JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Stability and Emulsion-Forming Ability of Water-Soluble Fish Myofibrillar Protein Prepared by Conjugation with Alginate Oligosaccharide

RYO SATO, SHIGERU KATAYAMA, TOMOO SAWABE, AND HIROKI SAEKI*

Graduate School of Fisheries Sciences, Hokkaido University, Minato 3, Hakodate, Hokkaido 041-8611, Japan

Carp myofibrillar protein (Mf) was conjugated with alginate oligosaccharide (AO) through the Maillard reaction under low relative humidity, and the functional properties of the Mf−AO conjugate were investigated under different NaCl concentrations and pH levels. Mf became highly solubilized at lower NaCl concentrations by conjugation with AO, with a slight loss of available lysine. The thermal stability of Mf was effectively improved by conjugation with AO. Heat treatment at 80 °C for 2 h had no effect on the solubility of the Mf−AO conjugate attached to 227 $\mu$g/mg of AO regardless of the NaCl concentration and pH. Furthermore, the Mf−AO conjugate showed excellent emulsion-forming ability regardless of NaCl concentration. The improved functionalities of Mf by conjugation with AO remained even at a nearly isoelectric point. These results indicate that conjugation with AO through the Maillard reaction is an effective way to prepare high-functional food material from fish muscle protein.

**KEYWORDS: Fish; myofibrillar protein; alginate oligosaccharide; neoglycoprotein; glycosylation; Maillard reaction; solubility; isoelectric point; thermal stability; emulsion-forming ability**

## INTRODUCTION

Fish meat is an abundant protein source and is widely used as a material for processed seafood. Fish myofibrillar protein (Mf) has excellent functional properties such as emulsion-forming ability, water-holding activity, and gel-forming ability. Various kinds of processed seafood are manufactured using the food functionality of fish myofibrillar protein. However, fish Mf is thermally and chemically unstable compared with that of other vertebrates (*1*), and the food functionality is impaired easily as protein denaturation progresses. Therefore, the suppression of protein denaturation during storage of materials and food processing is important to the manufacture of high-quality processed seafood. Processing technologies such as a low-temperature storage to keep fish flesh at a temperature close to freezing (partial freezing) (*2*), mixing with edible cryoprotectants (*3*), and rapid neutralization of meat by washing with alkaline solution (*4*) have been developed for the suppression of protein denaturation during processing. These findings are significant for utilizing the functional properties of fish myofibrillar protein at an intrinsic level, and they have contributed to the quality control of processed seafood.

Recently, the neoglycoprotein−synthetic system using the Maillard reaction has been focused on an effective method to improve the functional property of food protein. The system requires no chemical reagent, and food proteins such as ovalbumin (*5*), $\beta$-lactoglobulin (*6*), phosvitin (*7*), lysozyme (*8*), and protamine (*9*) are conjugated with various reducing sugars through the Maillard reaction to obtain new food functionalities. The obtained neoglycoproteins are superior to native proteins in terms of food functionality, for example, emulsion-forming ability, antioxidant effect, and antimicrobial action. Characteristics of fish Mf have also been improved by glycosylation using the Maillard reaction. That is, fish Mf became water-soluble by reaction with monosaccharides such as glucose (*10*) and ribose (*11*), and the thermal stability and emulsion-forming ability were improved effectively by conjugation with polysaccharide (*12*). However, the neoglycoprotein−synthetic system impairs the nutritional value of protein because the reducing sugars are attached to $\epsilon$-amino groups of lysine residues of protein. On the other hand, when conjugated with alginate oligosaccharid (AO), the solubility of fish Mf in a low ionic strength medium was markedly improved without a significant loss of available lysine, which was not the case in the reaction with monosaccharides (*13*). This finding suggests that AO is a good material to prepare high-functional fish neoglycoprotein using the Maillard reaction.

The objective of this study is to investigate the stability of characteristics of fish Mf conjugated with AO. Water-soluble fish myofibrillar protein was prepared by conjugation with alginate oligosaccharide, and its thermal stability and emulsion-forming ability were examined under different NaCl concentrations and pH levels.

## MATERIALS AND METHODS

**Materials.** A cultured live carp (*Cyprinus carpio*) was obtained at a local fish market. Sodium alginate from brown algae was purchased from Kanto Chemicals Co., Inc. (Tokyo, Japan). Bovine serum albumin

* Author to whom correspondence should be addressed (e-mail saeki@fish.hokudai.ac.jp; fax +81-138-40-5515).

