Denaturation and Change in SH Group of Actomyosin from Milkfish (*Chanos chanos*) during Storage at -20 °C

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The formation of disulfides, effects of the addition of reductants before freezing, and denaturation of milkfish (*Chanos chanos*) actomyosin (AM) during storage at -20 °C were investigated. In the freezing process, aggregation-denaturation was mainly caused by the formation of hydrogen, hydrophobic, and disulfide bonds; during storage, more disulfides formed. The addition of $NaNO_2$ and $NaBH_4$ before freezing accelerated the denaturation of AM during storage. However, when added to freeze-denaturated AM, $NaNO_2$ and $NaBH_4$ recovered AM's solubility in 0.6 M KCl and the total SHs and decreased the sum of $NaBH_4$-soluble and -insoluble fractions. These data suggested that the formation of disulfides affected the denaturation of AM during freezing and frozen storage.

Because of the denaturation of myofibrillar proteins during frozen storage, some fish, if ever frozen, cannot be produced high-quality products (Okada and Nakayama, 1961; Okada et al., 1974; Tahata et al., 1975; Jiang, 1977; Nosaki et al., 1978; Kurokawa, 1979; Chou and Chou, 1980). Connell (1959) reported that the side-to-side aggregation occurred in myosin of frozen cod. The same evidence was observed through electron microscopic studies on carp actomyosin (Tsuchiya et al., 1975; Oguni, 1975), milkfish actomyosin (Jiang et al., 1987c), trout myosin (Buttkus, 1970), and cod actomyosin (Jarenback et al., 1975). Many hypotheses and postulations are currently proposed to explain the bonding in aggregated proteins (Sikorski et al., 1976; Sikorski, 1978, 1980; Jiang et al., 1987a-c). Connell (1959, 1960, 1965) postulated that the aggregation of proteins was caused by hydrogen, hydrophobic, and other bonds instead of by the formation of disulfides. Buttkus (1970, 1971) found that the total sulfhydryls (SHs) of myosin prepared from trout and rabbit decreased during storage at 0 °C and subsequently concluded that the aggregation was due to the exchange between SS and SH. Jiang et al. (1986) conjectured that the protein denaturation of frozen mackerel and cod was mainly caused by the formation of disulfides.

This study aimed to investigate the formation of disulfides in actomyosin during storage at -20 °C, whether the formation of disulfides and protein denaturation are related, and effects of added reductants ($NaBH_4$, $NaNO_2$) on the recovery of total SH, solubility, and ATPase activity in freeze-thawed actomyosin.

MATERIALS AND METHODS

Preparation of the Actomyosin. Actomyosin (AM) was extracted from milkfish (*Chanos chanos*) dorsal muscle according to Noguchi and Matsumoto (1970). $NaBH_4$ and $NaNO_2$ (18 μmol/g of protein) were added to the extracted AM (6.03 mg/mL) to investigate the effects of reductants on protein denaturation. The mixtures (30 mL) were put in plastic tubes, stoppered, and stored at -20 °C for 10 weeks. At definite time intervals, samples were removed, thawed to 0 °C with running tap water (about 25 °C), and subjected to the following analyses.

Determination of SHs of Actomyosin. The total SHs of AM were determined according to 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, 8 M urea; pH 8.0), and the resultant mixture was stirred for 30 min at 25 °C. To 3 mL of the mixture was added 0.02 mL of 0.01 M 5,5'-dithiobis(2-nitrobenzoic acid), and the resultant mixture was incubated at 40 °C for 15 min. The absorbance at 412 nm was measured to calculate the total SHs according to Ellman (1959).

Reactive SHs were determined by incubating the AM at 5 °C for 1 h, in the absence of urea according to Buttkus (1971). The total and reactive SHs were expressed as moles/5×10^5 g of protein.

