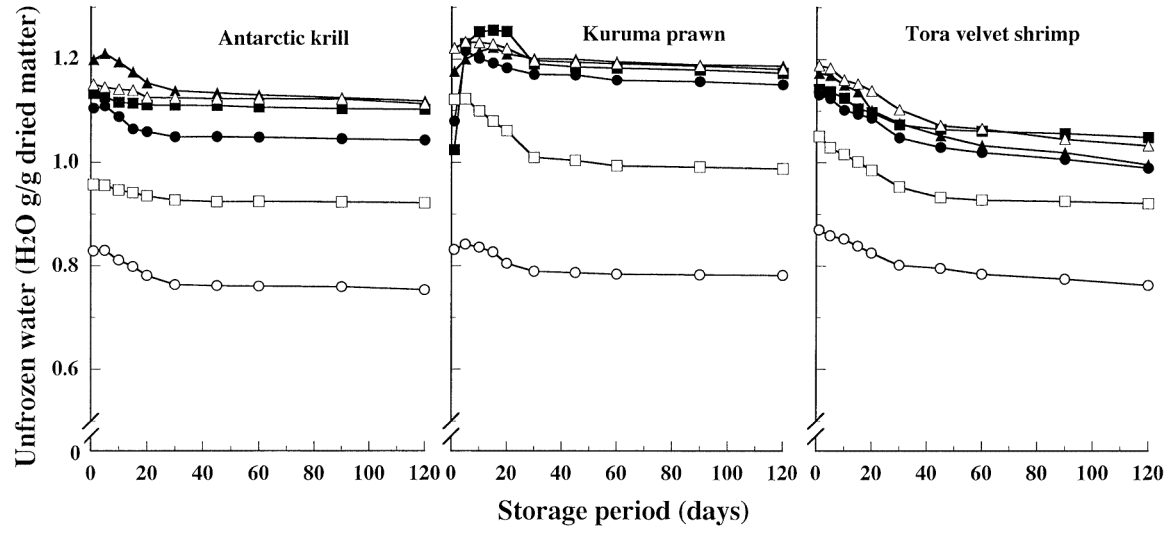

Fig. 3. Changes in the amount of unfrozen water in myofibrils during frozen storage at –25°C in the presence of various hydrolysates at varying concentrations. Symbols are the same as in Fig. 2.

results clearly showed that the hydrolysate increased the amount of unfrozen water in myofibrils and that the Antarctic krill hydrolysate increased unfrozen water levels to a degree comparable to that of Kuruma prawn and Tora velvet shrimp.

Relationship between the inactivation of myofibrillar Ca-ATPase and the amount of unfrozen water The above results indicated that hydrolysate addition increased the amount of unfrozen water and simultaneously suppressed the inactivation of

