JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Solubility Improvement of Shellfish Muscle Proteins by Reaction with Glucose and Its Soluble State in Low-Ionic-Strength Medium

SHIGERU KATAYAMA, JUNJI SHIMA, AND HIROKI SAEKI*

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

When myofibrillar proteins of scallop striated adductor muscle were reacted with glucose through the Maillard reaction, the change in the solubility of myofibrillar proteins in 0.05−0.5 M NaCl solutions during glycosylation and their soluble states were investigated. The solubility in low-ionic-strength media increased greatly with the progress of the Maillard reaction. The solubility in 0.1 M NaCl reached 83% when more than 60% of lysine residues in myofibrillar proteins were modified by glucose. However, the excess progress of the Maillard reaction impaired the improved solubility of myofibrillar proteins in a low-ionic-strength medium. Myosin, actin, and paramyosin in glycosylated myofibrillar proteins were solubilized independently regardless of NaCl concentration. In addition, the glycosylated myosin lost its filament-forming ability and existed as a monomer in 0.1 M NaCl.

KEYWORDS: Scallop; myofibrillar proteins; myosin; glycosylation; Maillard reaction; solubility; glucose

INTRODUCTION

Fish and shellfish are important protein resources and are widely used as materials for processed foods. Fish meat is highly nutritional, and its myofibrillar proteins have excellent functional properties, such as emulsifying properties, gel-forming ability, and water-holding capacity. Various technologies for dealing with fish meat, such as freeze-drying, spray-drying, extrusion cooking, and high-hydrostatic-pressure processing, have been developed and applied to the seafood industry (*1*−*4*). Attempts have also been made to solubilize fish muscle meat in water by proteolytic enzymes and by acid hydrolysis for human consumption, animal feed, and liquid fertilizers (*5*−*8*). However, the excellent functional properties of myofibrillar proteins are completely lost by protein degradation. In addition, the production of peptides caused by protein hydrolysis often causes a bitter and unacceptable taste. Recently, Saeki and his colleagues undertook research to improve the functional properties of fish myofibrillar proteins by glycosylation and found that the water-soluble myofibrillar proteins with an enhanced emulsifying property can be prepared by modifying lysine residues with monosaccharides (*9*−*11*).

Shellfish meat is high in nutritional value and has the same good functional properties as fish. However, there have been few attempts to develop new technology for the increased utilization of shellfish meat. There are biochemical differences between fish and shellfish muscles: shellfish muscle contains paramyosin, which forms the core protein of the thick filaments (*12*), and the role of the regulatory proteins in muscle contraction is different from that of fish muscle (*13, 14*). Some researchers have also reported that the functionality of shellfish myofibrillar proteins as foodstuff is different from that of fish myofibrillar proteins (*15, 16*). However, if the food functionality of shellfish meats could be improved by protein glycosylation, the information would contribute to the development of a new method of utilization of shellfish resources.

The object of this work is to investigate the effect of glycosylation on the solubility of shellfish myofibrillar proteins. In this study, we report the preparation of water-soluble myofibrillar proteins from scallop meat by reaction with glucose through the Maillard reaction. In addition, the soluble state of glycosylated myofibrillar proteins in low- and high-ionic-strength media will be discussed.

MATERIALS AND METHODS

Materials. A cultured live scallop (*Pecten yessoensis*) was obtained at a local fish market. Bovine serum albumin (fraction V) was obtained from Merck Co. Ltd. (Darmstadt, Germany). A fructosamine test calibration kit (glycosylated human serum) was purchased from Japan Roche Co. Ltd. (Tokyo, Japan). Sephacryl S-500 was purchased from Pharmacia LKB Biotechnology (Uppsala, Sweden). All chemicals (reagent grade) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of Scallop Myofibrils, Actomyosin, and Myosin. Striated adductor scallop muscle was washed and homogenized using a homogenizer (model AM-6, Nissei Co. Ltd., Tokyo, Japan) in 8 vol of 50 mM NaCl for 1 min at 15 000 rpm. The homogenate was centrifuged at 10 000*g* for 10 min to collect myofibrils, and the precipitate was resuspended in 50 mM NaCl and further centrifuged four times. Finally, myofibrils were obtained by filtration through a nylon cloth. Scallop actomyosin and myosin were prepared from the

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

10.1021/jf011717o CCC: $22.00 © 2002 American Chemical Society
Published on Web 06/18/2002

striated adductor muscle according to the method of Barany and Barany (*17*). Actomyosin formation of the myofibrillar proteins in NaCl solutions was confirmed by measuring the superprecipitation activity (*18*). All preparation steps were carried out below 8 °C. The protein concentration was determined by the biuret method (*19*) using bovine serum albumin as a standard.