Solubility. Solubility of AM was determined according to Hamada et al. (1977). Samples were thawed to 0 °C with running tap water (25 °C) and centrifuged at 15000*g*, 0 °C, for 1 h. The collected supernatant was defined as salt-soluble fraction (I), which was considered to be native proteins. To the precipitate was added 5 mL of a mixture solution (containing 8 M urea, 6 mM EDTA, and 0.6 M KCl solution) and the mixture stirred for 30 min at 25 °C. The supernatant obtained after centrifuging this mixture at 15000*g*, 15 °C, for 1 h was defined as urea-soluble fraction (II), which was considered to be aggregated by formation of hydrogen and hydrophobic bonds. Finally, 5 mL of a mixture solution (containing 0.5% $NaBH_4$, 6 mM EDTA, 8 M urea, and 0.6 M KCl) was added to the precipitate and the mixture stirred for 30 min at 25 °C. The supernatant obtained after centrifuging this mixture at 15000*g*, 15 °C, for 1 h was defined as $NaBH_4$-soluble fraction (III), which was aggregated by formation of disulfides, and the final residue as insoluble fraction (IV).

Protein concentration was measured by the micro-biuret method (Itzhaki and Gill, 1964). The solubility was expressed as the ratio of the quantity of soluble protein to that of original AM.

Ca-ATPase Activity. To 1 mL of 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 finally 0.5 mL of 20 mM adenosine 5'-triphosphate (ATP) solution (pH 7.0). After ATP was added, the releasing rate of inorganic phosphate at 25 °C within 3-min reaction was measured. A 5-mL portion of 15% trichloroacetic acid was added to stop the reaction; the quantity of resulting inorganic phosphate was measured according to the method of Arai (1974). The Ca-ATPase activity was defined as micromoles of inorganic phosphate liberated/milligram of protein within 1 min for the reaction at 25 °C.

Mg(EGTA)-ATPase Activity. To 1 mL of AM solution (1-5 mg/mL) were added 1.0 mL of 0.02 M $MgCl_2$,

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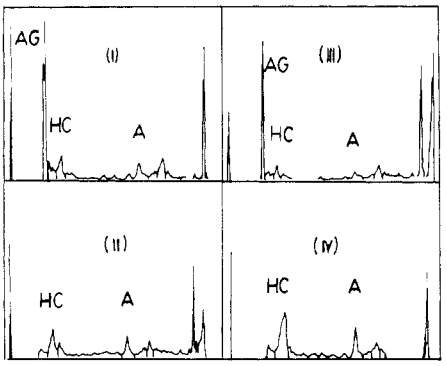

Figure 1. Electrophoretic separation of freeze-thawed actomyosin after 6 weeks of storage at −20 °C: I, AM with sodium dodecyl sulfate added; II, AM with sodium dodecyl sulfate and mercaptoethanol added; III, AM with sodium dodecyl sulfate and $NaBH_4$ added; IV, AM with sodium dodecyl sulfate, mercaptoethanol, and NaBH added; A, actin; HC, myosin heavy chain; AG, aggregate.

1.0 mL of 5 mM ethylene glycol bis(2-aminoethyl ether) tetraacetic acid (EGTA), 1.0 mL of 0.2 M Tris–maleate buffer (pH 7.0), 0.5 mL of 0.1 M $CaCl_2$, 7.5 mL of deionized water, and finally 0.5 mL of 20 mM ATP solution (pH 7.0). After ATP was added, the releasing rate of inorganic phosphate at 25 °C within a 3-min reaction was measured. A 5-mL portion of 15% trichloroacetic acid was added to stop the reaction; the quantity of resulting inorganic phosphate was measured according to the method of Arai (1974). The Mg(EGTA)–ATPase activity was defined as micromoles of inorganic phosphate liberated/milligram of protein within 1 min for the reaction at 25 °C.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoretic Analyses (SDS-PAGE). After being dialyzed overnight against 62.5 mM Tris-HCl buffer (pH 6.8) at 5 °C, the AM solution was incubated at 100 °C for 3 min in a buffer consisting of 2% SDS, 5% 2-mercaptoethanol, and 62.5 Tris-HCl (pH 6.8). The solubilized AM was analyzed with disc-SDS-PAGE according to Laemmli and Favre (1973). The concentration of polyacrylamide gel was 10%.

Distance scanning at a wavelength of 585 nm, at which the staining solution has maximum absorbance, was performed to analyze the band size of the gel on a UV–vis microprocessor-controlled spectrophotometer system (2600, Gilford Instrument).

Statistical Analysis. Duncan's multiple-range test was used for statistical analyses.