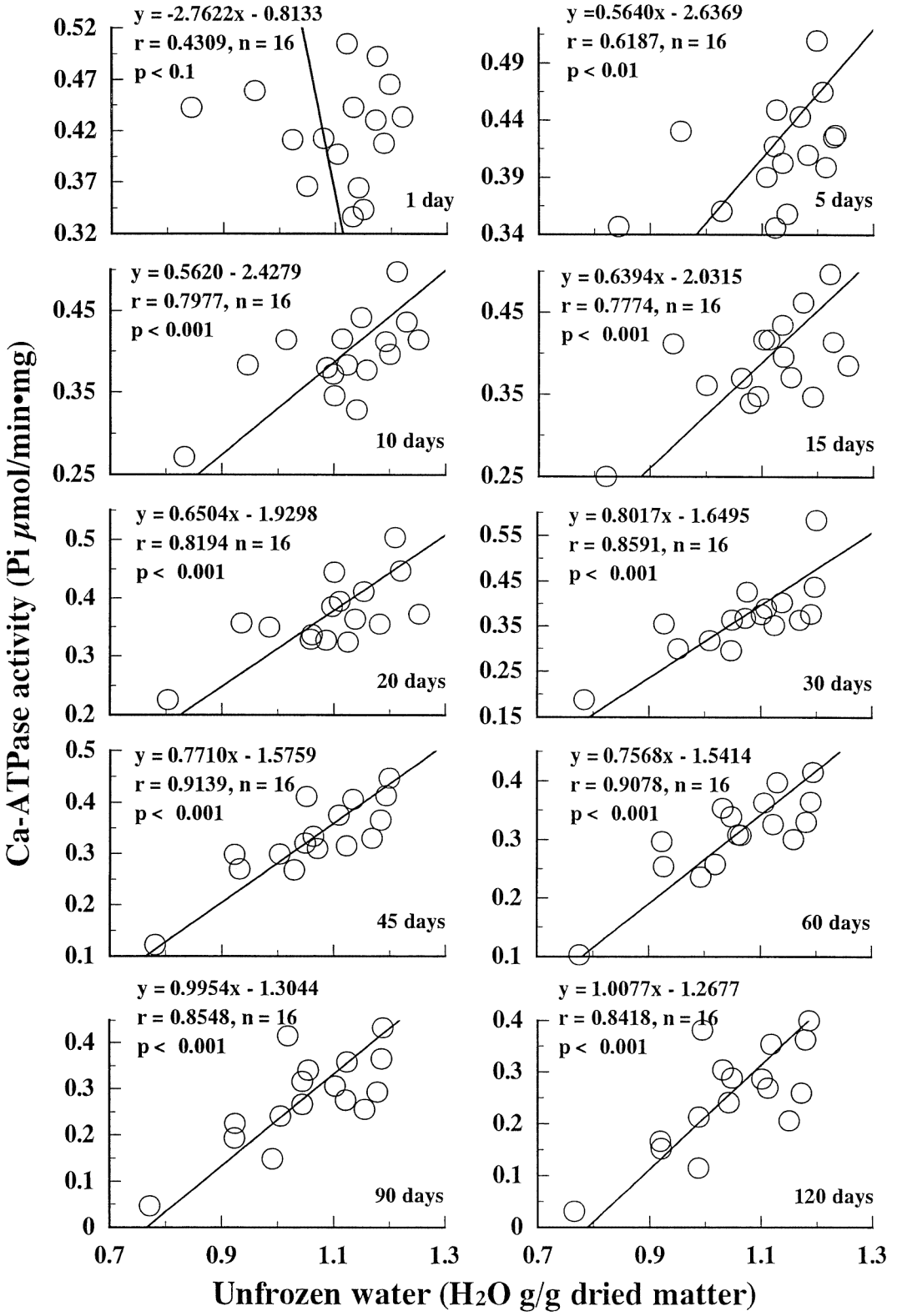

Fig. 4. Correlation between Ca-ATPase activity and the amount of unfrozen water in myofibrils in each storage period. Hydrolysates were added to 100 g myofibrils at 2.5–12.5%. Control had no additions.

myofibrillar Ca-ATPase under frozen storage. A correlation appeared to exist between these effects. To confirm this, the relationship between ATPase activity and the amount of unfrozen water was investigated. As shown in Fig. 4, although a low correlation was observed at the initial stage, a high correlation was observed between these variables in later stages despite the differences. These results indicated that the denaturation of myofibrillar Ca-ATPase was correlated with the amount of frozen water, irrespective of the presence or absence of added hydrolysate.

Discussion

In the present study, the effects of hydrolysate prepared from Antarctic krill on the denaturation of proteins and the amount of unfrozen water in frozen myofibrils were studied. For comparison, hydrolysates from Kuruma prawn, which is used in expensive dishes, and from Tora velvet shrimp, which is used in ordinary dishes in the Nagasaki district, were also examined.

The time-dependence of freeze-denaturation of fish myofibrils varies with fish species, concentration of denaturation-inhibiting substances, and pH (Matsumoto *et al*., 1985). Fishes exhibiting rapid denaturation show biphasic changes whereas those exhibiting slow denaturation show monophasic changes. The lizard fish myofibrils used in the present study showed rapid denaturation in 15 days, which slowed down afterwards during frozen storage, indicating that the myofibrils of this fish show biphasic changes similar to those of carp (Matsumoto *et al*., 1985), sardines (Ooizumi *et al*., 1981), and mackerel (Ooizumi *et al*., 1981). When hydrolysate was added, the time-dependent denaturation was also biphasic. However, the denaturation for myofibrils with hydrolysate proceeded more slowly than that of the control (without hydrolysate) prior to 30–45 days of frozen storage. This indicates that the addition of the hydrolysate may suppress freeze-denaturation. Conversely, our previous research has shown that the addition of amino acids with strong freeze-denaturation-inhibiting activity, such as sodium asparaginate and sodium glutamate, caused a monophasic denaturation of myofibrils during 150 days of frozen storage (Nozaki, 1987). The hydrolysates in the present study, however, were less effective than the amino acids used in the previous study.

