Hubert, P. L. Riviera, TX, personal communication, 1983.
Kim, H. L. Texas A&M University, unpublished data, 1982.
Leete, E. *Bioorg. Chem.* **1977**, *6*, 273.
Tschesche, R.; Kometani, K.; Kowtiz, F.; Snatzke, G. *Chem. Ber.* **1961**, *94*, 3327.
Wenkert, E.; Cochran, D. W.; Hagaman, E. W.; Schell, F. M.; Neuss, N.; Katner, A. S.; Potier, P.; Kan, C.; Plat, M.; Koch, M.; Mehri, H.; Poisson, J.; Kunesch, N.; Rolland, Y. *J. Am. Chem. Soc.* **1973**, *95*, 4990.
Wieland, H.; Ishimasa, M. *Ann.* **1931**, *491*, 14.
Wieland, H.; Koschara, W.; Dane, E.; Renz, J.; Schwartze, W.; Linde, W. *Ann.* **1939**, *540*, 103.

Received for review April 30, 1984. Revised manuscript received July 10, 1986. Accepted September 12, 1986. This paper is approved as TA No. 19752 by the Director, Texas Agricultural Experiment Station. Presented in part at the 186th National Meeting of the American Chemical Society, Washington, DC, Aug 1983; AGFD 144.

Protein Denaturation and Changes in Nucleotides of Fish Muscle during Frozen Storage

Shann-Tzong Jiang,* Bao-Shyung Hwang, and Ching-Yu Tsao

Changes in quantity and composition of adenosine nucleotides and their relation to protein denaturation of fish muscle during frozen storage at −20 °C were studied. The protein denaturation was evaluated by the measurements of extractability of actomyosin (AM) and Ca-ATPase and Mg(EGTA)-ATPase activities of AM. The electrophoretic analysis of AM was also performed. No changes in electrophoretic separation of AM were observed during frozen storage. However, the molecular weight of myosin heavy chain (MHC) and actin decreased during frozen storage. The fish muscle with the highest levels of inosine (HxR) and hypoxanthine (Hx) and with the lowest levels of adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), and inosine 5′-monophosphate (IMP) was the most unstable in this study.

The deteriorative changes in texture as a consequence of long-term storage are considered to be due to protein denaturation during frozen storage (Dyer, 1951; Sikorski et al., 1976; Sikorski, 1978, 1980; Matsumoto, 1979, 1980; Shenouda, 1980; Andou et al., 1979, 1980; Noguchi, 1982; Acton et al., 1983; Jiang and Lee, 1985). For chub mackerel, pacific mackerel, and amberfish, much more denaturation of muscle proteins in fish frozen during postrigor than those frozen during prerigor was observed (Tsao et al., 1980; Fukuda et al., 1984). Many biochemical changes such as hydrolysis of lipids, oxidation of lipids, development of peptides and free amino acids, changes in nucleotide profile, etc., were involved in postmortis of fish muscle. The lipids and their derivatives, free amino acids, and peptides did affect the stability of muscle proteins during frozen storage (Noguchi, 1974; Sikorski et al., 1976; Sikorski, 1978, 1980; Matsumoto, 1979, 1980; Shenouda, 1980; Jiang, 1984; Jiang and Lee, 1985). However, the effect of adenosine nucleotides and interactions between proteins and nucleotides during frozen storage have not yet been studied. The present study aims to investigate the effect of adenosine nucleotides on protein denaturation so as to clarify the nature and mechanism of protein denaturation of frozen fish muscle.

As indicated in the *Data for Biochemical Research* (Burton, 1982; Symons, 1982), the pK values for phosphate in adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), and inosine 5′-monophosphate (IMP) were 6.00–6.95, 6.10–6.70, 6.20–6.40, and 1.54–6.04, respectively. the pK values for the adenosine base in these nucleotides were 4.00, 3.95, 3.74, and 8.90, respectively. However, the pK values for the adenosine base in inosine (HxR) and hypoxanthine (Hx) were as follows: HxR, 1.20, 8.90; Hx, 1.98, 8.94, 12.10. According to the above data, ATP, ADP, AMP, and IMP should hold four, three, two, and two negatively charged groups at the pH condition of fish muscle (6.5–7.0), respectively. It is, accordingly, hypothesized that the repulsion force among protein molecules interacting with adenosine nucleotides with more than two negatively charged groups will be intensified and consequently will prevent protein denaturation during frozen storage, while that interacting with HxR and Hx will increase a little, but the possibility of hydrophilic interactions among protein molecules will increase due to the adenosine base.