RESULTS AND DISCUSSION

Electrophoretic Analyses of the Freeze-Denatured Actomyosin. According to Hamada et al. (1977), the insoluble fraction (IV) was caused by bonding other than hydrophobic, hydrogen, and disulfide. To examine whether disulfides exist in insoluble fraction (IV) or not, freeze-denatured AM (after 6 weeks of storage at −20 °C) was treated with four kinds of solutions: 2% sodium dodecyl sulfate (SDS), 2% SDS and 5% 2-mercaptoethanol (ME), 2% SDS and 0.5% $NaBH_4$, and finally 2% SDS, 0.5% $NaBH_4$, and 5% ME. The resultant AMs were then analyzed with disc-SDS-PAGE. As shown in Figure 1, aggregates were obviously observed in samples treated with 2% SDS and with the mixture of 2% SDS and 0.5% $NaBH_4$ (Figure 1, parts I and III) but disappeared after being treated with 5% ME in 2% SDS and with 5% ME in a mixture of 2% SDS and 0.5% $NaBH_4$ (Figure 1, parts II and IV). This phenomenon indicated that 0.5% $NaBH_4$ could not reduce the disulfides completely. Accordingly, both $NaBH_4$-soluble (III) and -insoluble fractions (IV) prepared from the method of Hamada et al. (1977) were the result of formation of the disulfides.

Table I. Effect of Frozen Storage on the Solubility (mg/mL) of Milkfish Actomyosin (AM), AM with $NaBH_4$, and AM with $NaNO_2$

storage time, wk		solubility[a] A	B	C
unfrozen		6.03 (100.0)	6.03 (100.0)	6.03 (100.0)
0	I[b]	4.42 (73.3) a[c]	4.35 (72.1) a	4.39 (72.8) a
	II	0.75 (12.4) b	0.92 (15.3) a	0.94 (15.6) a
	III	0.28 (4.6) c	0.37 (6.2) a	0.31 (5.1) b
	IV	0.58 (9.6) a	0.39 (6.5) b	0.39 (6.5) b
	III + IV	0.96 (14.2) a	0.76 (12.7) b	0.70 (11.6) c
2	I	4.33 (71.8) a	4.17 (69.2) c	4.26 (70.6) b
	II	0.90 (14.9) c	1.08 (17.9) a	1.02 (16.9) b
	III	0.21 (3.5) b	0.36 (6.0) a	0.33 (5.5) a
	IV	0.59 (9.8) a	0.42 (7.0) b	0.42 (7.0) b
	III + IV	0.80 (13.3) a	0.78 (13.0) a	0.75 (12.5) a
4	I	4.06 (67.3) a	4.12 (68.3) a	3.94 (65.3) b
	II	0.90 (14.9) b	1.04 (17.3) a	1.09 (18.1) a
	III	0.50 (8.3) a	0.42 (7.0) b	0.43 (7.1) b
	IV	0.57 (9.5) a	0.45 (7.5) b	0.57 (9.5) a
	III + IV	1.07 (17.8) a	0.87 (14.5) c	1.00 (16.6) b
6	I	4.05 (67.2) a	3.85 (63.8) b	3.82 (63.3) b
	II	0.64 (10.6) c	1.03 (17.1) b	1.24 (20.6) a
	III	0.64 (10.6) b	0.69 (11.4) a	0.63 (10.4) b
	IV	0.70 (11.6) a	0.46 (7.6) b	0.34 (5.6) c
	III + IV	1.34 (22.2) a	1.15 (19.0) b	0.97 (16.0) c
8	I	3.73 (61.9) a	3.50 (58.0) b	3.44 (57.0) b
	II	0.51 (8.5) b	0.53 (8.8) b	0.77 (12.8) a
	III	0.76 (12.6) b	0.74 (12.3) b	0.84 (13.9) a
	IV	1.08 (17.9) b	1.26 (20.9) a	0.98 (16.3) c
	III + IV	1.84 (30.5) b	2.00 (33.2) a	1.82 (30.2) b
10	I	3.55 (58.9) a	3.00 (49.7) b	2.99 (49.6) b
	II	0.54 (8.9) b	0.46 (7.6) c	0.99 (16.4) a
	III	0.71 (11.8) b	0.79 (13.1) a	0.78 (12.9) a
	IV	1.23 (20.5) b	1.74 (28.9) a	1.27 (21.1) b
	III + IV	1.94 (32.3) c	2.53 (42.0) a	2.05 (34.0) b

[a] Key: A, control; B, AM with $NaBH_4$; C, AM with $NaNO_2$. [b] Key: I, salt soluble; II, urea soluble; III, $NaBH_4$-soluble; IV, insoluble; III + IV, sum of III and IV. [c] Values in parentheses were the percent ratios relative to the unfrozen one; values in the same row bearing unlike letters differ significantly ($P < 0.01$).

