Bifunctional Lysozyme-Galactomannan Conjugate Having Excellent Emulsifying Properties and Bactericidal Effect

Soichiro Nakamura,† Akio Kato,*,‡ and Kunihiko Kobayashi‡

Department of Biochemistry, Yamaguchi University, Yamaguchi 753, Japan, and Department of Food and Nutrition, Ube College, Yamaguchi 755, Japan

Lysozyme-galactomannan conjugate prepared through controlled Maillard reaction revealed excellent emulsifying properties and antimicrobial properties. The lytic activity of the conjugate remained about 80% that of native lysozyme when measured by using *Micrococcus lysodeikticus* as a substrate. The emulsifying properties of the conjugate were superior to those of commercial emulsifiers. The emulsifying activity and stability of the lysozyme-galactomannan conjugate were not affected in the presence of 0.2 M NaCl and in acidic pH, while those of commercial emulsifiers were decreased. In addition, the lysozyme-galactomannan conjugate exhibited a lethal antimicrobial effect against Gram-negative bacteria.

INTRODUCTION

Protein-polysaccharide conjugates have been proposed to be useful as a new functional biopolymer having excellent emulsifying properties and antimicrobial effect (Kato et al., 1988, 1989, 1990; Nakamura et al., 1991). We (Kato et al., 1990) reported that a safe ovalbumin-dextran conjugate can be prepared by covalent binding of the ϵ-amino groups in the protein to the reducing-end carbonyl group in the polysaccharide through controlled Maillard reaction without using any chemical reagents. The emulsifying properties of this conjugate were comparable to those of commercial emulsifiers, and the emulsion stability at high salt concentration and in acidic pH condition was much higher than those of commercial emulsifiers (Kato et al., 1990). In the previous paper, we reported that a lysozyme-dextran conjugate revealed better emulsifying properties than ovalbumin-dextran conjugate (Nakamura et al., 1991). Another interesting finding was that the soluble dextran conjugate provided lysozyme with a novel bifunctional effect both on Gram-negative and on Gram-positive bacteria. However, although dextran has wide therapeutic use as a substitute for plasma, it is not suitable as a food additive because of its high cost. Accordingly, if dextran can be replaced by other polysaccharides suited for foodstuffs, it would be possible to develop a safe lysozyme-polysaccharide conjugate that is available as a food additive having excellent emulsifying properties and antimicrobial properties. Guar gum is a favorable polysaccharide that is utilized as a thickener, binder, and stabilizing agent in foods, and its mannase hydrolysate has recently been developed by Yamamoto et al. (1990) as a soluble dietary fiber. Thus, the mannase hydrolysate of guar gum (galactomannan) was applied as a polysaccharide to conjugate with lysozyme.

The present paper reveals that the lysozyme-galactomannan conjugate prepared through naturally occurring Maillard reaction showed excellent emulsifying properties and significant bactericidal effects on Gram-negative bacteria.

MATERIALS AND METHODS

Materials. Mannase hydrolysate of guar gum (galactomannan, average molecular weight of 15 000) was supplied from Taiyo Chemicals Co. Sepharose CL 6B was purchased from Pharmacia. *Micrococcus lysodeikticus* cells for lysozyme assay were from Wake Pure Chemicals. Bactotryptone and bactoyeast extract were from Difco Laboratories, and MacConkey medium was from Nissui Seiyaku Co. Other chemicals were all of analytical grade.

Preparation of Lysozyme Conjugate with Galactomannan. Lysozyme was crystallized from fresh egg white at pH 9.5 in the presence of 5% sodium chloride, and it was recrystallized five times. Galactomannan was dialyzed against deionized water for 2 days at 4 °C. Lyophilized lysozyme-galactomannan mixtures in a given weight ratio were stored at 60 °C for 3 weeks under relative humidities of 65% and 79%, as previously described (Kato et al., 1990; Nakamura et al., 1991). The resulting lysozyme-polysaccharide conjugate was separated from the unreacted lysozyme by gel filtration using a Sepharose CL 6B column (65 × 2.0 cm) equilibrated and eluted with 50 mM acetic acid-sodium acetate buffer (pH 5.0). The degree of conjugation of protein with polysaccharide was determined from the absorption spectrum for lysozyme at 280 nm and for galactomannan at 490 nm after color development with the phenol-sulfuric acid reaction. All fractions containing lysozyme-galactomannan conjugate were collected together, dialyzed against deionized water, and lyophilized. The resulting conjugates were stored at 4 °C.

