Effect of Free Amino Acids on the Denaturation of Mackerel Myofibrillar Proteins in Vitro during Frozen Storage at −20 °C

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To investigate how the free amino acids affect the stability of muscle proteins, actomyosin (AM) was extracted from mackerel dorsal muscle and suspended in 0.10 M KCl solution. Histidine, lysine, taurine, glycine, and proline known to be relatively highly contained in mackerel, amberfish, and mullet muscle and glutamic acid, an acidic amino acid, were added to AM, and the resultant mixture was stored at −20 °C. The protein denaturation was evaluated by measuring the changes in solubility and Ca-ATPase and Mg(EGTA)-ATPase activities and by electrophoretic analysis of AM during storage. According to the infrared spectral analysis, the NH_3 group of these amino acids interacted with C=O groups on proteins and subsequently weakened the C=O stretching frequency after being added. Histidine, lysine, and taurine accelerated while glutamic acid and proline prevented the protein denaturation, as compared to the control samples.

Marked deteriorative changes in quality of seafoods have frequently taken place after prolonged frozen storage. These undesirable changes are recognized to be resulting from the deteriorative biochemical reactions occurring in lipids and proteins (Sikorski, 1978, 1980; Matsumoto, 1979, 1980; Suzuki, 1981; Noguchi, 1982). However, changes in texture as a consequence of long-term storage are recognized to be due to severe alterations of muscle proteins, usually termed denaturation–aggregation (Dyer, 1951; Sikorski et al., 1976; Sikorski, 1978, 1980; Matsumoto, 1979, 1980; Shenouda, 1980; Andou et al., 1980; Noguchi, 1982; Jiang and Lee, 1985). Most of the alterations occur in the myosin–actomyosin system (Sikorski et al., 1976). Some studies revealed that myosin, upon standing in solutions or frozen solutions, tended to aggregate almost exclusively side-to-side and to form dimers, trimers, and high molecular weight polymers (Lowey and Holtzer, 1959; Connell, 1959, 1960, 1962, 1963; Buttkus, 1970). Many studies also indicated that the myofibrillar proteins of fish muscle aggregated into high molecular weight polymers during frozen storage (King, 1966; Anderson and Ravesi, 1970; Childs, 1973; Jarenback and Liljemark, 1975; Jiang, 1977, 1984; Jiang and Lee, 1985). Although interactions between myofibrillar proteins and other constituents have been investigated to elucidate the mechanism of protein denaturation during storage or frozen storage (Andou et al., 1979, 1980, 1981a, 1981b; Braddock and Dugan, 1973; Braun and Radin, 1969; Childs, 1973, 1974; Fullington, 1969; Hironaka et al., 1976; Jarenback and Liljemark, 1975; Karel et al., 1975; King et al., 1962; Kostuch and Sikorski, 1977; Noguchi, 1974; Noguchi et al., 1976; Ooshiro et al., 1976; Robenson, 1966; Takama, 1974a, 1974b; Jiang, 1984), little is know about the roles of some muscle components such as free amino acids on various protein denaturation (Shenouda, 1980). Previously, Jiang and Lee (1985) reported that the content and composition of free amino nitrogen in fish muscle were related to the stability of muscle proteins. In free amino acids of mackerel, amberfish, and mullet muscle, the histidine, lysine, taurine, glycine, and proline were relatively higher than others. This study was carried out to investigate the effect of these free amino acids and glutamic acid, an acidic amino acid, on protein denaturation and the interactions between these free amino acids and myofibrillar proteins in vitro during frozen storage at −20 °C.

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MATERIALS AND METHODS

Preparation of the Actomyosin. Actomyosin (AM) was extracted according to Noguchi (1974). Ten grams of mackerel dorsal muscle (*Scomber lapeinocephalus*) was blended by using an Waring Blendor subjoined with a baffle plate for 2 min with 90 mL of chilled 0.6 M KCl solution (pH 7.2). The extract was centrifuged at 5000*g*, 0 °C for 20 min. AM was precipitated by diluting with 2 vol of chilled distilled water and collected by centrifuging at 5000*g*, 0 °C for 20 min. The AM was then suspended in chilled 0.10 M KCl solution (pH 7.2), an ionic strength almost equivalent to that in fish muscle, and mixed with a magnetic stirrer for 1 h at 0 °C. The protein solution was made to 300 mL with chilled 0.10 M KCl solution for model test, resulting in a concentration of 2.8 mg/mL.