Effect of Frozen Storage on the Solubility of Actomyosin. Immediately after freezing, the percent ratios of salt-soluble fraction (I) of AM (sample A), AM with $NaBH_4$ (sample B), and $NaNO_2$ (sample C) decreased to 73.3, 72.1, and 72.8%, respectively, compared with the unfrozen samples (Table I). The percent ratios of 8 M urea-soluble fraction (II) were significantly higher in samples B and C than in sample A ($P < 0.01$). The sum of $NaBH_4$-soluble (III) and -insoluble fractions (IV) of sample A was lower than that of samples B and C. These suggested that, during freezing, the denaturation of AM was mainly caused by formation of hydrogen, hydrophobic, and disulfide bonds; more hydrogen and hydrophobic bonds and less disulfides formed in AM with $NaBH_4$ and $NaNO_2$ than in AM alone (Table I).

The percent ratio of salt-soluble fraction of all samples decreased with duration of storage (Table I). During storage, the decreasing rate of salt-soluble fraction (I) was higher in samples B and C than in sample A (Table II).

The quantity of urea-soluble fraction (IV) of samples A–C increased gradually during the first 4, 6, and 6 weeks storage, respectively, and then decreased during prolonged storage. The percent ratios of urea-soluble fraction were significantly higher in samples B and C than in sample A ($P < 0.01$) (Table I). This might be due to the formation of disulfides during prolonged storage, which subsequently decreased the solubilization in 8 M urea. This indicated formation of hydrogen and hydrophobic bonds during frozen storage. An increase in the sum of $NaBH_4$-soluble

Table II. Effect of Frozen Storage on the Change Rate of Solubility and Ca-ATPase Activity of Milkfish Actomyosin (AM), AM with $NaBH_4$, and AM with $NaNO_2$

	change rate[a]		
	A[b]	B	C
salt-soluble	−0.088 b (−2.0)[c]	−0.129 a (−3.0)	−0.137 a (−3.1)
III + IV[d]	+0.126 c (+2.1)	+0.220 a (+3.0)	+0.179 b (+2.3)
Ca-ATPase act.	−0.024 a (−5.6)	−0.007 b (−1.9)	−0.010 b (−2.6)
total SH	−0.648 c (−1.6)	−1.322 a (−3.1)	−0.909 b (−2.1)

[a] The change rates were expressed as follows: solubility, mg/mL per wk; Ca-ATPase activity, (P_i) μmol/min per mg protein per wk. [b] Refer to footnote *a* in Table I. [c] Values in parentheses were the percent change rates relative to zero week one; values in the same row bearing unlike letters differ significantly ($P < 0.01$). [d] III + IV = the sum of $NaBH_4$-soluble and -insoluble fractions.

Table III. Effect of Frozen Storage on the Total SHs[a] of Milkfish Actomyosin (AM), AM with $NaBH_4$, and AM with $NaNO_2$

storage time, wk	total SH		
	A[b]	B	C
unfrozen	40.1 a (100.0)[c]	46.0 a (100.0)	42.5 a (100.0)
0	38.2 b (95.3)	40.2 b (87.4)	40.4 b (95.1)
2	37.1 b (92.5)	32.8 c (71.3)	34.2 c (80.5)
4	33.7 c (84.0)	30.4 d (66.1)	28.5 e (67.1)
6	34.2 c (85.3)	29.8 d (64.8)	30.8 d (72.5)
8	32.8 cd (81.8)	23.8 f (51.7)	30.4 d (71.5)
10	31.6 d (78.8)	27.2 e (59.1)	29.5 d (69.4)

[a] The total SH groups were expressed as moles of SH group/5 × 10^5 g of protein. [b] Refer to footnote *a* in Table I. [c] Values in parentheses were the percent ratios relative to the unfrozen one; values in the same column bearing unlike letters differ significantly ($P < 0.01$).