10.1021/jf021023v CCC: $25.00 
Published on Web 06/13/2003

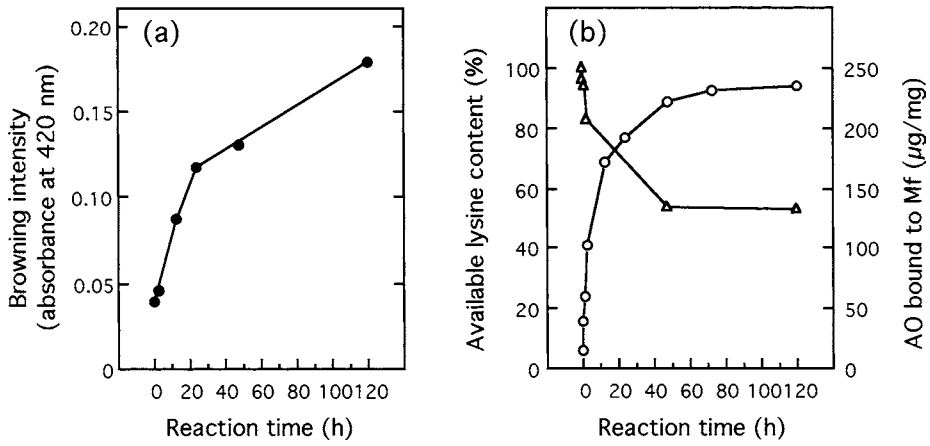


**Figure 1.** Monitoring of reaction between Mf and AO. Browning intensity (●), available lysine content (△), and amount of AO bound to Mf (○) were investigated during the Maillard reaction at 50 °C and 35% relative humidity.

(fraction V) was obtained from Merck Co. Ltd. (Darmstadt, Germany). All other chemicals (reagent grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**Preparation of Myofibrillar Protein and Alginate Oligosaccharide.** Carp Mf was prepared from ordinary muscle of a cultured live carp according to the method of Saeki (*14*) and suspended in 50 mM NaCl. The protein concentration was measured by the biuret method (*15*) using bovine serum albumin as a standard. As previously described, AO was prepared by degrading sodium alginate using alginate lyase, which was purified from the culture medium of *Pseudoalteromonas elyakovii* (IAM 14594) (*16*). The average degree of polymerization of AO was 6.1.

**Glycosylation of Myofibrillar Protein with Alginate Oligosaccharide.** Mf was conjugated with AO using the Maillard reaction between the $\epsilon$-amino group of the lysine residue in Mf and the end-carbonyl group of AO. Mf suspended in a 50 mM NaCl solution containing 0.6 M sorbitol was mixed with AO. The final concentrations of Mf and AO were 6 and 54 mg/mL, respectively. Five milliliters of the mixture was placed in a test tube (diameter = 16 mm), frozen at −40 °C for 1 h, and immediately lyophilized using a freeze-dryer (FDU-506, Tokyo Rika Co. Ltd., Tokyo, Japan). The lyophilized Mf and AO mixture was incubated at 50 °C and 35% relative humidity for 0−120 h. A humidity cabinet (model PR-1G, Tabai Espec Corp., Tokyo, Japan) was used to control the temperature and the relative humidity. The Mf conjugated with AO (Mf−AO conjugate) thus obtained was immediately dissolved in various solutions by the following process.

**Browning Intensity.** Browning intensity is a simple indicator of the progress of the Maillard reaction. The browning intensity of the Mf−AO conjugate was determined by measuring the absorbance at 420 nm. The samples were dissolved in 10% (w/w) sodium dodecyl sulfate (SDS) at 5 mg/mL of the final protein. This concentration reduced scattering due to protein aggregates.

**Available Lysine Content.** The Mf−AO conjugate was dissolved in 0.5 M NaCl containing 40 mM Tris-HCl (pH 7.5), and available lysine content in the protein was determined by spectrophotometric analysis using *o*-phthalaldehyde and *N*-acetyl-L-cysteine (*17*). Before the analysis of available lysine, the protein was precipitated with 7.5% trichloroacetic acid (at the final concentration) to remove the Tris buffer and was redissolved in 50 mM phosphate buffer (pH 9.5) containing 2% SDS.

**Amount of AO Bound to Mf.** Sixty percent of saturated ammonium sulfate at the final concentration was added to the Mf−AO conjugate dissolved in 0.5 M NaCl containing 40 mM Tris-HCl (pH 7.5) at 2 °C. The Mf−AO conjugate was collected as a precipitate by centrifugation and redissolved in 0.5 M NaCl (pH 7.5) three times to remove unreacted AO. The amount of AO bound to Mf was determined according to the phenol−sulfuric acid method (*18*).