Bound water contained in fish myofibrils is strongly affected by the surface of protein molecules, which forms part of the polymer, whereas partially bound water is under a physical or chemical constraint, which restricts its movement. In the present study, the amount of water that was not frozen at –40°C was measured using a differential scanning calorimeter (the DSC method) (Wakamatsu & Sato, 1979). The value obtained most likely included part of the partially bound water as well as the bound water. Correlation between the myofibrillar Ca-ATPase and the unfrozen water level was low within a 20-day storage period, however, a closer correlation was observed afterwards up to 120 days. These results suggest that aside from the role that hydrolysates play in preventing ice crystal formation around myofibrils to inhibit denaturation of myofibrillar proteins, some other factor may have a function in its cryoprotective effect. The level of unfrozen water was considered indicative of the state of water (degree of constraint) in fish myofibrils.

Our results showing that the addition of hydrolysate to myofibrils increased the amount of unfrozen water may indicate that water in myofibrils is affected by hydrolysate. The hydrolysate may have captured water molecules surrounding the myofibrils, which were excluded by the hydrophobic residues of the protein molecules. As a result, the structure of the water molecules in the entire solution changed so that the hydration water on the surface of the protein molecules was stabilized, leading to an increase in the amount of unfrozen water. Our results also showed, however, that the amount of unfrozen water did not increase above a specific hydrolysate concentration of 5.0%. This can be explained by the concentration-dependent increase in the stability of hydration water up to a threshold hydrolysate concentration that corresponds to the capacity of the myofibrillar protein to exclude the hydrolysate. Above the threshold, the free hydrolysate molecules surrounding the myofibril proteins take up the hydration water, resulting in changes in the hydration structure (Gekko, 1981; Gekko & Koga, 1983).

Although peptides are believed to be the main components involved in hydration of myofibrils and inhibition of freeze-denaturation of proteins, effects of other components such as sugar cannot be ignored. More detailed analysis of the peptides awaits future studies. The use of hydrolysate has the advantage of using all the materials without waste. Therefore, hydrolysates from seldom-used fish such as Antarctic krill are expected to be highly effective. In particular, use of krill hydrolysate for extracting functional substances and as moistening agents, due to their high hydrating capacity, is promising. However, because the smell, taste, and color of hydrolysates may limit their application to food, further studies are required before they are widely used as food material.

References

Akiba, M. (1973). Denaturation of protein and water activity. In "Foods and Water," ed. by Jpn. Soc. Fisheries Sci., Koseisha Koseikaku, Tokyo, pp. 101–116 (in Japanese).

Arai, K., Takahasi, H. and Saito, T. (1970). Studies on muscular proteins of fish-III. *Nippon Suisan Gakkaishi*, **36**, 232–236 (in Japanese).

Arai, K., Watanabe, T. and Kinumaki, T. (1976). Studies on the utilization of Antarctic krill-I. *Tokai-ku Suisan Kenkyusho Kenkyu Hokoku*, **85**, 1–12 (in Japanese).

Gekko, K. (1981). Enthalpy and entropy of transfer of amino acids and diglycine from water to aqueous polyol solutions. *J. Biochem*., **90**, 1643–1652.

Gekko, K. and Koga, S. (1983). Increased thermal stability of collagen in the presence of sugars and polyols. *J. Biochem*., **94**, 199–205.

Gornall, A. G., Bardawill, C. T. and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem*., **177**, 751–766.

Hanafusa, N. (1973). Biological macromolecules and water, In "Foods and Water," ed. by Jpn. Soc. Fisheries Sci., Koseisha Koseikaku, Tokyo, pp. 9–24 (in Japanese).

Hansen, R.P. and Meiklen, S.M. (1970). Isoprenoid fatty acides in Antarctic krill (*Euphausia superba*). *J. Sci. Food Agric*., **21**, 203–206.

Iwamoto, M., Fujiwara, R. and Yokoyama, M. (1991). Immunological effects of BM-2, an enzymatic digestive extract of chub mackerel. *J. Jpn. Soc. Cancer Ther*., **26**, 939–947 (in Japanese).

Katoh, N., Uchiyama, H., Tsukamoto, S. and Arai, K. (1977). A biochemical study on fish myofibrillar ATPase. *Nippon Suisan Gakkaishi*, **43**, 857– 867 (in Japanese).

Kauzman, W. (1959). Some factors in the interpretation of protein denaturation. *Adv. Protein Chem*., **14**, 1–63.

Kavanau, J.L. (1965). "Structure and Function in Biological Membranes." Vol.1, Holden-Day, San Francisco, pp.171–248.

Kuwano, K. and Mitamura, T. (1977). On the Antarctic krill protein concentrate (KPC)-I. *Nippon Suisan Gakkaishi*, **43**, 559–565 (in Japanese).

Matsuda, Y. (1973). Water adsorption and stability of quality of fish meat powder, In "Foods and Water," ed. by Jpn. Soc. Fisheries Sci., Koseisha Koseikaku, Tokyo, pp. 117–123 (in Japanese).