MATERIALS AND METHODS

Milkfish (*Chanos chanos*; body weight 310–380 g, length 34–38 cm) were obtained from a commercial culture farm in southern Taiwan. After being netted, the fish samples were iced and transported immediately to the laboratory. All fish were gutted, eviscerated, and headed. After the carcases were washed, fish samples were divided into three groups. Sample I was packed in polyethylene bags with five fish to each bag and stored at −20 °C. Samples II and III were stored at room temperature (25 °C) for 6 and 12 h and then in a −20 °C freezer for 18 weeks. at definite time intervals, one bag of each group was removed and thawed by immersion in running water (20–25 °C), until the body temperature reached 0 °C. The body temperature was measured by inserting a thermocouple into the center of dorsal portion. Five freeze–thawed fish from each

Department of Marine Food Science, National Taiwan College of Marine Science & Technology, Keelung, Taiwan 200, R.O.C.

0021-8561/87/1435-0022$01.50/0

group were deboned, skinned, and pooled together. Three samples from each group were subjected to the following measurements.

Determination of the Composition of Adenosine Nucleotides. The nucleotides were extracted according to Ehira et al. (1974). The composition of these nucleotides was analyzed by ion-exchange chromatography (resin AG 1X4, Cl^- type; mesh 200–400) according to Kato et al. (1974). The elute was passed through a UV detector (single-path monitor UV-1, Pharmacia Fine Chemicals) and collected on a fractional collector (FRAC-100, Pharmacia Fine Chemicals). The concentration of nucleotide was computed automatically on the basis of peak area. The K value was expressed as the percentage ratio of the quantity of HxR and Hx to that of total adenosine nucleotides.

Determination of the Protein Denaturation of Fish Muscle. the extractability of actomyosin (AM), Ca-ATPase and Mg(EGTA)-ATPase [EGTA = ethylene glycol bis(2-aminoethyl ether)-tetraacetic acid] activities of AM, and changes in the electrophoretic patterns of AM were determined for evaluating the extent of protein denaturation of fish muscle during frozen storage.

Extraction of Actomyosin. The AM was extracted according to Noguchi and Matsumoto (1970). Concentration of AM was determined by using the Biuret method modified by Umemoto (1966). Extractability of AM was expressed as milligrams of AM/grams of muscle.

Ca-ATPase Activity of Actomyosin. 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 distilled water, and 0.5 mL of 20 mM Na_2ATP solution (pH 7.0) in that order. The rate of release of inorganic phosphate at 25 °C within 3 min of reaction was determined after the addition of ATP. Five milliliters of 15% trichloroacetic acid was added to stop the reaction and the inorganic phosphate determined according to the method of Arai (1974). The Ca-ATPase-specific activity was shown as micromoles of inorganic phosphate released per milligram of AM within 1 min for the reaction at 25 °C. The Ca-ATPase total activity was expressed as micromoles of phosphate released per minute from AM solution extracted from 10 g of muscle.

Mg(EGTA)-ATPase Activity. To 1 mL of AM solution (1–5 mg/mL) were added 1.0 mL of 0.02 M $MgCl_2$, 1.0 mL of 0.005 M EGTA, 1.0 mL of 0.2 M Tris–maleate buffer (pH 7.0), 5.0 mL of distilled water, and 1.0 mL of 20 mM Na_2ATP solution (pH 7.0) in that order. The rate of release of inorganic phosphate at 25 °C within 3 min of reaction was determined after the addition of ATP. Five milliliters of 15% trichloroacetic acid was added to stop the reaction and the inorganic phosphate determined according to the method of Arai (1974). The Mg(EGTA)-ATPase-specific activity was shown as micromoles of inorganic phosphate released per milligram of AM within 1 min for the reaction at 25 °C.

Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoretic Analyses. For understanding the conformational changes of the AM, the SDS polyacrylamide gel electrophoresis was monitored according to Weber and Osborn (1969). The AM solution was dialyzed overnight at 5 °C against 0.01 M sodium phosphate buffer containing 0.1% SDS (pH 7.2). The dialyzed protein was incubated at 40 °C for 2 h in a solubilizing solution consisting of 0.001 M sodium phosphate buffer (pH 7.2), 1% SDS, 25% glycerin, and 2% mercaptoethanol. On the top of polyacrylamide gel, 0.02 mL of 0.05% bromophenol blue and 0.06 mL of the solubilized protein sample were pipetted. After the electrophoretic run in 0.1% sodium phosphate buffer (8 mA each gel), the gels were stained with a 0.12% Coomassie blue–50% methanol–9.2% acetic acid solution for 3 h. Destaining was done by immersion in a mixture of 50% methanol and 7.5% acetic acid for 8–10 h, as recommended by Seki (1974). The concentration of polyacrylamide was 7.5%.