**Solubility.** The Mf−AO conjugate was dissolved in 0.05−0.5 M NaCl containing 40 mM glycine−HCl (pH 2.5), 40 mM citrate−NaOH (pH 3.5−5.0), 40 mM Tris−maleate−NaOH (pH 5.5−6.5), and 40 mM Tris−HCl (pH 7.5) at 1.0 mg/mL of the final protein with a homogenizer (Ultra-turrax T25/N-8G, IKA-Laboatechnik, Staufen, Germany) operating at 13500 rpm for 60 s. Samples were immediately centrifuged at 15000*g* for 30 min at 4 °C. The supernatant and the total protein solution before centrifugation were mixed with an equal volume of 2.0 M NaOH, and their protein concentrations were determined according to the Bradford method (*19*) using bovine serum albumin as a standard. The solubility was expressed as the ratio (percent) of protein in the supernatant to that of the total protein solution. In the preliminary experiment, we confirmed that unreacted AO and sorbitol have no effect on the solubility of the Mf−AO conjugate.

**Isoelectric Point.** The isoelectric points (p*I*) of carp myosin and actin, the main protein components of Mf, were estimated with DNA/protein analytical software (DNAsis, version 3.5, Hitachi Software Co. Ltd., Tokyo, Japan). The primary structure of carp myosin (*20*) and actin (*21*) was used to estimate the p*I* of the proteins.

**Thermal Stability.** The thermal stability of the Mf−AO conjugate was examined by monitoring the solubility change by heat treatment. After the removal of unreacted AO and sorbitol by the ammonium sulfate fractionation, the Mf−AO conjugate (2.0 mg/mL) was dissolved in 0.05 and 0.5 M NaCl solutions containing 40 mM Tris−maleate−NaOH (pH 5.5) or 40 mM Tris−maleate (pH 7.5) and immediately heated at 50−80 °C for 2 h with a water bath. After being cooled in ice−water, each soluble fraction was obtained by centrifugation at 15000*g* for 30 min at 4 °C. The solubility was expressed as the ratio (percent) of protein concentration before and after heating. The protein concentration was determined according to the Lowry method (*22*).

**Emulsion-Forming Ability.** The emulsion-forming ability of the Mf−AO conjugate was determined according to the method of Pearce and Kinsella (*23*). The protein concentration of the Mf−AO conjugate dissolved in 0.05 and 0.5 M NaCl (pH 5.5 or 7.5) was adjusted to 3.0 mg/mL. A total of 2.1 mL of the protein solution was added to 0.7 mL of corn oil in a test tube (diameter = 10.5 mm), and an oil-in-water emulsion was prepared by using a homogenizer (Ultra-turrax T25/N/8G) operating at 13500 rpm for 1 min in ice−water. After 1 min of standing, 0.15 mL of the emulsion was taken from the bottom of the test tube and diluted with 2.85 mL of 0.1% SDS at 25 °C. The turbidity of the diluted emulsion was immediately measured at 500 nm in a 1 cm path cuvette. The emulsifying activity was expressed by the turbidity of the diluted emulsion after 1 min of standing. Additionally, the stability of the Mf−AO/oil emulsion was investigated by measuring the turbidity decrease of the emulsion at 2 °C. The diluted turbidity was measured at 5, 10, 15, 30, and 45 min and further half-hourly. The measurement was stopped when the absorbance of the turbidity decreased to below 50% of the initial one. The half-life of the initial value of the turbidity of the diluted emulsion was measured and expressed as the emulsion stability. The Mf−AO conjugate heated at 80 °C for 2 h was also examined to investigate the effect of heating on emulsion-forming ability. The significance of differences among samples was determined by Tukey's multiple-range test (*24*).

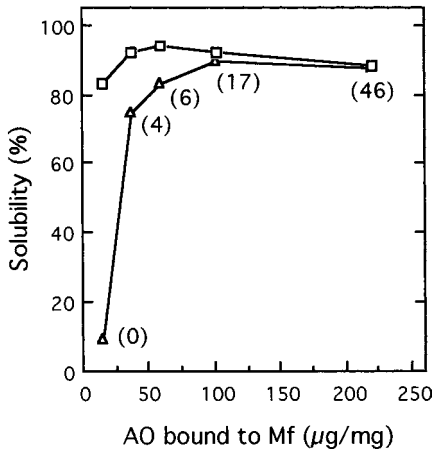


**Figure 2.** Effect of conjugation with AO on solubility of Mf. The relationship between the solubility in 0.05 M (△) and 0.5 M (□) NaCl (pH 7.5) and the amount of AO bound to Mf was investigated during the preparation of the Mf–AO conjugate at 50 °C and 35% relative humidity. The number in parentheses is the available lysine reacted with AO (%).