Table IV. Effect of Frozen Storage on the Reactive SH[a] Groups of Milkfish Actomyosin (AM), AM with $NaBH_4$, and AM with $NaNO_2$

storage time, wk	reactive SH		
	A[b]	B	C
unfrozen	32.2 a[c]	34.1 a	37.4 a
0	21.8 b	21.1 b	21.1 b
2	20.5 b	20.9 b	20.7 b
4	21.6 b	20.3 b	21.5 b
6	22.6 b	16.8 c	21.6 b
10	22.4 b	17.6 c	21.2 b

[a] The reactive SH groups were expressed as moles of SH group/5 × 10^5 g of protein. [b] Refer to footnote *a* in Table I. [c] Values in the same column bearing unlike letters differ significantly ($P < 0.01$).

Table V. Effect of Frozen Storage on the Ca-ATPase Activity[a] of Milkfish Actomyosin (AM), AM with $NaBH_4$, and AM with $NaNO_2$

storage time, wk	Ca-ATPase act.		
	A[b]	B	C
unfrozen	0.469 (100.0) aA[c]	0.386 (100.0) aC	0.407 (100.0) aB
0	0.431 (96.4) b	0.380 (98.4) a	0.405 (99.5) a
2	0.445 (99.6) b	0.356 (92.2) b	0.368 (90.4) b
4	0.437 (97.8) b	0.346 (89.6) b	0.363 (89.2) b
6	0.301 (67.3) c	0.334 (86.5) b	0.316 (77.6) c
8	0.296 (63.1) c	0.305 (79.0) c	0.311 (76.4) c
10	0.210 (47.0) d	0.309 (80.1) c	0.304 (74.7) c

[a] The Ca-ATPase activity was expressed as micromoles of inorganic phosphate released within 1-min reaction at 25 °C/milligram of protein. [b] Refer to footnote *a* in Table I. [c] Values in parentheses were the percent ratios relative to the unfrozen one; values in the same column bearing unlike lowercase letters differ significantly ($P < 0.01$); values in the same row bearing unlike uppercase letters differ significantly ($P < 0.01$).

and -insoluble fractions in all samples was observed during storage (Table I). The increasing rate of $NaBH_4$-soluble and -insoluble fractions was the highest in sample B and then samples C and D in this order (Table II). These data suggested that protein denaturation during freezing and subsequent storage was mainly caused by formation of hydrogen, hydrophobic, and disulfide bonds.

Effect of Frozen Storage on the Total and the Reactive SHs. The total SHs of all samples decreased with duration of storage. After 10 weeks of storage, the total SHs of samples A–C decreased 21.2%, 40.9%, and 30.6%, respectively (Table III). During storage, the decreasing rate of the total SHs was the highest in sample B and then samples C and A in this order (Table II).

The reactive SHs of all samples decreased 32–44% immediately after freezing; but no distinct change was observed during storage (Table IV). During freezing, the decrease in the reactive SHs was considered, owing to the oxidation of SHs (Tables I and III). In addition, the structural changes in AM, due to formation of hydrogen and hydrophobic bonds (Table I), could mask the reactive SHs in molecules. During storage, the tertiary structure of AM changed, by formation of disulfide, hydroen, and hydrophobic bonds (Tables I and III); consequently, the reactive SHs masked in molecules were gradually exposed and oxidized to disulfides. The rate of exposure and oxidation of the reactive SHs might reach an equilibrium state during storage when the amounts of reactive SHs no longer change. On the basis of changes in the total SHs and the sum of $NaBH_4$-soluble and -insoluble fractions, disulfides formed in all samples during freezing and storage.

Effect of Frozen Storage on ATPase Activity. Ca-ATPase activities of AM decreased after $NaBH_4$ and $NaNO_2$ were added (Table V). This might be due to the change in conformation of AM after the addition of reductants. During storage, Ca-ATPase activity of all samples decreased (Table V). The decreasing rate of Ca-ATPase activity was the fastest in sample A and then samples C and B (Table II). Although Ca-ATPase activity of AM decreased significantly after $NaBH_4$ (sample B) and $NaNO_2$ (sample C) were added, the decreasing rate of Ca-ATPase of samples B and C was slower than for sample A (Table II).