Glycosylation of Scallop Myofibrillar Proteins with Glucose. Glucose at a final concentration of 0.6 M was added to the scallop myofibrils suspended in 50 mM NaCl, and the protein concentration was adjusted to 6.0 mg/mL. Each 5 mL of the mixture was placed in a test tube (diameter 16 mm), frozen at −40 °C, and immediately lyophilized using a freeze-dryer (FDU-506, Tokyo Rika Co. Ltd.,Tokyo, Japan). The lyophilization was stopped when the sample temperature reached 15−18 °C. The lyophilized protein powder was stored at −25 °C and examined within 30 days of preparation. The lyophilized powder was incubated at 40−60 °C and 35% relative humidity in a humidity cabinet (model LHU-112, Tabai Espec Corp., Tokyo, Japan) to react the proteins with glucose through the Maillard reaction. The glycosylated myofibrillar proteins thus obtained were dissolved in different concentrations of NaCl containing 40 mM Tris−HCl (pH 7.5) and used for subsequent experiments and analysis. In this study, myofibrillar proteins with 0.6 M sorbitol were lyophilized and incubated under the same conditions as the control.

Determination of Available Lysine and Fructosamine Contents. Available lysine and fructosamine assays were carried out to evaluate the protein glycosylation. Available lysine content was determined by the spectrophotometric analysis using *o*-phthalaldehyde and *N*-acetyl-L-cysteine (*20*). Before the analysis, the protein was precipitated with 7.5% (at a final concentration) trichloroacetic acid to remove Tris buffer and unreacted glucose. The protein was redissolved in 50 mM phosphate buffer (pH 9.5) containing 2% sodium dodecyl sulfate (SDS) at room temperature. The fructosamine was assayed by the method of Johnson et al. (*21*), and glycosylated human serum was used as a standard for determining the fructosamine content. Before the analysis of fructosamine, the protein solution was dialyzed in 0.1 M NaCl containing 40 mM Tris−HCl (pH 7.5) at 4 °C for 16 h to remove unreacted glucose. Available lysine and fructosamine assays were performed within 48 h after the protein glycosylation. Each value of available lysine and fructosamine is the mean of four replicates that were reproducible within ± 5%.

Solubility of Glycosylated Myofibrillar Proteins. The glycosylated myofibrillar proteins were suspended in 0.05−0.5 M NaCl containing 40 mM Tris−HCl (pH 7.5) at 1.0−1.5 mg/mL of the final protein concentration with a homogenizer (Ultra-turrax T 25/N-8G, IKA-labortechnik, Staufen, Germany) operating at 13 500 rpm for 1 min and immediately centrifuged at 15 000*g* for 30 min at 4 °C. The supernatant and the total protein solution were precipitated with 7.5% (at a final concentration) trichloroacetic acid and redissolved in 1.0 M NaOH. Their protein concentrations were determined by the microbiuret method (*22*) using bovine serum albumin as a standard. The solubility of the glycosylated myofibrillar proteins was expressed as the percent of the protein concentration in the supernatant with respect to that of the protein solution before centrifugation. In the preliminary experiment, we confirmed that unreacted glucose has no effect on the solubility of the glycosylated myofibrillar proteins. Each value of solubility is the mean of three replicates that were reproducible within ± 4%.

Stability of Glycosylated Myofibrillar Proteins. The stability of the glycosylated myofibrillar proteins was followed by monitoring changes in the solubility of the protein solution during cold storage. Lyophilized myofibrillar proteins with glucose were reacted at 50 °C and 35% relative humidity for 24 h. After being solubilized in 0.1 or 0.5 M NaCl containing 40 mM Tris−HCl (pH 7.5) and centrifuged at 15 000*g* for 30 min, the soluble fractions were dialyzed in the same NaCl solution at 4 °C for 16 h. The protein solution (2 mg/mL) was kept at 4 °C for 7 days with 0.05% sodium azide as a preservative. The solubility was measured in the manner described above.