Histidine (His), lysine (Lys), taurine (Tau), proline (Pro), and glycine (Gly) were found to be relatively highly contained in the free amino acids of mackerel, amberfish, and mullet muscle (Jiang and Lee, 1985). In order to investigate the effects of these free amino acids and the acidic amino acid glutamic acid (Glu) on protein denaturation, each amino acid was added to an AM solution and the resultant solution stored at −20 °C for 12 weeks. Each AM solution had a final concentration of 3 μmol of amino acid/mL. At definite time intervals, samples were removed. One part was freeze-dried on a freeze-dryer (Refrigeration for Science, Inc.) at a plate temperature of 30 °C and subjected to the infrared spectral analysis. The rest of each sample was thawed at room temperature (25 °C) and subjected to the solubility, Ca-ATPase activity, Mg(EGTA)-ATPase activity, and SDS polyacrylamide gel electrophoresis analyses.

Infrared Spectral Analysis. Two milligrams of freeze-dried protein powder was ground with pestle and mortar; 200 mg of potassium bromide was then added. This mixture was ground to a very fine particle size of 200–300 mesh. The mixture was then placed on a tabulet former with a vacuum pump providing a pressure of 0.1 Torr and pressed into a transparent disk (13 × 1 mm) under 600 kg/cm^2 pressure for 30 s.

The infrared spectra of the samples were recorded from 400 to 4000 cm^{-1} on an infrared spectrophotometer (Hitachi Model 260-30; Hitachi Ltd., Tokyo, Japan). The changes in amides I and II were used for evaluating the conformational change of protein molecules after the addition of these amino acids, during frozen storage and freeze-drying.

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Solubility. Potassium chloride solution (1.2 M) was added to the freeze–thawed suspension before homogenization to bring the salt concentration to 0.6 M KCl. After being mixed by a magnetic stirrer for 30 min at 0–5 °C, the resultant solution was centrifuged at 5000*g*, 0 °C for 20 min. The supernatant was used for solubility and Ca-ATPase and Mg(EGTA)-ATPase [EGTA = ethylene glycol bis(2-aminoethyl ether)tetraacetic acid] activity analyses.

The protein concentrations of these supernatants were determined by using the Biuret method modified by Umemoto (1966). The solubility was expressed as milligrams of protein/milliliter and the percentage ratio of the quantity of soluble protein to that of original quantity of AM.

Ca-ATPase Activity. To 1 mL of AM solution (1–3 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 finally 0.5 mL of 20 mM Na_2ATP solution (pH 7.0) in that order. The rate of release of inorganic phosphate within 3 min of reaction at 25 °C 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 liberated by 1 mg of protein within 1 min of reaction at 25 °C.

Mg(EGTA)-ATPase Activity. To 1 mL of AM solution (1–3 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 finally 1.0 mL of 20 mM Na_2ATP solution (pH 7.0) in that order. The rate of release of inorganic phosphate within 3 min of reaction at 25 °C 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 activity was shown as micromoles of inorganic phosphate liberated by 1 mg of protein within 1 min of reaction at 25 °C.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE) Analysis. For investigation of the changes in electrophoretic separation of myofibrillar proteins during frozen storage. SDS PAGE was performed according to Weber and Osborn (1969). After the salt concentration of freeze–thawed suspensions was made 0.6 M KCl, protein solutions before precipitation of the denatured proteins and after centrifugation at 5000*g*, 0 °C for 20 min, to precipitate the denatured part, were dialyzed overnight at room temperature against 0.01 M sodium phosphate buffer containing 0.1% SDS (pH 7.2) and then incubated at 40 °C for 2 h in a solubilizing solution consisting of 0.01 M sodium phosphate buffer, 1% SDS, 25% glycerin, and 2% mercaptoethanol (pH 7.2). About 0.02 mL of 0.05% bromophenol blue and 0.06 mL of the solubilized protein sample were dropped on the top of gel with micropipet in turn.

The polyacrylamide gel was prepared basically as described by Weber and Osborn (1969). The concentration of polyacrylamide was 7.5%. After the electrophoretic run in 0.1% SDS–0.1 M sodium phosphate buffer (8 mA each gel), the gels were stained for 3–4 h with 0.12% Coomassie blue and 50% methanol in 9.2% acetic acid solution. They were destained with 50% methanol in 7.5% acetic acid solution for 8–10 h, as recommended by Seki (1974).