Distance scanning at the wavelength 585 nm, for which the staining solution has maximum absorbance, was employed to analyze the bands 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

Changes in Adenosine Nucleotides during Frozen Storage. In order to investigate the effect of nucleotide composition of fish muscle on protein denaturation, milkfish, iced for about 4 h after being caught, was stored at room temperature (25 °C) for 0, 6, and 12 h. The sum of the quantity of ATP, ADP, AMP, and IMP decreased from 8.51 μmol/g of meat in samples killed and eviscerated immediately to 7.92 and 7.32 μmol/g of meat in samples stored at 25 °C for 6 and 12 h, respectively. The sum of HxR and Hx increased from 1.40 to 1.98 and 2.55 μmol/g of meat after 6- and 12-h storage at 25 °C, respectively (Table I). The contents of HxR and Hx were high in fresh samples. This might be due to the struggling during netting and transporting and might accelerate the degradation of adenosine nucleotides. Samples with different levels of adenosine nucleotides were subjected to the frozen storage at −20 °C for 18 weeks. The levels of ATP, ADP, AMP, and IMP decreased gradually during storage. The ATP in sample I disappeared after 3 weeks of storage, while that in samples II and III disappeared after freezing. The development of HxR and Hx in all samples was observed during 18 weeks of storage. The K values calculated from these data were 14.1, 20.0, and 25.8% in samples stored at 25 °C for 0, 6, and 12 h, respectively. After 18 weeks of storage, they increased to 18.3, 24.9, and 32.4%, respectively.

According to these data (Table I), it appears that the changes in levels of adenosine nucleotides are much faster in room-temperature than in frozen storage. As indicated in Table I, the increase in K value of samples stored at 25 °C for 6 h was almost the same as that of samples stored at −20 °C for 18 weeks. It suggests the importance of temperature in handling, transportation, storage, and processing of fish muscle.

Protein Denaturation during Frozen Storage. The degree of protein denaturation was evaluated by measuring the extractability of actomyosin (AM) and Ca-ATPase and Mg(EGTA)-ATPase activities of AM during frozen storage. The extractable AM values were 91.1, 88.5, and 90.2 mg/g of meat in samples stored at 25 °C for 0, 6, and 12 h, respectively (Table II). No significant difference in extractability of AM was observed during storage at 25 °C for 12 h. However, significant deterioration in Ca-ATPase specific activity and total activity was obtained during storage at 25 °C. They were 0.563, 0.440, and 0.402 μmol of P_i/min per mg of protein and 512.9, 392.1, and 362.6 μmol of P_i/min per 10 g of meat after 0, 6, and 12 h of storage, respectively.

AM is considered to be the protein group responsible for the gel strength, or ashi, of minced fish products. As shown in Table II, insolubilization of AM was observed in all samples during frozen storage. The highest AM insolubilization was found in sample III, which had the

Table I. Changes in the ATP and Its Derivatives of Milkfish during Frozen Storage at -20 °C

