Short Paper

Change in Solubility of Carp Myofibrillar Protein by Glycosylation with Ribose

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Glycosylation of protein through the Maillard reaction is an effective method for improving functional properties of proteins. The functionality of food proteins such as bovine serum albumin,¹⁾ ovalbumin,²⁾ trypsin,³⁾ and water-soluble protein fraction of fish muscle⁴⁾ has been improved by glycosylation with glucose and its derivatives. In addition, Saeki and Inoue⁵⁾ reported that solubility of carp myofibrillar protein (Mf) in media of low ionic strength was effectively improved by modifying lysine residue with glucose through the Maillard reaction. However, no improvement was observed in the solubility of Mf in conjugation with polysaccharide, although its thermal stability and emulsifying properties were enhanced.⁶⁾ In this study, the effect of glycosylation with ribose on the solubility of carp Mf in low ionic strength media was investigated.

Mf (6.0 mg/ml) prepared from live carp ordinary muscle⁵⁾ was mixed with ribose at a final concentration of 0.3 M and lyophilized. In order to glycosylate protein with ribose through the Maillard reaction, the lyophilized protein powder was incubated at 30°C and 65% relative humidity for 0–8 h. The glycosylated proteins thus obtained were immediately mixed with 0.05–0.5 M NaCl containing 40 mM Tris-HCl (pH 7.5) at a final protein concentration of 1.5 mg/ml. The proteins were then homogenized with a high-speed homogenizer. The homogenates were dialyzed against the same NaCl solution at 4°C for 16 h to remove unreacted ribose, and then they were centrifuged at 15,000 g at 4°C for 30 min. The total solubility of Mf was expressed as the ratio of the protein concentration of the supernatant to that of the homogenate before centrifugation determined by the Biuret method.⁷⁾ The solubilities of myosin and actin were investigated by SDS-polyacrylamide gel electrophoresis with an image scanner.⁵⁾ The available lysine⁸⁾ and ketoamine⁹⁾ contents of Mf were also determined during the reaction.

Figure 1 shows the changes in NaCl concentration in relation to total solubility upon glycosylation of Mf. Before glycosylation, the total solubility in 0.01–0.1 M NaCl solutions was 9–12%, and a marked increase was observed in the NaCl concentration range of 0.16–0.2 M. On the other hand, the total solubility of the glycosylated Mf increased remarkably in the NaCl concentration range of 0.05–0.16 M. Seventy-three percent of the proteins were solubilized in 0.16 M NaCl when Mf was reacted for 2 h, and 21% of

Fig. 1. Change in NaCl concentration dependence of total solubility of carp Mf.

Glycosylation time: 0 h (○), 2 h (△), 4 h (□), and 8 h (●).

the lysine residue was modified by ribose. Further, the total solubility of Mf in 0.01–0.05 M NaCl increased as glycosylation progressed. The positive effect of glycosylation on protein solubility in NaCl solutions was obtained by the reaction at 35–50°C and confirmed in KCl solutions (data not shown). These results indicate that the solubility in low ionic strength media was improved by glycosylation with ribose through the Maillard reaction. Thus glycosylation with monosaccharides would be an effective method for solubilizing Mf protein in low ionic strength media.

Glycosylation with ribose improved the solubility of proteins at lower reaction temperatures than glycosylation with glucose, in which 61% of myosin and 82% of actin of Mf were solubilized in 0.16 M NaCl at 40°C for 12 h. (17% of available lysine was modified.⁵⁾) In the case of glycosylation with ribose, 72% of myosin and 80% of actin were solubilized in 0.16 M NaCl when Mf was modified with ribose at 30°C for 2 h (23% of available lysine was modified), as shown in Table 1. However, as the reaction advanced, a marked decrease was observed in the amount of solubilized myosin with a decrease in ketoamine content, whereas actin decreased slowly. This indicates that the decrease in the total solubility of the glycosylated Mf

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Table 1. Changes in available lysine and ketoamine contents and solubility of protein components in Mf during glycosylation

Reaction time (h)	Available lysine ($\mu\text{mole/g}$)	Ketoamine ($\mu\text{mole/g}$)* ¹	Ratio of soluble protein (%)** ²	
			Myosin heavy chain	Actin
0	666	0	0	12
2	526	40	72	80
4	439	56	60	72
8	286	25	19	57

*¹ Fructosamine equivalent. **² See Reference No. 5.

shown in Fig. 1 was caused by the insolubilization of myosin as the Maillard reaction progressed. Therefore, glycosylation by the Maillard reaction in the early stage is quite important for improving the solubility of Mf by glycosylation with ribose.

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