Electrophoretic Analysis and Densitometry of Myosin, Actin, and Paramyosin. Relative amounts of myosin, actin, and paramyosin dissolved in 0.1 M NaCl containing 40 mM Tris−HCl (pH 7.5) were examined by SDS−polyacrylamide gel electrophoresis (SDS−PAGE) and quantified by densitometry. SDS−PAGE was performed in a 7.5% slab gel according to the method of Laemmli (*23*). A total of 0.5 mL of protein solutions was added to 0.5 mL of 2% SDS, 8 M urea, and 2% β-mercaptoethanol solution with 40 mM Tris−HCl (pH 8.0) and was heated in boiling water for 2 min. Each 6-μL sample was loaded in each gel lane, and 0.25% Coomassie Brilliant Blue R was used for protein staining. The image of each protein band stained on the gel was captured on a TIFF image file at 144 dpi and 256 gray scale by using a Macintosh model 7200/90 personal computer with an Epson GT-8000 scanner. NIH-image, version 1.55 (computer software written by W. Rasband, U.S. National Institutes of Health, 1994), was used to determine the relative intensity of each protein band. The solubility of myosin was determined by measuring the relative intensity of its heavy chain.

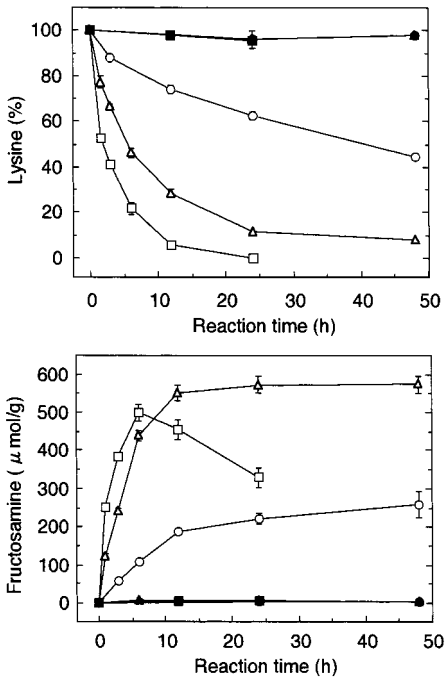

Figure 1. Changes in available lysine and fructosamine contents of scallop myofibrillar proteins during reaction with glucose at different temperatures. Myofibrillar proteins mixed with glucose (○, △, □) or sorbitol (●, ▲, ■) were lyophilized and incubated at 40 °C (○, ●), 50 °C (△, ▲), and 60 °C (□, ■) and 35% relative humidity. Data are means ± SD, $n = 4$.

Gel Permeation Chromatography of Glycosylated Myofibrillar Proteins. Lyophilized myofibrillar proteins with glucose were reacted at 50 °C and 35% relative humidity for 0 and 24 h. After being solubilized in 0.1 or 0.5 M NaCl containing 40 mM Tris−HCl (pH 7.5) and centrifuged at 15 000*g* for 30 min, 3 mg of the soluble fractions was applied to a gel filtration column (Sephacryl S-500, HR-16/100) equilibrated with the same buffer containing 1.0 M sorbitol, and eluted at a flow rate of 0.4 mL/min. Each 2 mL of the fractions was collected, and the protein concentration was determined by the Bradford method (*24*).

RESULTS AND DISCUSSION

Improvement of Solubility in a Low-Ionic-Strength Medium by Glycosylation. Figure 1 shows the effect of temperature on the reaction between lysine residues of scallop myofibrillar proteins and glucose. When lyophilized myofibrillar proteins with glucose were incubated at 40, 50, and 60 °C and 35% relative humidity, the available lysine content (0.097 g/g of protein) decreased, and fructosamine was produced simultaneously at all temperatures. These changes occurred rapidly with an increase in the reaction temperatures. On the other hand,