sample[a]	storage time, weeks	μmol/g of meat ATP	ADP	AMP	IMP	HxR	Hx	K, %
I	F[b]	0.14	0.43	0.21	7.73	0.84	0.56	14.1
		(8.4)[c]	(5.6)	(9.2)	(3.4)	(6.5)	(5.4)	
	0	0.13	0.31	0.35	7.81	0.84	0.53	13.7
		(9.2)	(8.7)	(6.3)	(2.8)	(4.8)	(6.9)	
	3		0.68	0.22	7.92	0.96	0.56	14.7
			(4.2)	(10.2)	(4.4)	(5.8)	(6.5)	
	6		0.62	0.20	7.45	0.84	0.73	16.0
			(6.4)	(9.5)	(3.8)	(6.7)	(3.2)	
	12		0.51	0.17	7.17	0.85	0.64	16.0
			(4.8)	(9.8)	(4.6)	(7.4)	(6.6)	
	18		0.43	0.19	6.93	1.01	0.68	18.3
			(7.8)	(8.5)	(5.2)	(6.9)	(8.4)	
II	F	0.03	0.41	0.26	7.22	1.34	0.64	20.0
		(11.2)	(7.6)	(9.8)	(4.3)	(5.7)	(9.5)	
	0		0.51	0.25	7.30	1.50	0.67	21.2
			(8.1)	(9.2)	(3.7)	(4.2)	(6.9)	
	3		0.44	0.13	6.88	1.37	0.75	22.2
			(6.5)	(12.1)	(7.4)	(8.9)	(10.0)	
	6		0.37	0.20	7.28	1.45	0.68	21.4
			(11.9)	(10.8)	(8.1)	(3.7)	(8.4)	
	12		0.40	0.13	6.83	1.50	0.76	23.5
			(9.6)	(13.9)	(6.6)	(5.9)	(7.7)	
	18		0.34	0.12	6.90	1.59	0.85	24.9
			(12.8)	(14.2)	(7.9)	(8.1)	(6.5)	
III	F	0.01	0.33	0.25	6.73	1.64	0.91	25.8
		(14.0)	(7.6)	(8.9)	(4.8)	(5.2)	(7.7)	
	0		0.49	0.16	6.44	1.75	0.85	26.8
			(8.8)	(12.5)	(3.1)	(5.9)	(7.1)	
	3		0.35	0.10	6.34	1.70	1.10	29.2
			(9.9)	(13.8)	(6.3)	(7.5)	(8.8)	
	6		0.27	0.22	6.60	1.87	1.16	29.9
			(11.4)	(9.8)	(7.4)	(8.6)	(5.7)	
	12		0.30	0.11	6.17	1.98	1.02	31.3
			(9.8)	(14.2)	(5.8)	(7.1)	(8.3)	
	18		0.21	0.23	6.06	2.07	1.04	32.4
			(10.9)	(12.7)	(8.4)	(6.5)	(9.1)	

[a] Sample I: samples immediately killed and eviscerated; K value of this sample 14.1%. Sample II: samples immediately killed, eviscerated, and stored at room temperature for 6 h; K value of this sample 20.0%. Sample III: samples immediately killed, eviscerated, and stored at room temperature for 12 h; K value of this sample 25.8%. [b] F = unfrozen. [c] Values in parentheses, percentage coefficient of variation.

Table II. Effect of the Freshness on Extractability of Actomyosin and Ca-ATPase Activity of Actomyosin of Milkfish during Frozen Storage at -20 °C

sample[a]	fresh[b]	storage time, weeks 0	3	6	9	12	18
		Extractability of Actomyosin, mg/g of Meat					
I	91.1	94.5	83.9	83.2	80.2	76.1	74.5
	(100)[a]	(103.7)[a]	(92.1)[a]	(91.3)[a]	(88.0)[a]	(83.5)[a]	(81.8)[a]
II	88.5	90.7	80.5	74.7	70.3	68.5	64.4
	(100)[a]	(102.5)[a]	(91.0)[a]	(84.4)[b]	(79.4)[b]	(77.4)[b]	(72.8)[b]
III	90.2	84.5	75.1	70.5	67.0	60.9	52.3
	(100)[a]	(93.7)[b]	(83.3)[b]	(78.2)[c]	(74.3)[c]	(67.5)[c]	(58.0)[c]
		Ca-ATPase-Specific Activity, μmol of P_i/min per mg of Protein					
I	0.563	0.432	0.417	0.330	0.335	0.305	0.295
	(100)[a]	(76.7)[b]	(74.1)[a]	(58.6)[a]	(59.5)[a]	(54.2)[a]	(52.4)[a]
II	0.443	0.314	0.285	0.240	0.180	0.175	0.106
	(100)[a]	(70.9)[c]	(64.3)[b]	(54.2)[b]	(40.6)[b]	(39.5)[b]	(23.9)[b]
III	0.402	0.322	0.258	0.200	0.142	0.121	0.078
	(100)[a]	(80.1)[a]	(64.2)[b]	(49.8)[c]	(35.3)[c]	(30.1)[c]	(19.4)[c]
		Ca-ATPase Total Activity, μmol of P_i/min per 10 g of Meat					
I	512.9	408.2	349.9	274.6	268.7	232.1	219.8
	(100)[a]	(79.6)[a]	(68.2)[a]	(53.5)[a]	(52.4)[a]	(45.3)[a]	(42.9)[a]
II	392.1	284.8	229.4	179.3	126.5	119.9	68.3
	(100)[a]	(72.6)[a]	(58.5)[b]	(45.7)[b]	(32.3)[b]	(30.6)[b]	(17.4)[b]
III	362.6	272.1	193.8	141.0	95.1	73.7	40.8
	(100)[a]	(75.0)[b]	(53.4)[c]	(38.9)[c]	(26.2)[c]	(20.3)[c]	(11.3)[c]

