Effect of sodium alginate on changes in the denaturation of fish myofibrillar protein and the state of water during frozen storage

HIROSHI MOTOMURA,¹ KINGDUEAN SOMJIT,^{2*} AND YUKINORI NOZAKI³

¹ Graduate School of Marine Science and Engineering, Nagasaki University (*jnan-h@edu-c.pref.nagasaki.jp*)

² Graduate School of Science and Technology, Nagasaki University, ³ Faculty of Fisheries, Nagasaki University, Bunkyo, Nagasaki, 852-8521, Japan

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INTRODUCTION

Alginic acid is a major polysaccharide and structural component in the cell wall of brown algae. The water holding capacity and gelling ability of alginic acid are particularly useful function in the food industries.¹⁾ The quality of fish meat gradually deteriorates upon freezing due to protein denaturation. In the previous study,⁴⁾ we added sodium alginate (Na-Alg) isolated from three species of brown algae to myofibrils (Mf) from lizard fish and observed changes in both the state of water molecules and the denaturation of protein caused by dehydration. Results showed the retention and denaturation-suppressing effects of Na-Alg. In this study, we investigated the concentration effects of Na-Alg on protein denaturation due to freezing and the amount of unfrozen water in fish myofibrils.

MATERIALS AND METHODS

Hijiki (*Hizikia fusiformis*), wakame (*Undaria pinnatifida*) and konbu (*Laminaria japonica*) and fresh lizard fish (*Saurida wanieso*) were obtained from Nagasaki's fish market. Na-Alg was prepared by the method of Haug.⁵⁾ The sample was freeze-dried and stored until use. The mannuronic acid /glucuronic acid ratio (M/G ratio) of Na-Alg was determined by the method of Morris *et al.*⁶⁾ Mf were prepared by the method of Katoh *et al.*⁷⁾ Na-Alg of 2.5-12.5% (w/w) dry weight was added to Mf and pH was adjusted to 7.0. After mixing, the samples were sealed in micro-tubes and stored at -25°C. Mf without Na-Alg was also processed and stored as control. The Mf were taken out from the freezer and thawed in a cold room to 5°C. The sample was homogenized in 30-fold 0.1 M KCl, 20 mM Tris-maleate buffer (pH 7.0) and centrifuged at 750 x g for 10 min. The sediment was washed twice and suspended in the same buffer. Mf Ca-ATPase was measured according to the method of Katoh *et al.*⁷⁾ The decay of Ca-ATPase activity during frozen storage was assumed to obey the first order reaction mechanism. The rate constant of denaturation of Mf Ca-ATPase (K_D) was calculated using the following equation.⁸⁾ $K_D = (\ln C_0 - \ln C_t)/t$, where C_0 and C_t denote the relative activity of Mf Ca-ATPase before and t days after

storage, respectively. The amount of unfrozen water was measured by the method of Wakamatsu and Sato,⁹⁾ using a differential scanning calorimeter (DSC-100, Seiko Electronics Inc.). The thawed sample about 20 mg was placed in a tightly sealed aluminum container. The amount of heat of fusion was measured by elevating the temperature from -40°C to 25°C at a rate of 1.0°C/min. The amount of unfrozen water expressed as g H₂O/g dried sample was calculated by subtracting the free water from the water content.

RESULTS

The mean molecular weights of Na-Alg from hijiki, wakame, and konbu were 20,342, 44,834, and 44,363, while the M/G ratio were 1.68, 2.19, and 2.05 respectively. Ca-ATPase inactivation during frozen storage of Mf with 2.5%-12.5% Na-Alg from 3 species of algae was examined. Table 1 shows the rate constant for inactivation of Mf Ca-ATPase. Despite the addition of Na-Alg, a biphasic decrease in activity was observed. However, Mf with 7.5%-12.5% hijiki Na-Alg, 10%-12.5% wakame Na-Alg, and 10%-12.5 % konbu Na-Alg showed monophasic. A rapid drop to about 35% of the original level in 15 days of storage was observed in the control followed by a gradual decrease in 120 days. While the sample contained Na-Alg, ATPase activity was maintained at a high level and decrease in both phases was slower than without the additive. However, Ca-ATPase of Mf with 7.5%-12.5% hijiki Na-Alg was slightly different, as the same tendency as