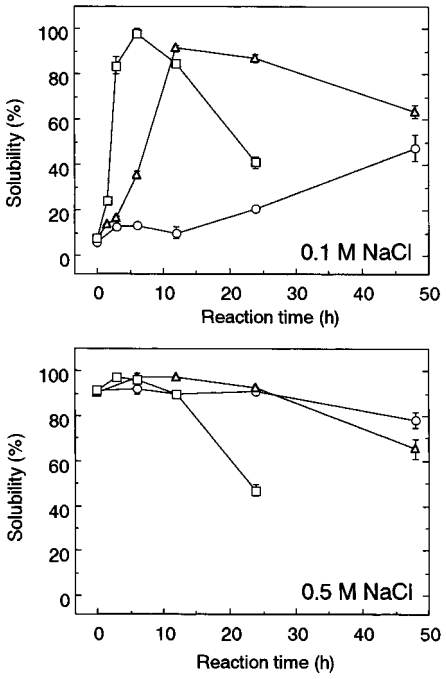

Figure 2. Solubility changes of myofibrillar proteins in 0.1 and 0.5 M NaCl during reaction with glucose at different temperatures. Reaction temperatures: 40 °C (○), 50 °C (△), and 60 °C (□). Data are means ± SD, $n = 3$.

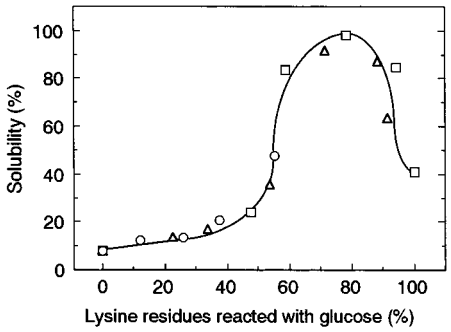

Figure 3. Relation between rate of reacted lysine residues and solubility in 0.1 M NaCl. Symbols are the same as those in Figure 2.

when the lyophilized proteins with sorbitol were incubated under the same conditions, the available lysine content remained unchanged, and no production of fructosamine was observed. These results indicate that the lysine residues in myofibrillar proteins were reacted with glucose through the Maillard reaction.

Figure 2 shows the changes in the solubility of myofibrillar proteins in 0.1 and 0.5 M NaCl during the reaction with glucose. The solubility in 0.1 M NaCl increased at all temperatures, and marked increases were observed in the reaction at 50 and 60 °C. When 72% (reaction at 50 °C for 12 h) and 79% (reaction at 60 °C for 6 h) of the lysine residues were reacted with glucose, the solubility reached 93% and 98%, respectively. On the other hand, no change in the solubility was observed in the lyophilized proteins with sorbitol incubated under the same conditions (data not shown). Thus, it is apparent that the solubility improvement of myofibrillar proteins occurred by the reaction with glucose through the Maillard reaction glycosylation.

The improved solubility in 0.1 M NaCl diminished with the progress of glycosylation, and the solubility in 0.5 M NaCl simultaneously decreased, as shown in **Figure 2**. **Figure 3** shows the relation between the amount of lysine residues reacted with

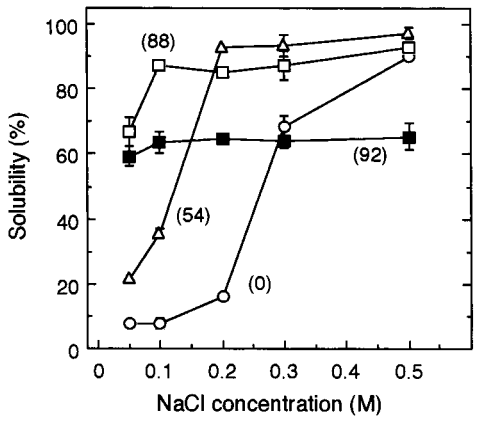

Figure 4. Changes in NaCl concentration dependence of solubility of glycosylated myofibrillar proteins. Myofibrillar proteins were reacted with glucose at 50 °C for 0 h (○), 6 h (△), 24 h (□), and 48 h (■). Numbers in parentheses are the rate at which lysine residues reacted with glucose (%). Data are means ± SD, $n = 3$.

glucose and the solubility in 0.1 M NaCl. The solubility in 0.1 M NaCl improved with an increase in the lysine residues reacted with glucose, and the marked increase was observed when 55−80% of lysine residues were reacted with glucose, regardless of reaction temperature. However, the improved solubility decreased when more than 90% of lysine residues were reacted with glucose. In addition, the solubility loss occurred with the decrease in the fructosamine content, as shown in **Figures 1** and **2**. It is known that fructosamine is the Amadori rearrangement product in the early stage of the Maillard reaction (*25*). Therefore, more than 60% of the lysine residues should be reacted with glucose without excess progress of the Maillard reaction to obtain a high solubility improvement in scallop myofibrillar proteins.