Suzuki (1967) and Hatano (1968) postulated that, during frozen storage, the loss of enzymic activity of AM was due to the tertiary structural changes caused by ice crystallization. However, Buttkus (1971) and Hamada et al. (1977) concluded that the decrease in Ca-ATPase was highly related to the oxidation of SHs. Decrease in the total SHs and increase in the sum of $NaBH_4$-soluble and -insoluble fractions during frozen storage (Tables I and III) indicated that the loss of Ca-ATPase activity might be due to the oxidation of SHs on the active site of AM.

The initial activities of Mg(EGTA)-ATPase of all samples were very low. The Mg(EGTA)-ATPase activity of samples A–C increased gradually during the first 8, 10, and 6 weeks of storage, respectively; however, the activity increased rapidly after 10 and 8 weeks of storage in samples A and C, respectively (Table VI). From these data, it appeared that loss of Ca sensitivity of AM occurred the fastest in sample C, and then samples A and B.

The Ca sensitivity of myofibrillar proteins is attributed to the activity of native tropomyosin (Ebashi et al., 1968). The loss of Ca sensitivity is considered the result of filamentation of myofibrils, effect of hydrolysis by proteases (Tokiwa and Matsumiya, 1969). However, according to the studies of Seki and Hasegawa (1978), Seki and Iwabuchi (1978), Shitamura and Seki (1978), and Seki et al. (1979), the loss of Ca sensitivity of myofibrillar proteins

Table VI. Effect of Frozen Storage on the Mg(EGTA)-ATPase Activity[a] of Milkfish Actomyosin (AM), AM with $NaBH_4$, and AM with $NaNO_2$

storage time, wk	Mg(EGTA)-ATPase act.		
	A[b]	B	C
unfrozen	0.014 e[c]	0.017 e	0.014 d
0	0.031 d	0.025 d	0.018 d
2	0.039 d	0.056 c	0.048 c
4	0.044 d	0.069 b	0.052 c
6	0.051 c	0.074 a	0.087 b
8	0.081 b	0.073 a	0.162 a
10	0.131 a	0.078 a	0.157 a

[a] The Mg(EGTA)-ATPase activity was expressed as micromoles of inorganic phosphate released within 1-min reaction at 25 °C/milligram of protein. [b] Refer to footnote *a* in Table I. [c] Values in the same column bearing unlike letters differ significantly ($P < 0.01$).

Table VII. Effect of Reductants on the Solubility of Milkfish Actomyosin after Storage at -20 °C for 6 and 8 Weeks

storage time, wk		solubility		
		control	$NaNO_2$ added	$NaBH_4$ added
6	I[a]	67.2 c[b]	73.8 b	75.7 a
	II	10.4 b	11.2 a	11.4 a
	III	10.6	3.8	3.6
	IV	11.6	11.2	9.3
	III + IV	22.2 a	15.0 b	12.9 c
8	I	61.9 c	73.9 a	70.6 b
	II	8.5 b	6.7 c	9.3 a
	III	12.6	3.4	4.5
	IV	17.9	16.0	15.6
	III + IV	30.5 a	19.4 b	20.1 b

[a] Refer to footnote *b* in Table I. [b] The values were the percent ratios relative to the unfrozen one; values in the same row bearing unlike letters differ significantly ($P < 0.01$).

during icing was an effect of the modification of actin-myosin interaction caused by oxidation of SHs in myosin, instead of the hydrolysis of tropomyosin and troponins by proteases. In this study, the increase in the sum of $NaBH_4$-soluble and insoluble fractions (Table I), the decrease in total SHs (Table III), and the loss of Ca sensitivity (Table VI) suggested that the oxidation of SHs occurred in AM molecules during frozen storage. This postulation is concordant with the studies by Jiang et al. (1986, 1987b).

Effect of Reductants on the Denaturation of Actomyosin. The addition of $NaBH_4$ and $NaNO_2$ before freezing decreased the solubilization and increased the sum of $NaBH_4$-soluble and -insoluble fractions during storage (Table I); it also decreased the total SHs when compared with AM alone (Tables II and III). However, Lan (1984), Lan et al. (1987), and Jiang et al. (1986) reported that the stability and gel strength of frozen surimi from frozen lizard fish, cod, and mackeral were improved by the addition of reductants on processing. This discordancy might be due to the difference in samples whether freeze-denatured or not.