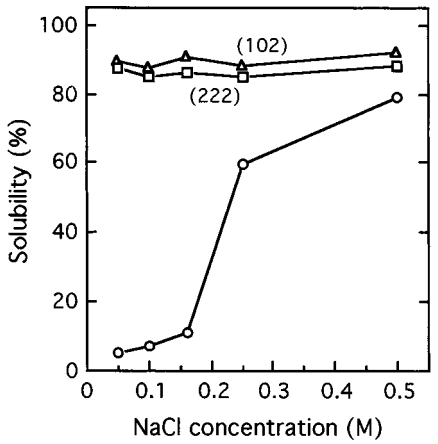


**Figure 3.** NaCl concentration dependence of solubility of the Mf–AO conjugate at pH 7.5: native Mf (○) and Mf–AO conjugate prepared by the reaction at 50 °C and 35% relative humidity for 3 h (△) and 48 h (□). The number of parentheses is the amount of AO bound to Mf (μg/mg).

**Table 1.** Changes in p*I* of Carp Myosin and Actin during Reaction with AO

| reaction time[a] (h) | lysine reacted with AO (%) | p*I* estimated | |
|---|---|---|---|
| | | myosin | actin |
| 0 | 0 | 5.52 | 5.10 |
| 0.5 | 4 | 5.38 | 5.03 |
| 3 | 17 | 5.04 | 4.89 |
| 48 | 46 | 4.59 | 4.62 |

[a] 50 °C and 35% relative humidity.

## RESULTS AND DISCUSSION

**Conjugation of Mf with AO through the Maillard Reaction.** When the lyophilized mixture of Mf and AO was incubated at 50 °C and 35% relative humidity, the browning intensity increased gradually with the progress of the reaction time as shown in **Figure 1a**. In addition, a decrease in available lysine and an increase in AO bound to Mf were observed simultaneously as presented in **Figure 1b**. These results clearly indicate that Mf was conjugated with AO through the Maillard reaction between the ϵ-amino group of the lysine residue in Mf and the end-carbonyl group of AO. When the available lysine content decreased to 53% of the initial value, the amount of the binding AO reached the maximum (235 μg/mg). This result indicates that ∼50% of the total lysine residues in Mf could react with AO in the reaction system. These lysine residues probably existed at the surface of the protein.

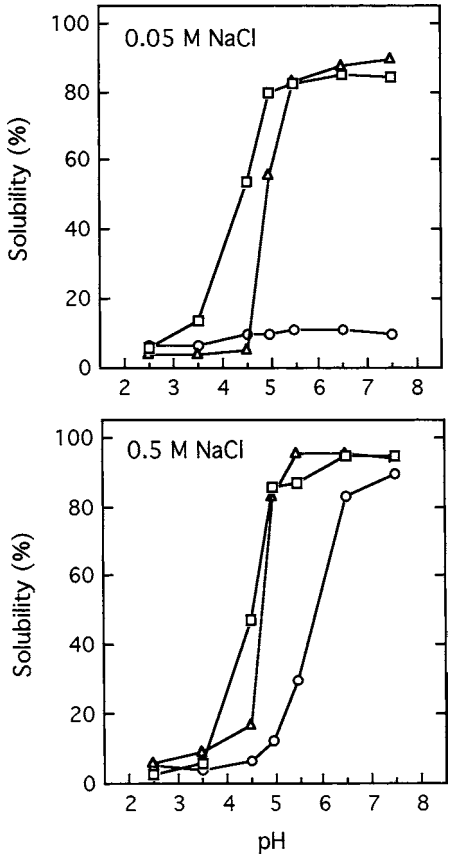


**Figure 4.** Effect of pH on solubility of the Mf–AO conjugate. Symbols are the same as in **Figure 3**.

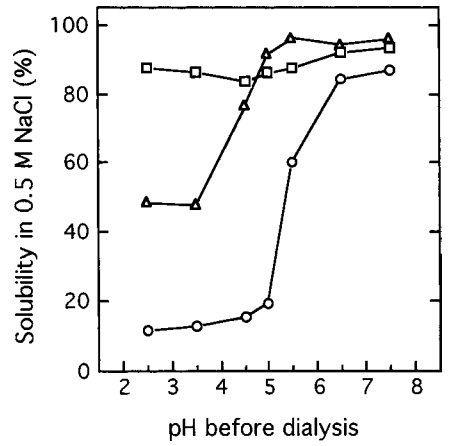


**Figure 5.** Effect of pH shift to 7.5 on the solubility of acid-treated Mf–AO conjugate. Acidic-treated Mf and Mf–AO conjugate were dialyzed against 0.5 M NaCl (pH 7.5) to change the pH. Symbols are the same as in **Figure 3**.