Solubility of Glycosylated Myofibrillar Proteins as affected by NaCl Concentration. Figure 4 presents the change in NaCl concentration dependence of the solubility of myofibrillar proteins during reaction with glucose. The solubility of native myofibrillar proteins (before glycosylation) increased gradually with the rise of NaCl concentration in the range of 0.2−0.5 M. On the other hand, when myofibrillar proteins were glycosylated at 50 °C for 6 h and 54% of available lysine was modified, the solubility increased markedly in the range of 0.05−0.2 M NaCl. Furthermore, the solubility in 0.1 M NaCl reached the highest value when myofibrillar proteins were glycosylated for 24 h and 88% of available lysine was modified by glucose. Although the highest solubility of myofibrillar proteins prepared by reaction for 48 h (92% of available lysine was modified) was lower than that of other glycosylated myofibrillar proteins, the NaCl concentration dependency of the solubility was completely lost. These results indicate that the NaCl concentration dependency of the solubility disappeared with the progress of glycosylation, and as a result, scallop myofibrillar proteins became solubilized in a medium containing a lower NaCl concentration than the native proteins.

Proteins Solubilized in a Low-Ionic-Strength Medium. SDS−PAGE analysis was performed to investigate the effect of glycosylation on protein components of myofibrillar proteins. **Figure 5** shows the changes in the protein subunits during glycosylation at 50 °C and the soluble fraction in 0.1 M NaCl. The electrophoretic mobility of myosin heavy chain, actin, and paramyosin decreased slightly with the progress of glycosylation. Although tropomyosin disappeared with the progress of glycosylation, no protein degradation was observed in the main

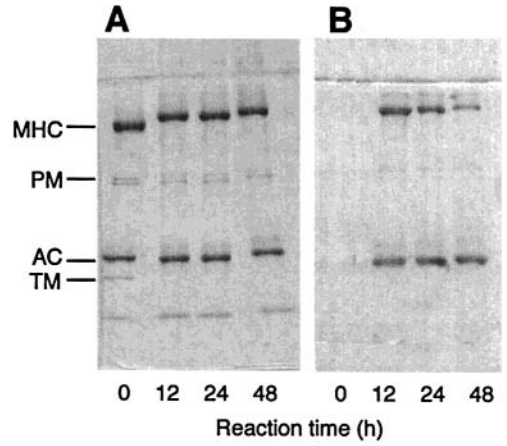

Figure 5. SDS–PAGE patterns of glycosylated myofibrillar proteins. Myofibrillar proteins were reacted with glucose at 50 °C. (A) Total fraction of myofibrillar proteins; (B) soluble fraction in 0.1 M NaCl. MHC, PM, AC, and TM denote myosin heavy chain, paramyosin, actin, and tropomyosin, respectively.

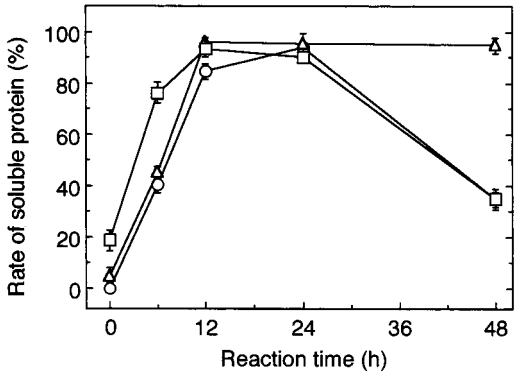

Figure 6. Changes in rate of protein components solubilized in 0.1 M NaCl during reaction with glucose. Myofibrillar proteins were reacted with glucose at 50 °C. Myosin (○), actin (△), and paramyosin (□). Data are means ± SD, $n = 3$.

components of myofibrillar proteins. The same changes were also observed in the reaction at 40 and 60 °C (data not shown).