[a] Refer to the footnote of Table I. [b] Values in parentheses were the percentage ratios relative to the orginal values. Values in the same column bearing unlike letters differ significantly ($P < 0.01$).

highest level of the sum of HxR and Hx and with the lowest level of the sum of ATP, ADP, AMP, and IMP, and then sample II and sample I in decreasing order. Protein quality is considered to be more sensitively reflected by the enzymatic activity than the extractability. This is because small microstructural changes in protein molecules

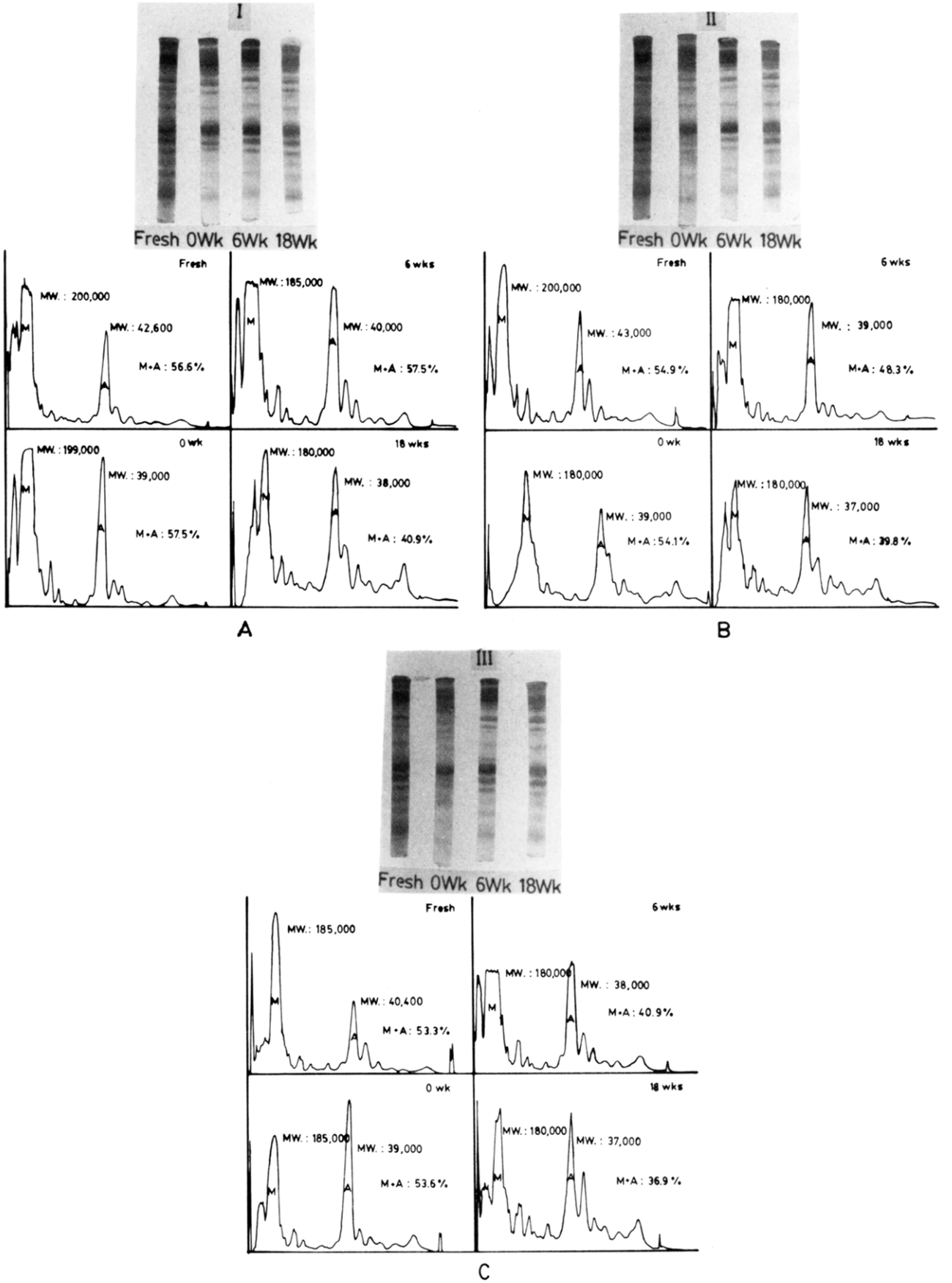

Figure 1. Electrophoretic changes in actomyosin from muscles with different levels of adenosine nucleotides during frozen storage at −20 °C: A, samples killed and eviscerated immediately; B, samples stored at 25 °C for 6 h; C, samples stored at 25 °C for 12 h.