**Improvement of Solubility of Mf by Conjugation with AO.** The solubility of Mf in a low ionic strength medium significantly increased with the progress of the conjugation with AO (**Figure 2**). The solubility in 0.05 M NaCl was greatly improved with an increase in the amount of AO bound to Mf. The solubility in 0.05 M NaCl reached 76% when the amount of bound AO was 38 μg/mg and the amount of available lysine residues reacted was 4% (reaction time of 0.5 h). When the amount of

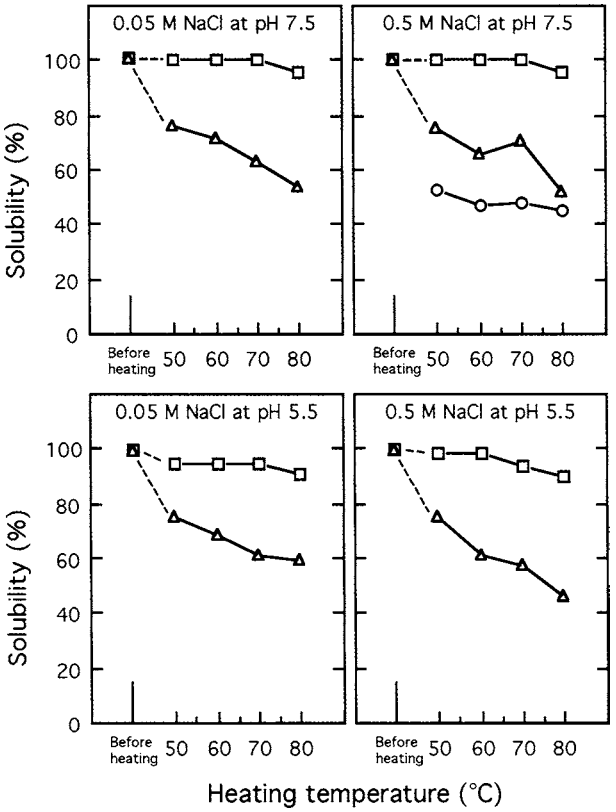


**Figure 6.** Effect of heating at different NaCl concentrations and pH levels on the solubility of the Mf–AO conjugate. Native Mf (○) and Mf–AO containing 120 (△) and 227 (□) μg/mg of AO were dissolved in 0.05 or 0.5 M NaCl (pH 5.5 and 7.5 in both cases) and heated at 50–80 °C for 2 h.

AO bound to Mf was 102 μg/mg (reaction time of 3 h), the solubility of the Mf−AO conjugate in 0.05 M NaCl reached 90% and equaled the solubility in 0.5 M NaCl. In this conjugate, 17% of available lysine residues in the Mf were reacted with AO. These results demonstrate clearly that attaching a small amount of AO to Mf improved the solubility of Mf in low NaCl concentration media without a significant loss of the available lysine.

**Figure 3** shows the NaCl concentration dependency of the solubility of Mf−AO conjugates. Mf was solubilized in lower NaCl solutions (pH 7.5) by the conjugation with AO. In the case of native Mf, the solubility increased gradually with the rise of NaCl concentration in the range of 0.16−0.25 M and reached the maximum value at 0.5 M NaCl. On the other hand, the solubility of the Mf−AO conjugate in 0.05 M NaCl reached 90% and the NaCl concentration dependency disappeared when 102 μg/mg of AO was bound to Mf (the reaction at 50 °C and 35% relative humidity was 3 h).

It is known that humidity affects the Maillard reaction, and the reaction proceeds quickly under 40−60 °C and 60−80% relative humidity (*25*, *26*). However, our previous study showed that protein heat denaturation occurred under these conditions and the improved high solubility of the Mf−AO conjugate was rapidly impaired with the progress of protein denaturation (*13*). On the other hand, we confirmed in the preliminary experiment that a significantly lower relative humidity (<40%) effectively suppressed the protein denaturation during reaction with AO. Indeed, as shown in **Figure 3**, the improved solubility of the Mf−AO conjugate prepared at 50 °C and 35% relative humidity remained unchanged even after reaction for 48 h (222 μg/mg of AO was attached to Mf). These results indicate that the Maillard reaction at low relative humidity is appropriate for the preparation of a neoglycoprotein with high solubility from fish Mf.