Electrophoresis in SDS Slab Polyacrylamide Gel. SDS slab polyacrylamide gel electrophoresis was conducted according to the method of Laemmli (1970) using 15% acrylamide separating gel and 5% stacking gel containing 0.1% SDS. Samples (0.1%) were heated at 100 °C for 3 min in Tris-glycine buffer (pH 8.8) containing 1% SDS. Electrophoresis was carried out at a constant current of 10 mA for 5 h using an electrophoretic buffer of Tris-glycine containing 0.1% SDS. The gels were stained for proteins and carbohydrates with Coomassie blue G-250 and Fuchsin, respectively.

Measurement of Emulsifying Properties. The emulsifying properties were determined according to the method of Pearce and Kinsella (1978). An emulsion was prepared by homogenization of 1.0 mL of corn oil and 3.0 mL of a 0.1% sample solution, using an Ultra Turrax machine (Hansen Co.) at 12 000 rpm for 1 min at 20 °C. One hundred microliters of emulsion was taken from the bottom of the test tube after standing for 0, 1, 2, 3, 5, 10, 20, and 40 min and diluted with 5.0 mL of 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted emulsion was then determined at 500 nm. The relative emulsifying activity was represented as the absorbance at 500 nm measured immediately after emulsion formation (0 min). The emulsion stability was estimated by measuring the half-life of the decay of emulsion, estimating from the turbidity curves of emulsion during standing for 40 min.

Determination of Free Amino Groups. The content of free amino groups in the lysozyme-galactomannan conjugate was determined according to the method of Haynes et al. (1967) using a specific reagent for amino groups, trinitrobenzenesulfonate.

* Author to whom correspondence should be addressed.
† Ube College.
‡ Yamaguchi University.

0021-8561/92/1440-0735$03.00/0

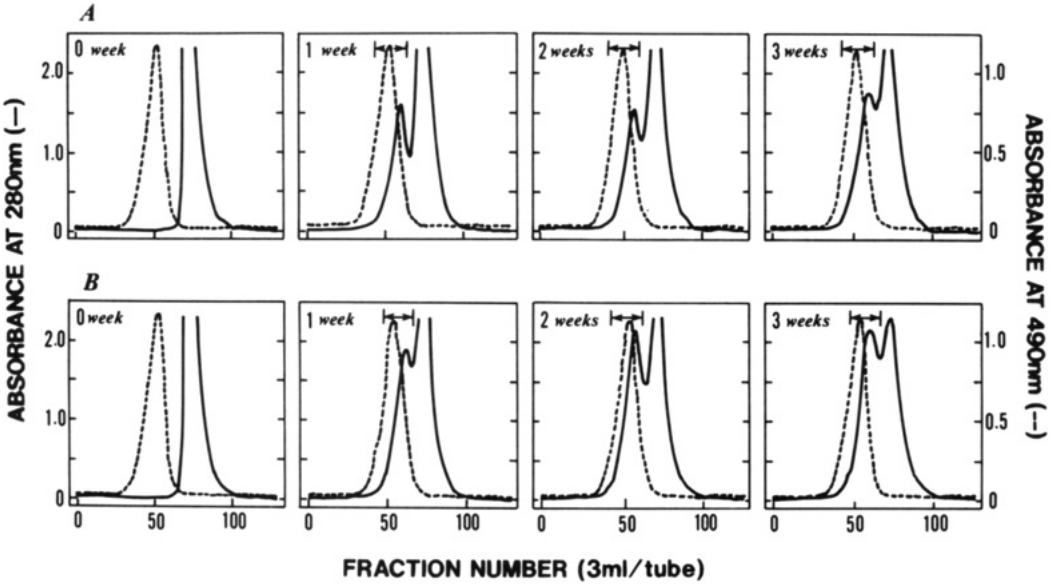

Figure 1. Changes in the gel filtration patterns of lysozyme and galactomannan mixtures (1:1) in 50 mM acetate buffer (pH 5.0) on a Sepharose CL 6B column during storage in dry state at 60 °C under 65% relative humidity (A) and under 79% relative humidity (B) for 0–3 weeks. The fractions indicated by horizontal arrows were pooled, dialyzed, and used for further experiments. (—) Absorbance at 280 nm for protein; (- - -) absorbance at 490 nm to follow the color development by the phenol–sulfate method for carbohydrate.