Distance scanning at the wavelength of 585 nm, at which the staining solution has maximum absorbance, was employed to analyze the bands of gel by using UV–vis microprocessor-controlled spectrophotometer system 2600 (Gilford Instrument Laboratory Inc.)

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

Table I. Effect of Free Amino Acids on the Solubility[a] of Actomyosin in Vitro during Frozen Storage at −20 °C

	weeks at −20 °C			
amino acid	0	4	8	12
control	1.518[c,b]	1.042[d]	0.731[d]	0.515[d]
	(54.2)[c]	(37.2)	(26.1)	(18.4)
histidine	1.389[d]	0.812[e]	0.574[e]	0.339[e]
	(49.6)	(29.0)	(20.5)	(11.4)
lysine	1.358[d]	0.781[e]	0.588[e]	0.331[e]
	(48.5)	(27.9)	(21.0)	(11.8)
taurine	1.434[cd]	0.583[f]	0.498[f]	0.294[e]
	(51.2)	(20.8)	(17.8)	(10.5)
glycine	2.074[b]	1.281[c]	0.868[c]	0.571[c]
	(73.1)	(45.8)	(31.0)	(20.4)
glutamic acid	2.355[a]	2.341[a]	1.756[a]	1.478[a]
	(84.1)	(83.6)	(62.7)	(52.8)
proline	2.295[a]	2.156[b]	1.260[b]	1.196[b]
	(82.0)	(77.0)	(45.0)	(42.7)

[a] The solubility of actomyosin was expressed as milligrams of soluble protein/milliliter of solution. [b] Values in the same column bearing unlike letters differ significantly ($P < 0.01$). [c] Values in the parentheses were the percentage ratios of the quantity of soluble protein to the original one.

RESULTS AND DISCUSSION

Effect of Free Amino Acids on the Denaturation of Actomyosin during Frozen Storage. The solubility of AM with Glu, Pro, and Gly was significantly higher than that of samples with His, Lys, and Tau and of control sample immediately after freezing (Table I). The percentage ratios of the soluble proteins to the original ones were 84.1, 82.0, and 73.1% in samples with Glu, Pro, and Gly, respectively, after freezing. They decreased to 52.8, 42.7, and 20.4%, respectively, after 12 weeks of storage. The solubilities of control sample and samples with His, Lys, and Tau were significantly lower than those of samples with Glu, Pro, and Gly (Table I).

The Ca-ATPase specific activity of AM after being extracted was 0.387 μmol of P_i/min per mg of AM. However, that of samples without (control) and with the addition of free amino acids significantly decreased, as compared to the unfrozen AM. After 12 weeks of storage, samples with Glu, Pro, and Gly revealed higher Ca-ATPase activity than control sample and samples with His, Lys, and Tau (Table II). Considering the changes in solubility and Ca-ATPase activity during freezing and frozen storage, AM with His, Lys, and Tau denatured more than the control sample. The addition of Glu, Pro, and Gly revealed a preventive effect on the denaturation of AM during freezing and frozen storage, as compared to the control one.

AM is recognized to be the protein group responsible for the gel strength, or ashi, of minced fish products. Several cryoprotectives have already been developed for increasing the stability of frozen surimi (or frozen minces). Sucrose and sorbitol are the most commonly used in frozen surimi processing (Arai et al., 1970; Okada et al., 1974). The sodium glutamate and Glu are also developed for preventing the protein denaturation of frozen surimi (Matsumoto, 1979, 1980). According to the data obtained from this study (Tables I and II), the Pro also has the potential use in frozen surimi processing as a cryoprotective to substitute for the sucrose and sorbitol. It is well-known that 5–10% of sucrose and sorbitol used in frozen surimi resulted in an oversweet taste, and consequently the utilization of this kind product is limited. If the Pro can be used in this product, the problem of oversweet taste will