**Effect of pH on Solubility of the Mf—AO Conjugate. Figure 4** shows the effect of pH on the solubility of native Mf and Mf−AO conjugates in 0.05 and 0.5 M NaCl. The solubility of native Mf decreased greatly in the pH range of 5.0−6.5 in 0.5 M NaCl. The p*I* values of carp myosin and actin estimated from the amino acid composition were pH 5.52 and 5.10, respectively (**Table 1**). Therefore, it is apparent that the loss of

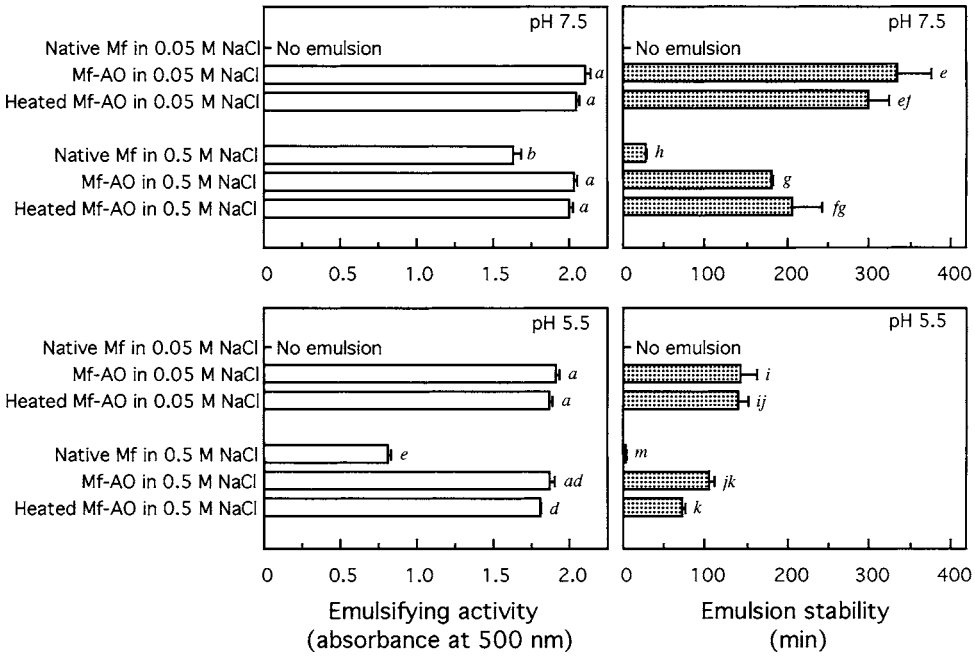


**Figure 7.** Emulsion-forming ability of the Mf–AO conjugate under different NaCl concentrations and pH levels. Native Mf and Mf–AO containing 227 μg/mg of AO were dissolved in 0.05 and 0.5 M NaCl (pH 5.5 and 7.5 in both cases), and the emulsion-forming ability was investigated by measuring the emulsifying activity and the emulsion stability. Samples heated at 80 °C for 2 h were also examined under the same condition. Data are means ± SD, and the same italic letter shows no significant difference ($n = 3$, $p < 0.01$).

net charge of proteins at nearly the p*I* is closely related to the solubility decrease of native Mf. On the other hand, Mf−AO conjugates showed high solubility at the range of pH 5.0−7.5, regardless of NaCl concentration. Furthermore, the solubility of the Mf−AO conjugate containing 222 $\mu$g/mg of AO remained at ∼50% even at pH 4.5. The p*I* of myosin and actin in Mf−AO conjugates seemed to shift to the acidic side because the positively charged lysine reacted with AO. Indeed, when the total available lysine loss reached 17% (the reaction period was 3 h) and 46% (48 h), the p*I* values of myosin/actin in Mf−AO conjugates were estimated to be 5.04/4.89 and to 4.59/4.62, respectively, as listed in **Table 1**. Thus, the result shown in **Figure 4** indicates that the Mf−AO conjugate was soluble at more acidic pH than the native Mf.

It is known that fish Mf denatures easily and loses its salt solubility in acidic conditions (*27*). Therefore, the structural change of Mf−AO conjugates in acidic conditions seems to differ from that of native Mf. To investigate the relationship between acid denaturation and solubility loss, the pH of native Mf and Mf−AO conjugates shown in **Figure 3** was shifted to neutral by dialyzing against 0.5 M NaCl (pH 7.5), and restoration of the solubility was observed. As presented in **Figure 5**, the solubility of native Mf impaired in acidic solutions was unchanged after the pH was shifted to 7.5. This result indicates that the significant structural change would have occurred with a decrease in the net charge of the proteins in acidic conditions. On the contrary, the low solubility of Mf−AO conjugates at pH 2.5−4.5 was effectively recovered by adjusting the pH to 7.5. In particular, the solubility of the Mf−AO conjugate containing 222 $\mu$g/mg of AO recovered completely regardless of the pH before dialysis. The result shown in **Figure 5** suggests that the conjugation with AO suppressed the structural change of the proteins under acidic conditions. The loss of the net charge could be mainly responsible for the solubility decrease of the Mf−AO conjugate under acidic conditions.