will cause alterations in enzymatic activity. As shown in Table II, the Ca-ATPase activity of AM in samples II and III lost 59.4 and 50.2% of the original value after 9 and 6 weeks of storage, respectively. The Ca-ATPase total activity was recognized to be more sensitive, to reflect the protein quality of fish muscle, than specific activity and

Table III. Effect of the Freshness on Rate of Quality Change of Milkfish during Frozen Storage at -20 °C

sample[a]	rate of change: extractable actomyosin, mg/g of meat per week	Ca-ATPase sp act., μmol of P_i/min per mg of AM per week	Ca-ATPase total act., μmol of P_i/min per 10 g per week
I	-1.011[b]	-8.08×10^{-3}[c]	-10.33[d]
II	-1.377[b]	-11.8×10^{-3}[c]	-11.88[d]
III	-1.709[b]	-13.6×10^{-3}[c]	-12.52[d]

[a] Refer to the footnote of Table I. [b] Regression equation: sample I, $y = 90.154 - 1.011x$, $r = -0.92$; sample II, $y = 85.867 - 1.377x$, $r = -0.94$; sample III, $y = 82.056 - 1.709x$, $r = 0.99$. x = storage time (week); y = extractable actomyosin (mg/g of meat). [c] Regression equation: sample I, $y = 0.4169 - 0.0081x$, $r = -0.90$; sample II, $y = 0.3110 - 0.0118x$, $r = -0.99$; sample III, $y = 0.2958 - 0.0136x$, $r = -0.97$. x = storage time (week); y = Ca-ATPase specific activity (μmol/min per mg of AM). [d] Regression equation: sample I, $y = 374.853 - 10.330x$, $r = -0.92$; sample II, $y = 263.077 - 11.880x$, $r = -0.97$; sample III, $y = 236.270 - 12.523x$, $r = -0.95$. x = storage time (week); y = Ca-ATPase total activity (μmol of P_i/min per 10 g of meat).

Table IV. Effect of the Freshness on Mg(EGTA)-ATPase Activity of Actomyosin of Milkfish during Frozen Storage at -20 °C

sample[a]	activity, μmol of P_i/min per mg of AM: fresh	0 weeks	3 weeks	6 weeks	9 weeks	12 weeks	18 weeks
I	0.071	0.071	0.094	0.251	0.248	0.197	0.189
II	0.082	0.064	0.203	0.200	0.198	0.151	0.132
III	0.083	0.090	0.250	0.245	0.208	0.194	0.197

[a] Refer to the footnote of Table I.

extractability of AM (Arai, 1976). The Ca-ATPase total activity of samples I–III decreased to below 50% of the original value after 12, 6, and 6 weeks of storage, respectively (Table II). Considering the loss in extractable AM and Ca-ATPase specific and total activities of AM, the insolubilization and inactivation rate was fastest in sample III, followed by sample II and sample I, respectively (Table III).

As indicated in Figure 1, parts A–C, the same patterns of electrophoretic separation of AM from the fresh state of all samples were obtained. However, decreases in molecular weights of myosin heavy chain (MHC) and actin were observed in sample III. This hinted that some autolysis of myofibrillar protein occurred on MHC and actin during storage at 25 °C. No changes in subunit composition of all samples was observed even after 18 weeks of storage at -20 °C. The percentage ratio of MHC and actin to the total subunits and molecular weights of MHC and actin changed during frozen storage. For sample I, the percentage of MHC and actin decreased from 56.6% to 40.9%, while the molecular weights of MHC and actin decreased from 200000 and 42600 Da to 199000 and 39000 Da after freezing and to 180000 and 38000 Da, respectively, after 18 weeks of storage (Figure 1A). As shown in Figure 1, parts B and C, the percentage of MHC and actin in samples II and III decreased from 54.9 and 53.3% to 39.8 and 36.9%, respectively, after 18 weeks of storage. The molecular weights of MHC and actin were decreased after freezing and further during frozen storage. These data suggested that some hydrolysis in MHC and actin of myofibrillar proteins occurred during freezing, frozen storage, and thawing.