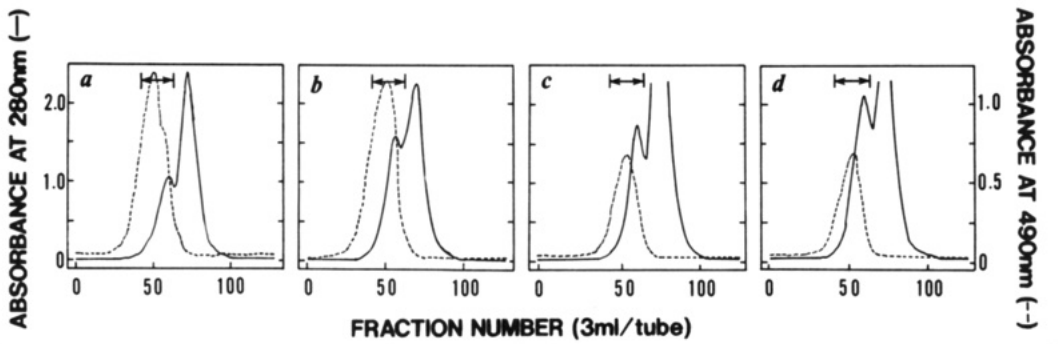

Figure 2. Changes in the gel filtration patterns of lysozyme and galactomannan mixtures in the weight ratios 1:2 (a, b) and 2:1 (c, d) in 50 mM acetate buffer (pH 5.0) on a Sepharose CL 6B column during storage in dry state at 60 °C under 65% relative humidity (a, c) and under 79% relative humidity (b, d) for 2 weeks. The fractions indicated by horizontal arrows were pooled, dialyzed, and used for further experiments. (—) Absorbance at 280 nm for protein; (- - -) absorbance at 490 nm to follow the color development by the phenol–sulfate method for carbohydrate.

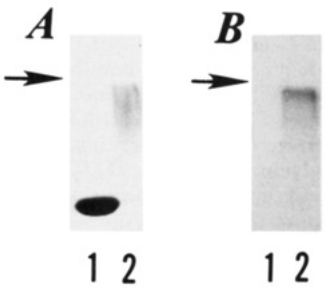

Figure 3. SDS slab polyacrylamide gel electrophoretic patterns of the purified lysozyme–galactomannan conjugate stained for protein (A) and carbohydrate (B). Arrows indicate the position of the boundary between staking (upper) and separating (lower) gels. (Lane 1) Native lysozyme; (lane 2) lysozyme–galactomannan conjugate.

Measurement of Lysozyme Activity. The lysozyme activity was measured by the lytic assay using *M. lysodeikticus* cells as a substrate. Cell suspension was prepared in 50 mM potassium phosphate buffer (pH 7.0). The absorbance at 660 nm was adjusted to 0.8, and then 0.1 mL of 0.003% lysozyme solution was added to 2.4 mL of the suspensions. The initial velocities were determined by measuring the decrease in the turbidity of the cells automatically monitored at 660 nm with a spectrophotometer (Hitachi Co., U-2000). The heat stability of lysozyme and the conjugate was measured at different temperatures from 37 to 95 °C at a rate of 1 °C/min. The heated lysozyme solutions (0.1 mL) were taken out at a given temperature and then immediately added to 2.4 mL of substrate solution incubated at 37 °C to measure lytic activity.