Table II. Effect of Free Amino Acids on the Ca-ATPase Specific Activity[a] of Actomyosin in Vitro during Frozen Storage at -20 °C

	weeks at -20 °C			
amino acid	0	4	8	12
control	0.194[c,b]	0.153[c]	0.082[d]	0.054[d]
	(50.1)[c]	(39.5)	(21.2)	(14.0)
histidine	0.175[d]	0.121[d]	0.071[e]	0.045[e]
	(45.2)	(31.3)	(18.3)	(11.6)
lysine	0.187[c,d]	0.135[d]	0.083[d]	0.044[e]
	(48.2)	(34.8)	(21.4)	(11.5)
taurine	0.167[e]	0.126[d]	0.072[e]	0.042[e]
	(43.2)	(32.6)	(18.7)	(10.8)
glycine	0.205[c]	0.163[c]	0.139[c]	0.098[c]
	(52.9)	(42.1)	(35.8)	(25.4)
glutamic acid	0.332[a]	0.319[a]	0.266[a]	0.240[a]
	(85.9)	(82.4)	(68.7)	(62.1)
proline	0.297[b]	0.264[b]	0.239[b]	0.211[b]
	(76.7)	(68.2)	(61.8)	(54.5)

[a] The Ca-ATPase specific activity was expressed as micromoles of P_i/minute per milligram of protein. [b] Values in the same column bearing unlike letters differ significantly ($P < 0.01$). [c] Values in the parentheses were the percentage ratios of the quantity of soluble protein to the original one.

Table III. Effect of Free Amino Acids on the Mg(EGTA)-ATPase Activity[a] of Actomyosin in Vitro during Frozen Storage at -20 °C

	weeks at -20 °C			
amino acid	0	4	8	12
control	0.054[c,b]	0.210[b]	0.221[a]	0.211[b]
histidine	0.049[c]	0.232[a]	0.211[b]	0.213[b]
lysine	0.062[c]	0.228[a]	0.221[a]	0.209[b]
taurine	0.048[c]	0.236[a]	0.229[a]	0.217[b]
glycine	0.066[d]	0.098[c]	0.206[b]	0.243[a]
glutamic acid	0.053[d]	0.067[c]	0.077[b]	0.225[a]
proline	0.069[c]	0.066[c]	0.086[b]	0.234[a]

[a] The Mg(EGTA)-ATPase activity was expressed as micromoles of P_i/minute per milligram of protein. [b] Values in the same row bearing unlike letters differ significantly ($P < 0.01$).

be overcome and then increase the utilization of this product. More detailed in situ experiments for using Pro in frozen surimi processing are doing in our laboratory now.

The Mg(EGTA)-ATPase activities of those samples without and with free amino acids were low during early stages of storage; however, that of the control sample and samples with His, Lys, and Tau rapidly increased after 4 weeks of storage and maintained high levels during prolonged storage (Table III). That of samples with the addition of Glu and Pro revealed low levels during 8 weeks of storage and rapidly increased after 12 weeks of storage, while that of samples with Gly rapidly increased after 8 weeks of storage (Table III). It suggested that the Ca sensitivity of AM of the control sample and samples with His, Lys, Tau, and Gly was lost sooner than in those samples with Glu and Pro. The addition of Glu and Pro showed good effect on retarding the loss of Ca sensitivity of myofibrillar proteins during storage. 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 filamentation of myofibrils caused by hydrolysis of protease (Tokiwa and Matsumiya, 1969). However, according to the studies by Seki and Hasegawa (1978), Seki and Iwabuchi (1978), Shitamura and Seki (1978), and Seki et al. (1979), the loss in Ca sensitivity of myofibrillar proteins is due to the modification of actin–myosin interaction by oxidation of the thiol groups of myosin moiety. The rapid increase in Mg(EGTA)-ATPase activity of these sample hinted that the oxidation of sulfhydryl groups into disulfide bonds was an important factor in denaturation of frozen muscle proteins. This can also support the previous studies by Jiang et al. (1984, 1986).