To investigate the effects of reductants on recovery of the total SHs and solubility of freeze-denatured AM, $NaBH_4$ and $NaNO_2$ were added to AM that had been stored at -20 °C for 6 and 8 weeks. Results such as the increase in salt-soluble fraction, decrease in sum of $NaBH_4$-soluble and -insoluble fractions, and recovery of the total SHs were obtained (Tables VII and VIII). It was considered that the change in conformation of native AM occurred after the addition of reductants that decreased the stability of AM. However, freeze-thawed lizard fish, cod, mackeral, and AM contained much disulfide, formed during freezing and subsequent storage. The reductants might be consumed to reduce the disulfides, leading to the recovery of solubility and the total SHs, as well as increased stability.

Table VIII. Effect of Reductants on the Recovery of Total SHs[a] of Milkfish Actomyosin after Storage at -20 °C for 6 and 8 Weeks

storage time, wk	recovery		
	control	$NaNO_2$ added	$NaBH_4$ added
unfrozen	40.1		
6	34.2 b (85.3)[b]	38.3 a (95.5)	37.5 a (93.5)
8	32.8 b (81.8)	37.7 a (94.0)	36.8 a (91.8)

[a] Refer to footnote *a* in Table III. [b] Values in parentheses were the percent ratios relative to the unfrozen one; values in the same row bearing unlike letters differ significantly ($P < 0.01$).

In summary, from an increase in sum of $NaBH_4$-soluble and -insoluble fractions and decrease in the total SHs of AM during frozen storage, it might be presumed that the formation of disulfides plays an important part in protein freeze–denaturation. Reductants added before freezing decreased the solubility and increased the sum of $NaBH_4$-soluble and -insoluble fractions of AM; but when added to freeze-denatured AM, reductants recovered the salt-soluble fraction and the total SHs.

Registry No. ATPase, 9000-83-3.

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Received for review August 12, 1987. Accepted January 20, 1988. This research work was supported by the National Science Council, ROC, under Grant No. NSC 77-0406-E019-01.

Oxygen-17 and Sodium-23 Nuclear Magnetic Resonance Studies of Myofibrillar Protein Interactions with Water and Electrolytes in Relation to Sorption Isotherms

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An NMR and water activity study of the interactions of myofibrillar proteins with sodium chloride and water is presented. Sorption isotherms are compared with high-field NMR data in an attempt to identify and quantitate different fractions of water. Salt "bound" to myofibrillar proteins in suspensions changed markedly the sorption isotherm; upon addition of 4% salt, the "monolayer" coverage, N_I, increased from 1.55 to 1.66 g of H_2O/100 g of solids; the total amount of "bound" water ($N_I + C$) increased from 22.6 to 27.2 g of water/100 g of solids. The fraction of Na^+ bound to the proteins increases with myofibrillar protein concentration up to a maximum of 500 mol of NaCl/10^6 g of protein. Above 50% (w/v) protein, the water activity decreases rapidly whereas the ^{17}O NMR line width increases only slightly with increasing concentration of myofibrillar proteins. Such data are interpreted in terms of protein activity that becomes dominant above 20% (w/v) myofibrillar protein. The effects of ion binding and charge fluctuations on myofibrillar protein–protein interactions are briefly discussed.

Per capita consumption of red meat, poultry, and fish in America has been in excess of 100 kg/year for the past decade (Meat Facts, 1986), hence the importance of research on meat and meat components. Current consumer trends indicate that more meat is being consumed in the form of further processed meat items; also, more meat items are being prepackaged and marketed with longer shelf life. This means that a better understanding of factors controlling meat shelf life is becoming increasingly important to the meat industry. Because meat contains approximately 75% water and 18–22% protein, the interactions between these two meat components are important in determining shelf life. Over half of the proteins in skeletal muscle are classified as myofibrillar or contractile proteins. The hydrated myofibrillar proteins are essentially those responsible for functional properties associated with meat. Binding and trapping of water in meat is of special interest in this context as the mechanism by

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0021-8561/88/1436-0437$01.50/0