Microorganisms. *Vibrio parahaemolyticus* IFO 12711, *Escherichia coli* IFO 12713, *Aeromonas hydrophila* IFO 13286, *Proteus mirabilis* IFO 13300, and *Klebsiella pneumoniae* IFO 14438 were used as test organisms in this study. These five Gram-negative strains were obtained from the Institute for Fermentation, Osaka. Stationary-phase cultures of these strains were grown in L-broth (10 g of bactotryptone, 5 g of bactoyeast extract, 5 g of NaCl, 1 g of glucose, and water to 1 L) as described previously (Nakamura et al., 1991).

Antibacterial Assays. Overnight cultured cells were harvested from the culture media by centrifugation and were resuspended in 50 mM potassium phosphate buffer (pH 7.0) after two washings with the same buffer. Washed cell preparations were diluted with the same buffer to give a concentration of 10^5 cells/mL by using a hematometer. Sample was added to the cell suspension to give a final lysozyme concentration of 0.05%. Five milliliters of the suspension was incubated at 50 °C in a water bath equipped with a Lab-thermo shaker (Advantic Co.) at 90 rpm to provide constant temperature and cell suspension. After a given heating time, the heated suspension was immediately added to a sterile tube immersed in an ice bath. After the suspensions were cooled to room temperature, decimal dilution was subsequently carried out in physiological saline solution adjusted to pH 7.2. A 100 μL portion was spread over MacCon-

Table I. Binding Mode of Lysozyme–Galactomannan Conjugate

	native lysozyme	conjugate	
		lysozyme	galactomannan
binding ratio			
weight		1	1.8[a]
molar		1	1.7
free amino groups			
no. per mole	7		5.3[a]

[a] The data represent the means of four determinations.

Table II. Lytic Activity of Lysozyme–Galactomannan Conjugate

	lytic activity, %
native lysozyme	100
lysozyme–galactomannan conjugate	78.9
(lysozyme–dextran conjugate)[a]	(13.3)

[a] Parentheses value is cited from the literature (Nakamura et al., 1991).

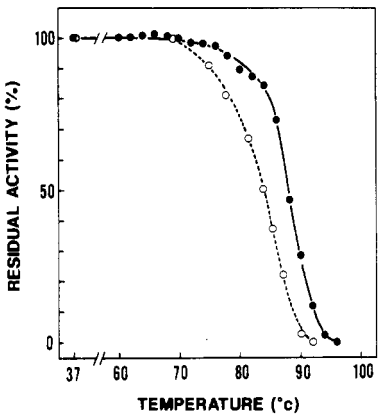

Figure 4. Denaturation curves of the purified lysozyme–galactomannan conjugate (●) and native lysozyme (○).

key agar plates. For halophilic bacteria, *V. parahaemolyticus*, the culture media supplemented with NaCl at the final concentration of 2.5% were used to ensure complete growth. The number of colonies formed after incubation at 35 °C for 24 h was measured to calculate the survival ratio.

Detection of Cell Destruction. The cell suspensions were treated at 20 °C for 60 min and at 50 °C for 30 min with and without 0.05% protein concentration of the lysozyme–galactomannan conjugate or native lysozyme, respectively. Each supernatant was scanned from 300 to 200 nm by UV absorbance after centrifuging at 15000*g* for 10 min.

RESULTS AND DISCUSSION

Figure 1 shows changes in elution profiles on a Sepharose CL 6B column of lysozyme–galactomannan mixtures in the weight ratio 1:1 during storage in 65% relative humidity (A) and in 79% relative humidity (B) at 60 °C for 0–3 weeks. Each fraction was analyzed for carbohydrate and protein to detect the formation of conjugate. As lysozyme was incubated with galactomannan, there were significant shifts in the protein peak from a smaller to a higher molecular weight fraction, suggesting that lysozyme was covalently attached to galactomannan. The data from elution patterns revealed that lysozyme was maximally linked with galactomannan in the weight ratio 1:1 by incubating at 60 °C under 79% relative humidity for 2 weeks. To examine further the optimal mixture ratio and relative humidity for the conjugate formation, Figure 2 shows changes in elution profiles of lysozyme–galactomannan mixtures in the weight ratios 1:2 (a, b) and 2:1 (c, d) during storage in 65% (a, c) and 79% relative humidity (b, d) at 60 °C for 2 weeks. All elution patterns in the