Effect of Free Amino Acids on the Electrophoretic Profiles of Actomyosin during Frozen Storage. No distinct changes in electrophoretic separation of AM was observed after the addition of free amino acids (I in Figure 1A–D). Considering the corresponding changes in electrophoretic profiles of those samples, the electrophoretic separation of samples before precipitation of the denatured proteins after 12 weeks of storage revealed almost the same patterns including the band size and subunit compositions as that of samples before freezing (I and II in Figure 1A–D). However, the electrophoretic separation of the soluble proteins of the control sample and samples with His, Lys, and Tau were considerably different from that of unfrozen samples: the myosin heavy chain (MHC) in soluble proteins of the control and samples with the addition of His, Lys, and Tau disappeared and subsequently formed many unknown components with molecular weights around 250 000–90 000 Da after 12 weeks of storage. The actin in soluble proteins of the control sample and those with His, Lys, and Gly markedly decreased after 12 weeks of storage, in comparison with the unfrozen samples (I and III in Figure 1A–C). With respect to the tropomyosin in samples with Glu, no distinct changes between samples before freezing and before precipitating the denatured part after 12 weeks of storage were observed. The band size of tropomyosin in samples before precipitation of the denatured part, with His, Lys, Tau, and Pro, considerably decreased after 12 weeks of storage, as compared to that of samples before freezing (I and II in Figure 1A–D). The band size of tropomyosin also markedly decreased in soluble protein (considered to be native) of all samples, especially in the control sample and those with His, Lys, Gly, and Pro after 12 weeks of storage. This phenomenon suggested that, in comparison with that of control samples, the addition of His, Lys, Gly, and Pro caused the denaturation not only on MHC and actin but on tropomyosin during frozen storage (II and III in Figure 1A–C).

According to those electrophoretic analyses, it can be proposed that the myofibrillar proteins of the control sample and those with His, Lys, and Tau denatured mainly on MHC and actin and that with His, Lys, Gly, and Pro further denatured on tropomyosin during frozen storage. This might be due to both dissociation and aggregation.

From the data depicted above, the Glu and Pro showed preventive effects on the denaturation of AM, whereas the His, Lys, and Tau accelerated the denaturation of AM during frozen storage, as compared to the control samples. The inhibitory effect on the protein denaturation appeared to be related to the isoelectric point (PI) and the functional groups the free amino acids possessed. The free amino acids where the PI values are lower than the pH in the protein solution showed protective effects on protein stability, whereas those where the PI are higher than the pH in protein solution revealed detrimental effects on the AM.

The preventive effect of Glu is considered to be due to the low PI, around 3.22, and its carboxyl group. The carboxyl groups will ionize and show a net negative charge in an environment with pH higher than PI. When this type of amino acid interacts with protein molecules, the net negative charge on protein molecules increases. It enhances the electrostatic repulsion force among protein molecules and subsequently prevents the aggregation of proteins. Pro, which is a nonpolar amino acid, also showed a marked inhibitory effect on protein denaturation in this study, compared to the other nonpolar amino acid Gly.