As shown in the SDS−PAGE pattern, large amounts of myosin, actin, and paramyosin were simultaneously solubilized in 0.1 M NaCl as glycosylation progressed. The changes in the rate of the proteins solubilized in 0.1 M NaCl were then measured (**Figure 6**). The solubility of myosin, actin, and paramyosin increased simultaneously with glycosylation: 93% of myosin, 95% of actin, and 90% of paramyosin were solubilized after reaction at 50 °C for 24 h, and actin remained at high solubility during reaction for 48 h. However, the amount of the solubilized myosin and paramyosin decreased simultaneously with the further progress of glycosylation. Myosin combines with actin to form actomyosin when they are mixed in solution. Thus, the result of **Figure 6** indicates that the interaction of myosin and actin was lost during glycosylation.

Gel permeation chromatography was performed to investigate the soluble state of glycosylated myofibrillar proteins. **Figure 7** shows the elution profiles of native and glycosylated myofibrillar proteins. The main peak eluted at the void volume (fraction 37−47), and a small peak (fraction 67−79) was observed when native myofibrillar proteins were dissolved in 0.5 M NaCl. The main and small peaks were identified as actomyosin and actin, respectively. The same elution pattern was observed in lyophilized myofibrillar proteins with glucose

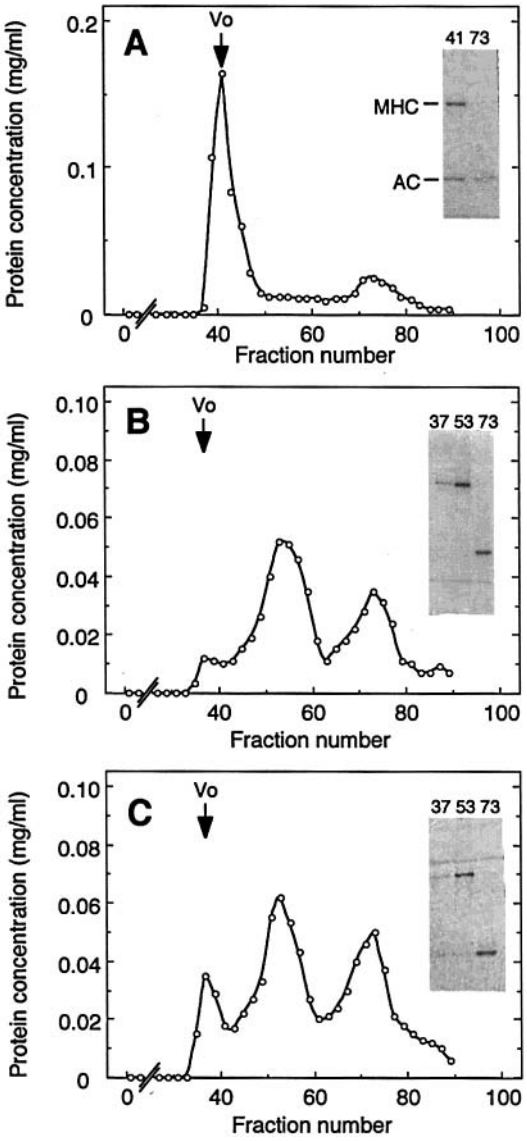

Figure 7. Elution profiles of glycosylated myofibrillar proteins. (A) Native myofibrillar proteins dissolved in 0.5 M NaCl; (B) glycosylated myofibrillar proteins dissolved in 0.5 M NaCl; (C) glycosylated myofibrillar proteins dissolved in 0.1 M NaCl. Myofibrillar proteins were reacted with glucose at 50 °C. Photos are SDS–PAGE patterns of each fraction. *Vo*: void volume determined by actomyosin. Abbreviations are the same as those in Figure 5.

(data not shown). However, the main peak diminished markedly and a new peak appeared (fraction 43−61) when myofibrillar proteins were reacted with glucose at 50 °C for 24 h and were dissolved in 0.5 M NaCl. The new peak coincided with that of the purified scallop myosin (data not shown). These results indicate that almost all of the myosin and actin were dissolved in 0.5 M NaCl without forming actomyosin and the solubilized myosin existed as a monomer. The same elution pattern was also obtained in the glycosylated myofibrillar proteins solubilized in 0.1 M NaCl. Further, the solubility of the glycosylated myofibrillar proteins remained unchanged for 7 days, as shown in **Table 1**. Therefore, it is apparent that the solubility improvement is not a tentative change and the myosin of the glycosylated myofibrillar proteins was dissolved consistently in 0.1 M NaCl without assembling insoluble filaments.