The initial levels of Mg(EGTA)-ATPase activity of all samples were very low (Table IV). No distinct changes in Mg(EGTA)-ATPase activity of all samples during early storage were obtained; however, rapid increases occurred in samples I–III after 6, 3, and 3 weeks of storage at -20 °C, respectively. A rapid increase in Mg(EGTA)-ATPase activity suggested that loss in Ca sensitivity occurred on the myofibrillar proteins (Seki et al., 1979). From these data, it seems likely that loss of Ca sensitivity of myofibrillar proteins occurred in samples II and III sooner than in sample I.

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 to be due to the

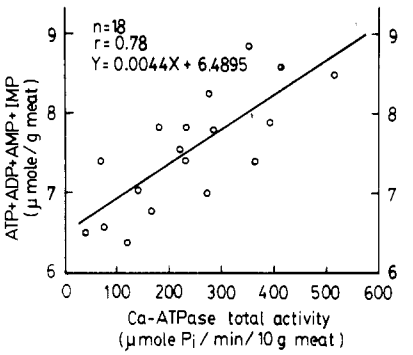

Figure 2. Relationship between the sum of the quantity of adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), and inosine 5′-monophosphate (IMP) and Ca-ATPase total activity ($r = 0.78$, $n = 18$, $y = 6.4898 + 0.0044x$).

filamentation of myofibrils caused by the hydrolysis of protease (Tokiwa and Matsumiya, 1969). 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 during ice storage is not due to the hydrolysis of tropomyosin and troponins by protease but is due to the modification of actin–myosin interaction by oxidation of the thiol groups of the myosin moiety. In this study, although the Ca sensitivity is lost after storage, the tropomyosin and troponin could still be clearly seen on the electrophoretic separation. This might also be because of the oxidation of thiol groups on protein molecules, consequently causing the loss of Ca-ATPase activity and Ca sensitivity during frozen storage. This result is concordant with the study by Jiang et al. (1986).

Since the extractable AM is considered to be native, the decrease in molecular weights of MHC and actin during frozen storage (Figure 1, parts A–C) hinted that some hydrolysis occurred on myofibrillar proteins. From the Mg(EGTA)-ATPase activity, however, oxidation of the thiol groups of extractable AM shall be intramolecular.

Relationship between the Content of Adenosine Nucleotides and Protein Denaturation. According to the statistical analysis, the decreases in the contents of ATP, ADP, AMP, and IMP and consequently increases in HxR and Hx were related to the instability of muscle proteins (Figures 2 and 3). The correlation between the

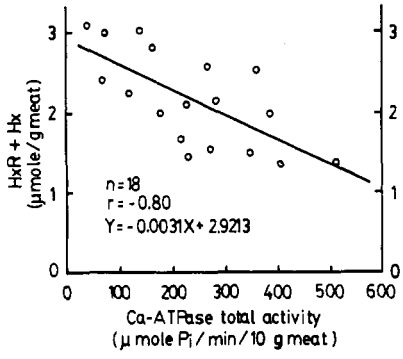

Figure 3. Relationship between the content of inosine (HxR) and hypoxanthine (Hx) and Ca-ATPase total activity ($r = -0.80$, $n = 18$, $y = 2.9213 - 0.0031x$).

contents of HxR and Hx and Ca-ATPase total activity of AM was −0.80, while that between the contents of ATP, ADP, AMP, and IMP and Ca-ATPase total activity of AM was 0.78. These correlations suggested that adenosine nucleotides might be involved in protein denaturation. For understanding the effects of adenosine nucleotides per se on denaturation of myofibrillar proteins, more detailed studies on the interaction between these nucleotides and protein molecules are necessary to elucidate the nature and mechanism of denaturation.

Registry No. ATP, 56-65-5; ADP, 58-64-0; AMP, 61-19-8; IMP, 131-99-7; Hx, 68-94-0; ATPase, 9000-83-3; inosine, 58-63-9.