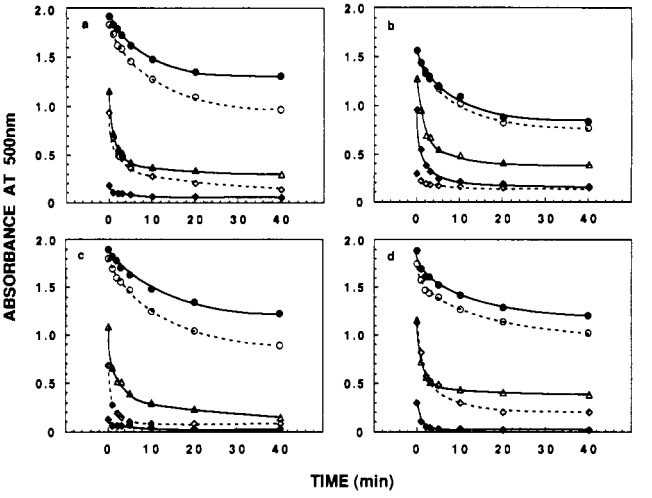

Figure 5. Emulsifying properties of lysozyme–galactomannan conjugates and commercial emulsifiers. (○) Purified lysozyme–galactomannan conjugates obtained from 2-week incubation at 60 °C under 65% relative humidity; (●) purified lysozyme–galactomannan conjugates obtained from 2-week incubation at 60 °C under 79% relative humidity; (♦) native lysozyme; (◇) commercial emulsifier, sunsoft SE11, sucrose fatty ester (HLB11), Taiyo Kagaku; (△) commercial emulsifier, sunsoft Q18S, decaglyceryl monoesterate (HLB12) Taiyo Kagaku. (a) In 1/15 M sodium phosphate buffer (pH 7.4); (b) in 1/15 M sodium citrate buffer (pH 3.0); (c) in 1/15 M sodium phosphate buffer (pH 7.4) containing 0.2 M NaCl; (d) samples heated to 90 °C at a rate of 3 °C/min in 1/15 M sodium phosphate buffer (pH 7.4).

different mixture ratios and relative humidities indicated that the conjugate formation in these conditions is lower than in the mixture ratio 1:1 in 79% relative humidity. Thus, the most effective conjugate formation was obtained from the mixture in the weight ratio 1:1 in 79% relative humidity. The higher molecular weight fractions were collected together and used in the subsequent experiments.

Evidence for covalent attachment of lysozyme to galactomannan was obtained from SDS slab polyacrylamide gel electrophoresis of the conjugate, as shown in Figure 3. The electrophoretic patterns of the lysozyme–galactomannan conjugate showed a broad band for both protein and carbohydrate stains near the boundary between stacking and separating gels, whereas the pattern of native lysozyme showed a single band. Thus, it was suggested that lysozyme was covalently attached to the polysaccharide.

Table I shows the binding mode of the lysozyme–galactomannan conjugate. The binding molar ratio was calculated on the assumption that the molecular weights of lysozyme and galactomannan were 14 000 and 15 000, respectively. On the basis of the binding weight ratio, about 2 mol of galactomannan appear to link to one molecule of lysozyme. The measurement of free amino groups in the conjugate was carried out to obtain further information on the binding mode. About two free amino groups per molecule were decreased in the conjugate. This result is consistent with the binding ratio of lysozyme–galactomannan conjugate estimated from the binding weight ratio between lysozyme and galactomannan, as described above. This suggests that lysozyme is linked with galactomannan through only one reducing-end group in the polysaccharide and amino groups in the protein without a polymerized network structure by the controlled Maillard reaction.