It is known that NaCl concentration dependence of the solubility of muscle reflects the biochemical characteristics of

Table 1. Comparison of Stability of Glycosylated Myofibrillar Proteins (values are means ± SD, $n = 3$)

specimen	medium[a]	storage time (days)	solubility (%)[b]
actomyosin	0.5 M NaCl	0	100.0
	0.5 M NaCl	7	88.8 ± 2.9
glycosylated myofibrillar proteins[c]	0.5 M NaCl	0	100.0
	0.5 M NaCl	7	98.9 ± 0.3
	0.1 M NaCl	7	99.5 ± 0.7

[a] Buffer contains 40 mM Tris–HCl (pH 7.5). [b] Solubility is normalized with the value at zero time as 100%. [c] Reaction was performed at 50 °C for 24 h.

myosin, which is the major constituent of myofibrillar proteins. Myosin aggregates and assembles into insoluble filaments when it exists in low ionic strength media and neutral pH (*26*). Myosin bears two fragments, the water-soluble head region (subfragment 1) and the water-insoluble rod region, and the rod regions are assembled into insoluble filaments (*27*). When myosin in the glycosylated myofibrillar proteins became solubilized with actin by glycosylation (**Figures 5** and **6**), the glycosylated myosin lost its filament-forming ability and existed as a monomer in 0.1 M NaCl (**Figure 7**). Therefore, the loss of filament-forming ability in the myosin rod region by glycosylation would contribute to the improved solubility of myofibrillar proteins in low-ionic-strength media.

Considering two lysine residues exist in the actin-binding site of subfragment 1 (*28*), the dissociation of myosin and actin by glycosylation could involve a structural impediment on the actin-binding site due to the attached glucose. Further study of the glycosylation process of myosin subfragment 1 is necessary to understand the molecular mechanism of the solubilization of the glycosylated myosin.

Saeki (*10*) has reported that the solubility improvement of fish myofibrillar proteins occurred when 17% of available lysine was reacted with glucose. However, in the case of scallop myofibrillar proteins, a 60% loss of the available lysine was required to achieve the solubility improvement. This result suggests that the addition of lysine may be required when the water-soluble scallop myofibrillar proteins are utilized as a protein resource. On the other hand, Sato et al. (*29*) reported that protein glycosylation with alginate oligosaccharide affected the same solubility improvement of fish myofibrillar proteins with a small amount of lysine loss (<10%). Thus, the application of other reducing sugars may reduce the nutritional loss occurring in the scallop myofibrillar proteins.

In conclusion, the results of this study revealed that water-soluble myofibrillar proteins can be prepared from shellfish meat by reaction with glucose through the Maillard reaction. This is the type of basic information needed to utilize shellfish meat as a liquid protein resource.

ACKNOWLEDGMENT

We thank Dr. Nobuo Seki, Dr. Kunihiko Konno, and Dr. Takao Ojima of the Graduate School of Fisheries Sciences, Hokkaido University, for their frequent, stimulating, and helpful discussions.

LITERATURE CITED

(1) Matsuda, Y. Influence of platen temperature of freeze-drying on the kamaboko-forming ability of lyophilized "Muen-Surimi". *Nippon Suisan Gakkaishi* **1971**, *37*, 130−134.

(2) Niki, H.; Deya, E.; Kato, T.; Igarashi, S. The process of producing active fish protein powder. *Nippon Suisan Gakkaishi* **1982**, *48*, 999−1004.

(3) Yu, S. Y.; Mitchell, J. R.; Abdullah, A. Production and acceptability testing of fish crackers ("keropok") prepared by the extrusion method. *J. Food Technol*. **1981**, *16*, 51−58.

(4) Fujimoto, K.; Endo, Y.; Cho, S.; Watebe, R.; Suzuki, Y.; Konno, M.; Shoji, K.; Arai, K.; Saito, S. A new method for producing Kamaboko from sardine meat powder by dehydration with a high osmotic pressure resin and defatting with liquefied carbon dioxide. *Nippon Suisan Gakkaishi* **1988**, *54*, 1071.