Matsumoto, I., Ooizumi, T. and Arai, K. (1985). Protective effect of sugar on freeze-denaturation of carp myofibrillar protein. *Nippon Suisan Gakkaishi*, **51**, 833–839 (in Japanese).

Migita, M., Matsumoto, J. and Saishu, T. (1956). On the denaturation of fish muscle proteins by dehydration. *Nippon Suisan Gakkaishi*, **22**, 433–439 (in Japanese).

Migita, M., Matsumoto, J. and Saishu, T. (1960). On the denaturation of fish muscle proteins by dehydration-II. *Nippon Suisan Gakkaishi*,

26, 925–930 (in Japanese).
Miyake, Y. (1982). Solubilization of fish scrap by enzyme treatment. *Nippon Shokuhin Kogyo Gakkaishi*, **29**, 117–122 (in Japanese).
Murata, Y., Hayashi T., Watanabe, E. and Toyama, K. (1991). Preparation of Skipjak spermary extract by enzymolysis. *Nipon Suisan Gakkaishi*, **57**, 1127–1132 (in Japanese).
Nemethy, G. and Scheraga, H. A. (1962). Structure of water and hydrophobic bonding in proteins-I. *J. Chem. Phys*., **36**, 3382–3399.
Niwa, E., Kohda, S., Kanoh, S. and Nakayama, T. (1987). Exposure of hydrophobic amino acid residues from myosin on freezing. *Nippon Suisan Gakkaishi*, **52**, 2127–2130 (in Japanese).
Nozaki, Y. (1987). Studies on the state of water and denaturation of fish protein. Doctoral Thesis, Hokkaido University, Sapporo (in Japanese).
Nozaki, Y., Ichikawa, H. and Tabata, Y. (1991). Effect of amino acid on the state of water and ATPase activity accompanying dehydration of fish myofibrils. *Nippon Suisan Gakkaishi*, **57**, 1531–1537 (in Japanese).
Nozaki, Y., Ichikawa, H. and Tabata, Y. (1993). Effect of amino acid addition on isosteric sorption heat during dehydration of fish myofibrils. *Nippon Suisan Gakkaishi*, **59**, 1209–1211 (in Japanese).
Ooizumi, T., Sakata, N. and Arai, K. (1981). Protective effect of sorbitol on freeze-denaturation of sardine and mackerel myofibrils. Abstracts of the Meeting of Japanese Society of Fisheries Science, Apr., p. 237 (in Japanese).
Sidhu, G.S., Montgomery, W.A., Holloway, G.L., Johnson, R. and Walker, D.M. (1970). Biochemical composition and nutritive value of krill (*Euphausia superba dana*). *J. Sci. Food Agric*., **21**, 293–296.
Suetsuna, K., Yamagami, M. and Kuwata, K. (1988). Inhibitory activity against angiotensin I-converting enzyme of peptides originating from fish and shellfish muscle. *Nippon Suisan Gakkaishi*, **54**, 1853 (in Japanese).
Sugiyama, K., Egawa, M., Onzuka, H. and Oba, K. (1991). Characteristics of sardine muscle hydrolysates prepared by various enzymic treatments. *Nippon Suisan Gakkaishi*, **57**, 475–479 (in Japanese).
Suzuki, T. (1971). Denaturation of fish muscle protein during dehydration. *Nippon Shokuhin Kogyo Gakkaishi*, **18**, 168–171 (in Japanese).
Suzuki, T. (1981). Utilization of krill for human consumption. In "Fish and Krill Protein," ed. by T. Suzuki, Applied Science Publishers, London, pp. 195–251.
Wakamatsu, T. and Sato, Y. (1979). Determination of unfreezable water in sucrose, sodium chloride and protein solutions by differential scanning calorimeter. *Nippon Nogeikagaku Kaishi*, **53**, 415–420 (in Japanese).
Watanabe, T., Sugii, K. Yuguchi, H. and Kinumaki, T. (1976). Studies on the utilization of Antarctic krill-II. *Tokai-ku Suisan Kenkyusho Kenkyu Hokoku*, **85**, 13–30 (in Japanese).
Yanase, M. (1979). Isopropyl alcohol extraction of krill to yield fish protein concentrate. *Tokai-ku Suisan Kenkyusho Kenkyu Hokoku*, **99**, 43–54 (in Japanese).
Yokoyama, K., Chiba, H. and Yoshikawa, M. (1992). Peptide inhibitors for angiotensin I-converting enzyme from thermolysin digest of dried bonito. *Biosci. Biotechnol. Biochem*., **56**, 1541–1545.