LITERATURE CITED

Acton, J. C.; Zieler, G. R.; Burge, D. L., Jr. *CRC Crit. Rev. Food Sci. Nutr.* **1983**, *18*(2), 99.
Andou, S.; Takama, K.; Zama, K. *Bull. Fac. Fish., Hokkaido Univ.* **1979**, *30*(4), 282.
Andou, S.; Takama, K.; Zama, K. *Bull. Fac. Fish., Hokkaido Univ.* 1980, *31*, 201.
Arai, K. In *Quality of Fish*; Japanese Society of Scientific Fisheries Education, Koseisha: Tokyo, 1974; pp 55–80.
Arai, K. *Refrigeration* **1976**, *51*, 195.
Burton, K. In *Data for Biochemical Research*; Dawson, R. M. C., Elliott, D. C., Elliott, W. H., Jones, K. M. Eds.; Clarendon: Oxford, 1982; pp 170–177.
Dyer, W. J. *Food Res.* **1951**, *16*, 522.
Ebashi, S.; Kodama, A.; Ebashi, F. *J. Biochem.* **1968**, *64*, 465.
Ehira, S.; Uchiyama, H.; Uda, F. In *Suisan Seibutsu-Kagaku Shokuhin Zikensho*; Saito et al., Eds.; Koseisha: Tokyo, 1974; p 19.
Fukuda, Y.; Tarakita, Z.; Arai, K. *Bull. Jap. Soc. Sci. Fish.* **1984**, *50*, 845.
Jiang, S. T. Doctoral Dissertation, University of Rhode Island, 1984.
Jiang, S. T.; Lee, T. C. *J. Agric. Food Chem.* **1985**, *33*, 839.
Jiang, S. T.; Lan, C. C.; Tsao, C. Y. *J. Food Sci.* **1986**, *51*, 310.
Kato, N.; Uchiyama, H.; Uda, F. In *Suisan Seibutsu-Kagaku Shokuhin Zikensho*; Saito et al., Eds.; Koseisha: Tokyo, 1974; pp 32–36.
Matsumoto, J. J. In *Proteins at Low Temperature*; fennema, O., Ed.; ACS Symposium Series 180; American Chemical Society: Washington, DC, 1979; pp 205–224.
Matsumoto, J. J. In *Chemical Deterioration of Proteins*; Whitkaker, J. R., Fujimai, M, Eds.; ACS Symposium Series 123; American Chemical Society: Washington, DC, 1980; pp 95–124.
Noguchi, S. Doctoral Dissertation, Sophia University, Tokyo, Japan, 1974.
Noguchi, S. *New Food Ind.* **1982**, *21*(4), 34.
Noguchi, S.; Matsumoto, J. J. *Bull. Jpn. Soc. Sci. Fish.* **1970**, *36*, 1078.
Seki, N. In *Suisan Seibutsu-Kagaku Shokuhin Zikensho*; Saito et al., Eds.; Koseisha: Tokyo, 1974; pp 124–132.
Seki, N.; Hasegawa, S. *Bull. Jpn. Soc. Sci. Fish.* **1978**, *44*, 71.
Seki, N.; Iwabuchi, S. *Bull. Jpn. Soc. Sci. Fish.* **1978**, *44*, 231.
Seki, N.; Ikeda, M.; Narita, N. *Bull. Jpn. Soc. Sci. Fish.* **1979**, *45*, 791.
Shenouda, S. Y. K. In *Adv. Food Res.* **1980**, *26*, 275.
Shitamura, M.; Seki, N. *Bull. Jpn. Soc. Sci. Fish.* **1978**, *44*, 231.
Sikorski, Z. *Int. J. Refrig.* **1978**, *1*(3), 173.
Sikorski, Z. In *Adv. Fish Sci. Tech.* **1980**, 78.
Sikorski, Z.; Olley, J.; Kostuch, S. *CRC Crit. Rev. Food Sci. Nutr.* **1976**, *8*(1), 97.
Symons, R. H. In *Data for Biochemical Research*; Dawson, R. M. C., Elliott, D. C., Elliott, W. H., Jones, K. M. Eds.; Clarendon: Oxford, 1982; pp 145–167.
Tokiwa, T.; Matsumiya, H. *Bull. Jpn. Soc. Sci. Fish.* **1969**, *35*, 1099.
Tsao, C. Y.; Chou, K. C.; Jiang, S. T. *J. Chin. Agric. Chem. Soc.* **1980**, *18*, 77.
Umemoto, S. *Bull. Jpn. Soc. Sci. Fish.* **1966**, *32*, 427.
Weber, K.; Osborn, M. *J. Biol. Chem.* **1969**, *244*, 4406.

Received for review February 21, 1986. Accepted October 5, 1986. This research work was supported by the National Science Council, Republic of China, under Grant No. NSC 74-0409-B019-03.