(5) Sugii, K.; Kinumaki, T. Studies on liquefied fish protein. Comparison of composition in different products by use of commercially available several proteolytic enzymes. *Bull. Tokai Reg. Fish. Res. Lab*. **1973**, *73*, 103−112.

(6) Archer, M. C.; Ragnarsson, J. O.; Tannenbaum, S. R.; Wang, D. I. C. Enzymatic solubilization of an insoluble substrate, fish protein concentrate: Process and kinetic considerations. *Biotechnol. Bioeng*. **1973**, *15*, 181−196.

(7) Miyake, Y. Solubilization of fish scrap by enzyme treatment. *Nippon Syokuhin Kogyo Gakkaishi* **1982**, *29*, 117−122.

(8) Piggot, G. M.; Tucker, B. W. *Seafood: Effects of Technology on Nutrition*; Dekker: New York, 1990; pp 223−227.

(9) 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.

(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 glycosylation with ribose. *Fish. Sci*. **1999**, *65*, 967−968.

(12) Elfvin, M.; Levine, R. C.; Dewey, M. M. Paramyosin in invertebrate muscles. I. Identification and localization. *J. Cell Biol.* **1976**, *71*, 261−272.

(13) Szent-Györgyi, A. G.; Szentkiralyi, E. M. The light chains of scallop myosin as regulatory subunits. *J. Mol. Biol.* **1973**, *74*, 179−203.

(14) Kendrick-Jones, J.; Szentkiralyi, E. M.; Szent-Györgyi, A. G. Regulatory light chains in myosins. *J. Mol. Biol.* **1976**, *104*, 747−775.

(15) Noguchi, S. Reito surimi no kagaku-I. *Suisan Neriseihin Gijutu Kenkyu Kaishi* **1976**, *5*, 356−363.

(16) Sano, T.; Noguchi, S.; Tsuchiya, T.; Matsumoto, J. Contribution of paramyosin to marine meat gel characteristics. *J. Food Sci*. **1986**, *51*, 946−950.

(17) Barany, M.; Barany, K. Myosin from the striated adductor muscle of scallop. *Biochem. Z.* **1966**, *345*, 37−56.

(18) Arai, K.; Takashi, R.; Saito, T. Studies on the muscular proteins of fish. V. On the superprecipitation of actomyosin from carp muscle. *Nippon Suisan Gakkaishi* **1970**, *36*, 487−490.

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

(20) Hernandez, M. J. M.; Alvarez-Coque, M. C. G. Available lysine in protein, assay using *o*-phthalaldehyde/*N*-acetyl-L-cysteine spectrophotometric method. *J. Food Sci*. **1992**, *57*, 503−505.

(21) Johnson, R. N.; Metcalf, P. A.; Baker, J. R. Fructosamine: A new approach to the estimation of serum glycosylprotein. An index of diabetic control. *Clin. Chim. Acta*. **1982**, *127*, 87−95.

(22) Itzhaki, R. F.; Gill, D. M. A micro-biuret method for estimating proteins. *Anal. Biochem.* **1964**, *9*, 401−410.

(23) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

(24) 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.

(25) Bunn, H. F.; Gabbay, K. H.; Gallop, P. M. The glycosylation of hemoglobin: relevance to diabetes mellitus. *Science* **1978**, *200*, 21−27.

(26) Huxley, H. E. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* **1963**, *7*, 281−308.
(27) Lowey, S.; Slayter, H. S.; Weeds, A. G.; Baker, H. Substructure of the myosin molecule. I. Subfragments of myosin by enzymic degradation. *J. Mol. Biol.* **1969**, *42*, 1−29.
(28) Nyitray, L.; Goodwin, E. B.; Szent-Györgyi, A. G. Complete primary structure of a scallop striated muscle myosin heavy chain. *J. Biol. Chem.* **1991**, *266*, 18469−18476.
(29) Sato, R.; Sawabe, T.; Kishimura, H.; Hayashi, K.; Saeki. 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.

Received for review December 28, 2001. Revised manuscript received April 29, 2002. Accepted May 9, 2002.

JF011717O