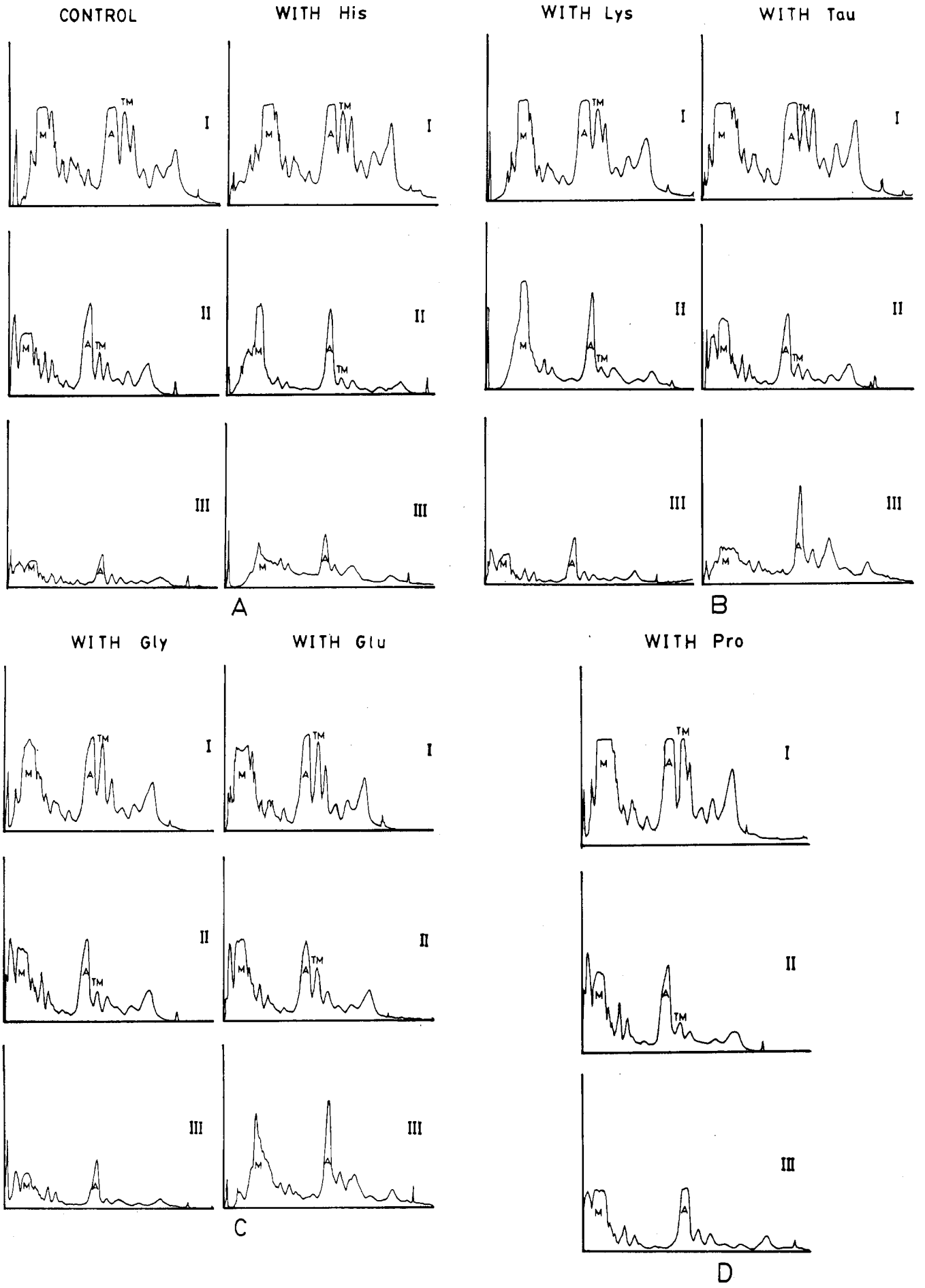

Figure 1. Effect of free amino acids on the electrophoretic profile of actomyosin extracted from mackerel dorsal muscle during frozen storage at −20 °C: I, sample before freezing; II, samples before precipitating the denatured proteins after 12 weeks of storage; III, soluble proteins after 12 weeks of storage; A, control sample and sample with histidine; B, samples with lysine and taurine; C, samples with glycine and glutamic acid; D, sample with proline.

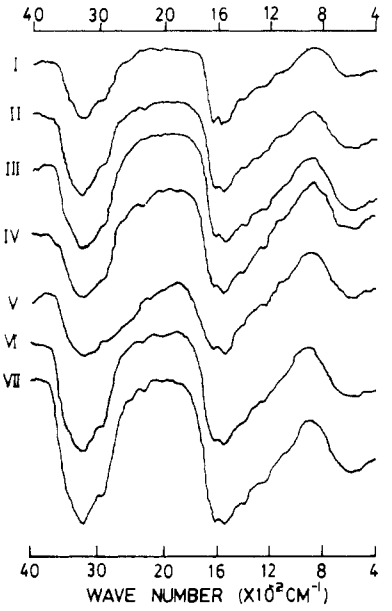

Figure 2. Effect of free amino acids on the infrared spectrum of actomyosin extracted from mackerel dorsal muscle: I, control sample; II, sample after the addition of histidine; III, sample after the addition of lysine; IV, sample after the addition of taurine; V, sample after the addition of glycine; VI, sample after the addition of glutamic acid; VII, sample after the addition of proline.

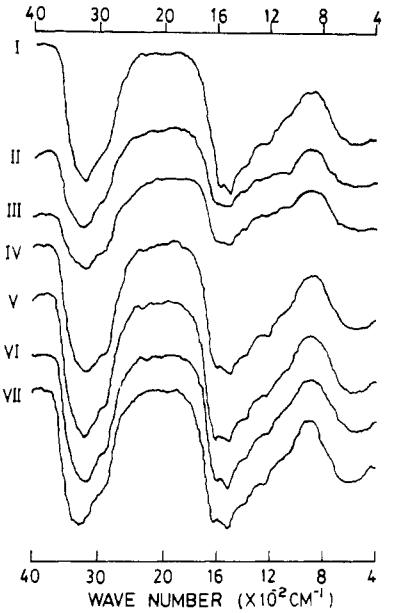

Figure 3. Effect of free amino acids on the infrared spectrum of mackerel actomyosin suspended in 0.10 M KCl solution after 12 weeks of storage at −20 °C: I, control sample; II, sample after the addition of histidine; III, sample after the addition of lysine; IV, sample after the addition of taurine; V, sample after the addition of glycine; VI, sample after the addition of glutamic acid; VII, sample after the addition of proline.