**Thermal Stability of the Mf—AO Conjugate**. **Figure 6** shows the thermal stability of the Mf−AO conjugate under different NaCl concentrations and pH levels. The solubility of native Mf dissolved in 0.5 M NaCl (pH 7.5) decreased to ∼50% of its original solubility when heated at 50−80 °C for 2 h. The solubility of heat-treated Mf−AO conjugate containing 120 $\mu$g/mg of AO in 0.05 and 0.5 M NaCl (pH 7.5) was significantly higher than that of native Mf, although the solubility tended to diminish as the heating temperature increased. Furthermore, heat treatment has no effect on the solubility of the Mf−AO conjugate containing 227 $\mu$g/mg of AO regardless of heating temperature or NaCl concentration. The improved thermal stability of Mf by conjugation with AO was also observed at pH 5.5, in which native Mf was insoluble at both NaCl concentrations as shown in **Figure 4.** It is confirmed in the preliminary experiment that the addition of an equal amount of AO has no protective effect on the protein solubility under all heating conditions (data not shown). Therefore, the results given in **Figures 4** and **6** show conclusively that Mf−AO conjugates have higher stability in a wide range of NaCl concentrations, pH levels, and thermal conditions than native Mf.

**Emulsion-Forming Ability of the Mf—AO Conjugate.** To investigate the effect of conjugation with AO on the emulsion-forming ability of Mf, the emulsifying activity and the emulsion stability of the Mf−AO conjugate containing 227 $\mu$g/mg of AO were investigated at different NaCl concentrations and pH levels. The results are shown in **Figure 7**. In the case of native Mf, the emulsion-forming ability was strongly affected by NaCl concentration and pH level. No emulsion was formed in 0.05 M NaCl (pH 5.5/7.5), and the emulsifying activity in 0.5 M NaCl (pH 7.5) became weak when the pH of the emulsion was nearly the p*I* of the proteins (pH 5.5). On the other hand, the NaCl concentration and pH had no effect on the emulsifying activity of the Mf−AO conjugate. The emulsifying activity of the Mf−AO conjugate was significantly higher than that of native Mf in pH 7.5 and particularly in pH 5.5. In addition, the emulsion stability of Mf was significantly improved by conjugation with AO. The emulsion stability of the Mf−AO conjugate showed the highest value in 0.05 M NaCl (pH 7.5), in which native Mf had no emulsion-forming ability. Although the emulsion stability of the Mf−AO conjugate decreased with an increase in NaCl concentration, it remained at a relatively high level even at pH 5.5. Furthermore, it was confirmed that the emulsion-forming ability of the Mf−AO conjugate was thermally stable as shown in **Figure 7**. The improved emulsion-forming ability described above was unchanged even after heating at 80 °C for 2 h, regardless of NaCl concentration and pH. These results clearly indicate that conjugation with AO is an effective way to improve the emulsion-forming ability of Mf.

**Conclusion.** Conjugation with AO was an effective way to improve the functional properties of fish Mf, such as solubility in low NaCl solutions, thermal stability, and emulsion-forming ability. The Mf−AO conjugate was solubilized in wide range of NaCl concentrations and pH levels with high stability and with excellent emulsion-forming ability. The result of this work can contribute to the high utilization of marine bioresources as functional food materials.

## LITERATURE CITED

(1) Yamashita, K.; Arai, K.; Nishita, K. Thermo-stabilities of synthetic actomyosins in various combinations of myosin and actin from fish, scallop, and rabbit muscles. *Bull. Jpn. Soc. Sci. Fish.* **1978**, *44*, 485−489.

(2) Chang, K. L. B.; Chang, J.; Shiau, C.; Pan, B. S. Biochemical, microbiological, and sensory changes of sea bass (*Lateolabrax japonicus*) under partial freezing and refrigerated storage. *J. Agric. Food Chem.* **1998**, *46*, 682−686.

(3) MacDonald, G. A.; Lanier, T. Carbohydrates as cryoprotectants for meats and surimi. *Food Technol.* **1991**, *45*, 151−159.

(4) Hashimoto, A.; Arai, K. The effects of pH and temperature on the stability of myofibrillar Ca-ATPase from some fish species. *Bull. Jpn. Soc. Sci. Fish.* **1978**, *44*, 1389−1393.

(5) Kato, A.; Sasaki, Y.; Furuta, R.; Kobayashi, K. Functional protein−polysaccharide conjugate prepared by controlled dry-heating of ovalbumin−dextran mixtures. *Agric. Biol. Chem.* **1990**, *54*, 107−112.

(6) Hattori, M.; Ogino, A.; Nakai, H.; Takahashi, K. Functional improvement of $\beta$-lactoglobulin by conjugating with alginate lyase-lysate. *J. Agric. Food Chem.* **1997**, *45*, 703−708.