Table 1. The rate constant (K_D) for inactivation of myofibrillar Ca-ATPase in the presence of various algal sodium alginates during frozen storage at -25°C

Type		Rate constant ($\times 10^3/\text{day}$)					
		control	2.5%	5%	7.5%	10%	12.5%
Hijiki	K_{D1}	64.9	19.7	13.7	5.4	3.9	4.2
	K_{D2}	12.7	6.5	5.2	-	-	-
Wakame	K_{D1}	64.9	25.3	17.5	13.6	7.6	9.1
	K_{D2}	12.7	11.4	9.2	6.9	-	-
Konbu	K_{D1}	64.9	38.4	24.0	20.4	7.7	8.7
	K_{D2}	12.7	15.9	6.3	7.5	-	-

those with 10%-12.5% wakame and konbu Na-Alg. The presence of Na-Alg delayed the decrease in liquid water in frozen Mf. The liquid water content in Mf decreased continuously while exhibiting an early fast phase followed by a second slower phase (Fig 1). The remaining liquid water in Na-Alg added samples was clearly greater than that in the control. However, the liquid water content in Mf with 7.5-12.5% hijiki Na-Alg was slightly different. The correlation between suppression of Mf ATPase and amount of unfrozen water by Na-Alg was investigated. A linear relationship existed between these two, although slopes varied among Na-Alg sources and concentrations (data not show).

DISCUSSION

Numerous reasons on the freeze-denaturation of Mf protein were observed. However, the destruction of hydrogen bonds and of the hydration layer surrounding polar residues of proteins due to ice crystal formation is considered to be the major cause of denaturation.³⁾ To utilize brown algae as a source of potential denaturation-suppressors, we isolated Na-Alg and evaluated their effects on denaturation during frozen storage in relation to the retention of liquid water. Alginic acid is a polysaccharide composed of D-mannuronic acid (M) and L-glucuronic acid (G), and its molecular weight, M/G ratio and the arrangement of M and G vary depending on the algal source, position in the algal body, season and site of gathering, extraction method, and measurement method.^{1,2)} It remains to be elucidated why the Na-Alg used in this study had lower molecular weight. The biphasic changes were observed when Ca-ATPase activity decreased rapidly, a slower decrease proceeded in a monophasic manner. This study, Ca-ATPase in Mf with Na-Alg was inactivated more slowly than control until 30-45 days of frozen storage and the Mf with 7.5%-12.5% hijiki Na-Alg, 10%-12.5% wakame Na-Alg, and 10%-12.5% konbu Na-Alg showed monophasic manner, indicating that Na-Alg clearly suppressed the frozen denaturation process. 10% hijiki Na-Alg showed the highest cryoprotective effect. This is caused by differences in the degree of Na-Alg polymerization. However, the mechanism behind the cryoprotective effects of Na-Alg has not been fully elucidated and it's a subject for future research. We estimated the amount of unfrozen water in fish Mf by differential scanning calorimetry. Our results clearly showed that Na-Alg delayed the decrease in liquid water content in Mf frozen with Na-Alg, and the absolute amount of unfrozen water in Mf with Na-Alg decreased as well as those without Na-Alg during frozen storage. This indicated that the freeze-denaturation of Mf progressed firstly during frozen storage, then decreasing the amount of unfrozen water in Mf. A close correlation exists between the remaining ATPase activity and the amount of unfrozen

water in frozen samples. These results suggest that the role of Na-Alg is to prevent ice crystal formation around Mf. The interaction of Na-Alg with hydrophilic residues such as OH^- in sucrose is believed to be responsible for this cryoprotection. However, the mechanism behind our results require further studies.

Fig.1 Effect of Na-Alg on the decrease in the amount of unfrozen water (gH₂O/g dried matter) in lizard fish Mf during frozen storage at -25°C for 120 days. Symbols: (○) control; (△) 2.5%; (■) 5%; (●) 7.5%; (▲) 10%; (□) 12.5%.

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