Table II shows the lytic activity of a lysozyme–galactomannan conjugate. When the lytic activity of the lysozyme–galactomannan conjugate was measured by using *M. lysodeikticus*, about 80% of enzymatic activity still remained, while only 13.3% activity remained in the

Table III. Emulsifying Properties of Lysozyme–Galactomannan Conjugate and Commercial Emulsifiers

condition		LG(RH65)[a]	LG(RH79)[b]	LZ[c]	SE11[d]	Q18S[e]
pH 7.4[f]	activity, OD_{500nm}	1.843	1.920	0.187	0.944	1.165
	stability, min	>40	>40	3.5	4.1	2.5
pH 3.0[g]	activity, OD_{500nm}	1.575	1.569	0.968	0.304	1.288
	stability, min	38	>40	1.7	5.1	3.8
0.2 M NaCl[h]	activity, OD_{500nm}	1.807	1.902	0.129	0.693	1.097
	stability, min	39	>40	3.0	0.8	2.3
heated[i]	activity, OD_{500nm}	1.757	1.892	0.299	1.142	1.168
	stability, min	>40	>40	0.9	2.4	1.8

[a] LG(RH65), lysozyme–galactomannan conjugate obtained from 2 weeks of incubation at 60 °C under 65% relative humidity. [b] LG(RH79), lysozyme–galactomannan conjugate obtained from 2 weeks of incubation at 60 °C under 79% relative humidity. [c] LZ, native lysozyme. [d] SE11, sucrose fatty acid ester (HLB11) from Taiyo Kagaku Co., Ltd. [e] Q18S, decaglyceryl monoesterate (HLB12) from Taiyo Kagaku. [f] 1/15 M sodium phosphate buffer. [g] 1/15 M sodium citrate buffer. [h] 1/15 M sodium phosphate buffer (pH 7.4) containing 0.2 M NaCl. [i] Samples were heated to 90 °C at a rate of 3 °C/min in 1/15 M sodium phosphate buffer (pH 7.4) and then immediately cooled to 20 °C.

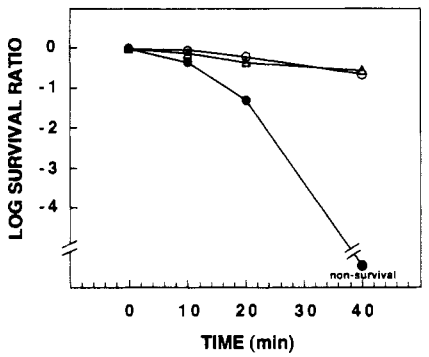

Figure 6. Antimicrobial effect of lysozyme–galactomannan conjugate on *E. coli* IFO 12713. (●) Lysozyme–galactomannan conjugate; (△) native lysozyme; (○) control (medium without conjugate or lysozyme).

case of lysozyme–dextran conjugate (Nakamura et al., 1991). A big difference in lytic activity between both conjugates may come from the steric hindrance due to the attached polysacchardies. The high lytic activity of lysozyme–galactomannan conjugate is preferable for use as a novel bactericidal agent.

Figure 4 shows the thermal denaturation curves of lysozyme–galactomannan conjugate obtained by monitoring the lysozyme activity. The heat stability of lysozyme was enhanced by conjugation with the galactomannan.

The emulsifying properties of lysozyme were remarkably increased by conjugation with galactomannan. Figure 5 shows the emulsifying properties of lysozyme–galactomannan conjugate obtained in a dry state at 60 °C for 2 weeks in 65% and 79% relative humidity and those of commercial emulsifiers. The turbidity of emulsion is plotted as ordinate and standing time after emulsion formation as abscissa. The conjugates prepared in 79% relative humidity revealed more excellent emulsifying properties than that prepared in 65% relative humidity. The emulsifying properties of the lysozyme–galactomannan conjugate were not affected in the presence of 0.2 M NaCl or acidic pH and by heating. Since high-salt condition, acidic pH condition, and heating processes were commonly used in industrial application, the lysozyme–galactomannan conjugate is suitable for foodstuffs. Table III shows the emulsifying activity and stability of the conjugate calculated from Figure 5. As shown in Table III, the emulsifying properties of the conjugate were much superior to those of commercial emulsifiers in various conditions. Thus, we have succeeded in developing a safe macromolecular emulsifier that can be used in practical food processing.