(7) Nakamura, S.; Ogawa, M.; Nakai, S.; Kato, A.; Kitts, D. D. Antioxidant activity of a Maillard-type phosvitin−galactomannann conjugate with emulsifying properties and heat stability. *J. Agric. Food Chem.* **1998**, *46*, 3958−3963.

(8) Shu, Y.; Nakamura, S.; Kato, A. The role of polysaccharide chain attachment to lysozyme in the excellent emulsifying properties of polymannosyl lysozyme. *Nahrung−Food* **1998**, *42*, 68−70.

(9) Matsudomi, N.; Tsujimoto, T.; Kato, A.; Kobayashi, K. Emulsifying and bactericidal properties of a protamine−galactomannan conjugate prepared by dry heating. *J. Food Sci.* **1994**, *59*, 428−431.

(10) Saeki, H.; Inoue, K. Improved solubility of carp myofibrillar proteins in low ionic strength medium by glycosylation. *J. Agric. Food Chem.* **1997**, *45*, 3419−3422.

(11) Saeki, H.; Tanabe, M. Change in solubility of carp myofibrillar protein by modifying lysine residue with ribose. *Fish. Sci.* **1999**, *65*, 967−968.
(12) Fujiwara, K.; Oosawa, T.; Saeki, H. Improved thermal stability and emulsifying properties of carp myofibrillar proteins by conjugation with dextran. *J. Agric. Food Chem.* **1998**, *46*, 1257−1261.
(13) Sato, R.; Sawabe, T.; Kishimura, H.; Hayashi, K.; Saeki, H. Preparation of neoglycoprotein from carp myofibrillar protein and alginate oligosaccharide: Improved solubility in low ionic strength medium. *J. Agric. Food Chem.* **2000**, *48*, 17−21.
(14) Saeki, H. Preparation of neoglycoprotein from carp myofibrillar protein by Maillard reaction with glucose: Biochemical properties and emulsifying properties. *J. Agric. Food Chem.* **1997**, *45*, 680−684.
(15) Gornall, A. G.; Bardwill, C. J.; David, M. M. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **1949**, *177*, 751−766.
(16) Sawabe, T.; Ezura, Y.; Kimura, T. Purification and characterization of alginate lyase from marine *Alteromonas* sp. *Nippon Suisan Gakkaishi* **1992**, *58*, 521−527.
(17) Medina Hernandez, M. J.; Guarcia Alvarez-Coque, M. C. Available lysine in protein, assay using *o*-phthalaldehyde/*N*-acetyl-L-cysteine spectrophotometric method. *J. Food Sci.* **1992**, *57*, 503−505.
(18) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−356.
(19) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(20) Hirayama, Y.; Watabe, S. Structural differences in the crossbridge head of temperature-associated myosin subfragment-1 isoforms from carp fast skeletal muscle. *Eur. J. Biochem.* **1997**, *246*, 380−387.
(21) Watabe, S.; Hirayama, Y.; Imai, J.; Kikuchi, K.; Yamashita, M. Sequences of cDNA clones encoding α-actin of carp and goldfish skeletal muscles. *Fish. Sci.* **1995**, *61*, 998−1003.
(22) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin−phenol reagent. *J. Biol. Chem.* **1955**, *193*, 265−275.
(23) Pearce, K. N.; Kinsella, J. E. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Food Chem.* **1978**, *26*, 716−723.
(24) Steel, R. G. D.; Torrie, J. K. Multiple comparisons. In *Principles and Procedures of Statistics, A Miometarical Approach*; McGraw-Hill: New York, 1986; pp 173−193.
(25) Lea, C. H.; Hannan, R. S. Studies of the reaction between proteins and reducing sugars in the "dry" state. I. The effect of activity of water, of pH, and of temperature on the primary reaction between casein and glucose. *Biochim. Biophys. Acta* **1949**, *3*, 323−325.
(26) Labuza, T. P.; Tannenbaum, S. R.; Karel, M. Water content and stability of low-moisture and intermediate-moisture foods. *Food Technol.* **1970**, *24*, 543−550.
(27) Hashimoto, A.; Arai, K. The effect of pH on the thermostability of fish myofibrils. *Bull. Jpn. Soc. Sci. Fish.* **1985**, *51*, 99−105.

**Received for review October 7, 2002. Revised manuscript received April 1, 2003. Accepted April 24, 2003. Part of this work was supported by Grants-in-Aid for Scientific Research, Ministry of Education, Science and Culture of Japan, and by the Hokkaido Northern Advancement Center for Science and Technology, Japan.**

JF021023V