This might be due to the formation of hydrophobic hydration on the pyrrolidine ring of Pro (Lee, 1983), subsequently reducing the freezing ratio of bound water during frozen storage, as the Pro interacts with protein molecules. The basic amino acids, e.g. His and Lys, appeared to accelerate the denaturation of AM. This is because these amino acids reduce the electrostatic repulsion force of protein molecules when they interact with proteins.

Effect of Free Amino Acids on the Infrared Spectrum of Actomyosin during Frozen Storage. The frequencies at 1640 and 1580 cm^{-1} in the infrared spectra of AM after being extracted were assigned to amides I and II, respectively (I in Figure 2). The amide I in samples after the addition of free amino acids was shifted to lower frequencies, 1620–1625 cm^{-1}. Frequencies for amides I and II are mainly caused by the C=O stretching and N—H bending, respectively (Fraser et al., 1970; Susi, 1972; Wallach and Oseroff, 1974). The amide I frequencies shifted lower in those samples, compared to the initial one, and hinted that the amino groups of these amino acids interacted with the C=O group on the protein molecules after being added (I–VII in Figure 2).

After 12 weeks of storage, amides I and II of all samples shifted to lower frequencies. This suggested the ionic interaction on both C=O and N—H groups of protein molecules occurred during frozen storage and caused protein aggregation–denaturation (I–VII in Figure 3). This phenomenon can be elucidated by the decrease in solubility and Ca-ATPase specific activity of these samples.

A great number of studies have so far published on the cryoprotective substances with the freezing denaturation of proteins, living cells, and tissues, though the basic mechanism of their action remains yet obscure. The Gly showed little or no effect on the stability of mackerel AM (present study) and carp AM (Noguchi, 1974). However, it prevented the denaturation of rabbit myosin during freezing and freeze-drying (Hanafusa, 1969). These suggest that an effective substance for one protein is perhaps not good for other proteins, as well as, whether a substance is effective or not depends on the treatments, i.e. frozen storage or freeze-drying.

Morichi et al. (1963) studied the survival of bacteria on freeze-drying with regard to the protective effect of many substances structurally related with glutamic acid. They concluded that, for maximum protection, the compounds had to possess (1) a functional group of high electronegativity such as NH_2 on the α-carbon, (2) the presence of two acid groups, and (3) close proximity between the carboxyl and electronegative group on α-carbon. The Glu was also excellent in preventing the denaturation of carp muscle proteins during frozen storage (Noguchi, 1974) and during freeze-drying and subsequent storage at room temperature (Matsuda, 1979). According to the present study, it prevented the denaturation of mackerel myofibrillar proteins during frozen storage.

Jiang et al. (1986) and Lan (1984) reported that the addition of cysteine during the early stage of grinding in surimi processing recovered the native myofibrillar proteins and increased the stability of surimi during frozen storage. According to Noguchi (1974), the cysteine and cystine, hydroxyproline, and proline protected the carp actomyosin against denaturation during frozen storage. Proline was also found to prevent the denaturation of mackerel myofibrillar proteins during frozen storage (present study).

Although histidine and lysine did not affect the stability of carp actomyosin during frozen storage (Noguchi, 1974), the present study showed that these amino acids accelerated the denaturation of mackerel actomyosin during frozen storage. This might be due to the difference in pH of the protein solutions.

The infrared spectral analysis in this study hinted that the NH_2 groups of added amino acids interacted with the C=O groups on protein molecules. Amino acids that possessed high negative net charge (such as Glu) or weak negative net charge but held a high hydration functional group (such as Pro) protected the actomyosin during frozen storage. However, amino acids with high positive net charge (such as His and Lys) accelerated the protein denaturation during frozen storage. From this study and other previous studies in this field, it seems likely that the

effect of amino acids on stability of muscle proteins is highly related to their isoelectric point.

Registry No. L-His, 71-00-1; L-Lys, 56-87-1; L-Glu, 56-86-0; L-Pro, 147-85-3; Gly, 56-40-6; ATPase, 9000-83-3; taurine, 107-35-7.

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