Since the commercial mannase hydrolysate of guar gum is contaminated with a considerable amount of free smaller molecular weight carbohydrates, the preferential conjugation of lysozyme with these oligomeric saccharides may cause lower emulsifying properties. It has been reported that the emulsifying properties of protein–glucose conjugate are lower than those of protein–polysaccharide conjugate (Kato et al., 1990). Therefore, before the preparation of the lysozyme–polysaccharide conjugate, the galactomannan was dialyzed against deionized water for 2 days at 4 °C. The yield of dialysate of the polysaccharide was 65% that of commercial sample, and the reducing power was also decreased to 27%, suggesting that low molecular weight carbohydrates were eliminated by dialysis.

Table IV. Antimicrobial Activity of Lysozyme–Galactomannan Conjugate for Five Laboratory Culture Gram-Negative Bacteria

tested strain	coexisting materials	survival ratio[a]
A. hydrophila IFO 13286	control[b]	−3.95
	conjugate[c]	nonsurvival
	lysozyme[d]	nonsurvival
V. parahaemolyticus IFO 12711	control	−1.14
	conjugate	nonsurvival
	lysozyme	−2.84
E. coli IFO 12713	control	−0.82
	conjugate	−2.48
	lysozyme	−0.79
P. mirabilis IFO 13300	control	−2.05
	conjugate	nonsurvival
	lysozyme	−2.15
K. pneumoniae IFO 14438	control	−0.59
	conjugate	−2.89
	lysozyme	−0.44

[a] Survival ratio when tested strains were incubated at 50 °C for 30 min. [b] In control medium. [c] In the medium supplemented with 0.05% (for protein) lysozyme–galactomannan conjugate. [d] In the medium supplemented with 0.05% native lysozyme.

Figure 6 shows the antimicrobial effect of lysozyme–galactomannan conjugate on *E. coli* IFO 12713 that is typical Gram-negative bacteria. The log survival ratio of the ordinate gives the survival decimal fraction based on the log values. The living cells were drastically decreased with heating time at 50 °C in the presence of lysozyme–galactomannan conjugate and had completely disappeared from the medium after 40 min of treatment. On the other hand, the bactericidal effects were not observed in the presence of native lysozyme and in control medium (without lysozyme and/or lysozyme–galactomannan conjugate). Table IV summarizes the antimicrobial effects of lysozyme–galactomannan conjugate induced by heat treatment at 50 °C for 30 min on five Gram-negative bacterial strains that are food-poisoning microorganisms. Although all tested strains were slightly affected by heating

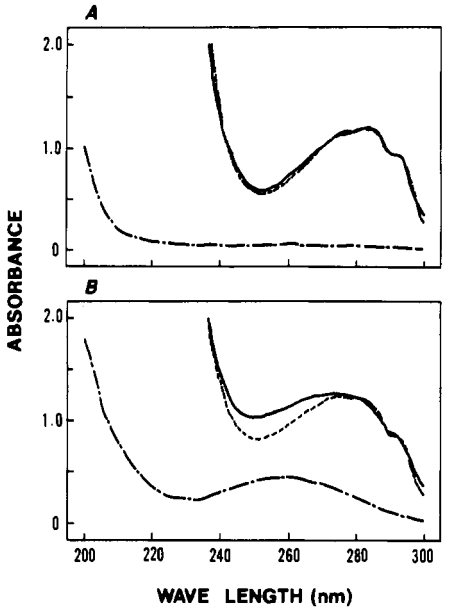

Figure 7. UV absorption characteristics of the supernatants of *E. coli* IFO 12713 suspensions treated with lysozyme–galactomannan conjugate at 20 °C for 60 min (A) and at 50 °C for 30 min (B). (—) Lysozyme–galactomannan conjugate; (- - -) native lysozyme; (– • –) control (medium without conjugate or lysozyme).

in the absence of the conjugate, the bactericidal effects for all strains appeared in the media supplemented with lysozyme–galactomannan conjugate. Thus, it is revealed that the lethal effect was effectively induced by exposure to lysozyme–galactomannan conjugate in medium as well as lysozyme–dextran conjugate (Nakamura et al., 1991).

Further evidence that the bactericidal effects were caused by the lysozyme–galactomannan conjugate was shown by using *E. coli*. As shown in Figure 7, the test strain was not affected with lysozyme–galactomannan conjugate or with native lysozyme by incubation at 20 °C. On the other hand, a significant increase of absorbance in the neighborhood of 260 nm was observed in the supernatant in the presence of the conjugate by incubation at 50 °C. This observation suggests that irreversible damage occurred to the cellular surface of *E. coli* in the presence of lysozyme–galactomannan conjugate, because the 260-nm-absorbing materials were regarded as the cellular materials leaked from damaged cells.

Lysozyme attacks only specific positions of glycosidic bonds between *N*-acetylhexosamines of the peptidoglycan layer in bacterial cell walls. However, since the cell envelope of these bacteria contains a significant amount of hydrophobic materials such as lipopolysaccharide (LPS) associated with the thin peptidoglycan layer, native lysozyme fails to lyse Gram-negative bacteria when it is simply added to the cell suspension in the native form. As discussed in the previous paper (Nakamura et al., 1991), cooperating factors such as the use of some detergents and heat treatment destabilize and consequently solubilize the outer membranes which consist mainly of LPS. Therefore, the excellent surfactant activity of lysozyme–galactomannan conjugate seems to result in the destruction of the outer membrane with synergistic effects of thermal stresses to lyse Gram-negative bacterial cells.

Many attempts have been made to develop food preservatives having superior antimicrobial effect without any toxicities. For this purpose, hen egg white lysozyme is the most promising reagent. We have made an attempt to extend the antimicrobial spectrum of hen egg white lysozyme to Gram-negative bacteria and succeeded in producing the lysozyme–dextran conjugate possessing novel bifunctional properties that act on both Gram-negative and Gram-positive bacteria (Nakamura et al., 1991). In this paper, it was shown that lysozyme–galactomannan conjugate revealed bactericidal effects comparable to those of lysozyme–dextran conjugate. In addition to the antimicrobial activity, lysozyme–galactomannan conjugate exhibited more stable emulsifying properties in various conditions than those of commercial emulsifiers. The conjugate prepared without the use of chemicals can be potentially applied to formulated foods as a safe multifunctional food additive. We have confirmed that lysozyme–galactomannan conjugate is nontoxic for oral administration test using rats and it is negative for Ames test and Rec assay (data not shown). In addition, the therapeutic effect of galactomannan is expected. Yamamoto et al. (1990) reported that the oral administration of galactomannan decreased total content of lipids in the liver of rats. Since galactomannan is not so expensive as dextran, lysozyme–galactomannan conjugate can be used as a food preservative and emulsifier.

LITERATURE CITED

Haynes, R.; Osuga, D. T.; Feeney, R. E. Modification of proteolytic enzyme. *Biochemistry* **1967**, *6*, 541–547.

Kato, A.; Murata, K.; Kobayashi, K. Preparation and characterization of ovalbumin–dextran conjugate having excellent emulsifying properties. *J. Agric. Food Chem.* **1988**, *36*, 421–425.

Kato, A.; Sato, T.; Kobayashi, K. Emulsifying properties of protein-polysaccharide complexes and hybrids. *Agric. Biol. Chem.* **1989**, *53*, 2147–2152.

Kato, A.; Sasaki, Y.; Furuta, R.; Kobayashi, K. Functional Protein-polysaccharide conjugate prepared by controlled dry-heating of ovalbumin-dextran mixture. *Agric. Biol. Chem.* **1990**, *54*, 107–112.

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

Nakamura, S.; Kato, A.; Kobayashi, K. New antimicrobial characteristics of lysozyme–dextran conjugate. *J. Agric. Food Chem.* **1991**, *39*, 647–650.

Pearce, K. M.; Kinsella, J. E. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Food Chem.* **1978**, *26*, 716–723.

Yamamoto, T.; Yamamoto, S.; Miyahara, I.; Matsumura, Y.; Hirata, A.; Kin, M. Isolation of a β-mannan hydrolyzing enzyme and hydrolysis of guar gum by the enzyme isolated. *Denpun Kagaku* **1990**, *37*, 99–105.

Received for review July 15, 1991. Revised manuscript received January 3, 1992. Accepted February 24, 1992.