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Review

Industrial Applications of Maillard-Type Protein-Polysaccharide Conjugates

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This review summarizes the properties of Maillard-type protein-polysaccharide conjugates through naturally occurring reaction in a dry state from the viewpoint of the development of new industrial proteins. Maillard-type protein-polysaccharide conjugates showed excellent emulsifying properties superior to conventional commercial emulsifiers, heat stability, and antimicrobial activity. Therefore, the conjugates can be useful for industrial applications as natural emulsifiers, antimicrobial agents devoid of toxicity. The possibility has also been proposed that conjugation of the allergen protein with polysaccharides may be effective to reduce the allergenicity. The molecular mechanism of the improvement of functional properties of proteins by attachment with polysaccharide was elucidated using the genetically glycosylated lysozyme constructed in the yeast expression system. The polymannosylation of lysozyme was effective in improving these functional properties, while oligomannosylation was not. Although single polyglycosylation of lysozyme was adequate to improve the functional properties, double polyglycosylation was even better.

Keywords: Maillard reaction, protein-polysaccharide conjugates, lysozyme, chitosan, emulsifying properties

1. Introduction

Several researchers have developed methods to improve the functional properties of proteins using chemical and enzymatic modifications to meet requirements for high quality proteins in foods. Transglutaminase and proteases have been successfully used for industrial applications (Nio *et al*., 1985; Motoki *et al*., 1986). In contrast, most chemical modifications have not been used for food applications, because of the potential health hazard or the appearance of detrimental products. Therefore, an idea different from a conventional approach is desirable to improve the functional properties of proteins in food systems. We investigated the utilization of a naturally occurring reaction, e.g., deamidation (Kato *et al*., 1987) and the Maillard reaction (Kato *et al*., 1990), without the use of a chemical reagent.

Proteins have various unique functional properties. However, the industrial applications of food proteins are limited, because proteins are generally unstable against heating, organic solvents and proteolytic attack. Therefore, if proteins can be converted into stable forms, the applications will be greatly broadened in food processing. Glycosylation of proteins is expected to overcome their instability to heating and to further improve the functional properties. This glycosylation was attempted using monosaccharides or oligosaccharides, but the effects were inadequate to make them industrially useful. Marshall and Rabinowitz (1976) reported that soluble protein-dextran conjugation by coupling proteins to cyanogen bromide activated dextran was dramatically effective to the heat stability of various enzymes. We also found that the soluble protein-dextran conjugates prepared by coupling with cyanogen bromide activated dextran showed excellent emulsifying properties in addition to heat stability (Kato *et al*., 1988). Based on these observations, we developed a novel method to conjugate proteins with polysaccharides by spontaneous Maillard reaction occurring in the controlled dry heating between the ε-amino groups in protein and the reducing-end carbonyl group in polysaccharide. The most striking characteristic of the resulting protein-polysaccharide conjugates is the excellent emulsifying properties which are superior to commercial emulsifiers (Kato *et al*., 1990, 1993; Kato & Kobayashi, 1991) Among many chemical and enzymatic modifications of proteins to improve functionality, this method could be one of the most promising approaches for food applications, because of its safety and other advantages. In addition to the dramatic improvement in emulsifying properties, this approach was efficient in improving the solubility of insoluble gluten (Kato *et al*., 1991), enhancement of the antioxidant effect of ovalbumin (Nakamura *et al*., 1992a) and broadening of the bactericidal effect of lysozyme (Nakamura *et al*., 1991, 1992b). Therefore, these Maillard-type protein-polysaccharide conjugates can be used as proteinaceous food additives such as emulsifiers, antibacterial agents and antioxidants. This review summarizes the properties of Maillard-type protein-polysaccharide conjugates from the viewpoint of the development of new types of food additives, medicines and cosmetics.

2. Preparation and Binding Mode of Protein-Polysaccharide Conjugates

Maillard-type protein-polysaccharide conjugates can be efficiently prepared during storage of the freeze-dried powders of protein-polysaccharide mixtures (molar ratio of 1:5) at 60˚C for a given day under either 65% or 79% relative humidity in a desiccator containing saturated KI or KBr solution in the bottom, respectively. Maillard reaction between the ε-amino groups in protein and the reducing-end carbonyl group in polysaccharide is accelerated in the low water activity described above. The rate of

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reaction for the formation of the conjugates seems to depend on the conformation of proteins. The typical unfolded protein αs-casein easily forms the polysaccharide conjugate and four lysyl residues in αs-casein are reacted with polysaccharide within only 24 h (Kato *et al*., 1992a). The folded lysozyme, in contrast, slowly forms the polysaccharide conjugate and only two lysyl residues are reacted with polysaccharide even after 2 weeks without loss of lytic activity (Nakamura *et al*., 1992b). This may be due to the difference in the reactivity of the lysyl residues exposed outside between folded and unfolded proteins.

The polysaccharides dextran, galactomannan, xyloglycan, pectin and chitosan can be used as partner saccharides. Galactomannan and xyloglucan, derived from guar gum and tamarind seed, respectively, are used as the model polysaccharide and oligosaccharide, because they are well characterized and favorable polysaccharides which are utilized as thickeners, binders and stabilizing agent in food applications. These polysaccharides have only one reactive residue, carbonyl group in the reducing end, which is easily attached to the ε-amino groups in proteins.

The binding mode was investigated using lysozyme-galactomannan conjugates (Kato *et al*., 1992b; Shu *et al*., 1996). The SDS polyacrylamide gel electrophoretic patterns display a single band for protein and carbohydrate stains near the boundary between stacking and separating gels, indicating the formation of the conjugate between ovalbumin or lysozyme and dextran. The molecular weight and the reduction in free amino groups of protein-polysaccharide conjugates suggest that about two moles of dextran are bound to ovalbumin or lysozyme, respectively. Since the binding ratio is expressed as average values, it is probable that proteins bound with one to three moles of polysaccharides may also exist in the conjugates. Analysis of the low-angle laser light scattering combined with HPLC suggests that these protein-polysaccharide conjugates easily form an oligomeric micelle structure in aqueous solution due to their amphiphilic property (Kato *et al*., 1992b). The binding reaction (panel A) and mode (panel B) for the formation of protein-galactomannan conjugates are proposed as shown in Fig. 1. Peptide analysis of the lysozyme-polysaccharide conjugate showed that an active reducing-end group in saccharide was attached to ε-amino group in the lysine residues at positions 1 and 97 in lysozyme (Shu *et al*., 1996). The limited number of bound polysaccharides may come from the steric hindrance of attached polysaccharide. This limitation is appropriate in designing the functional properties of proteins, because the functions of proteins are deteriorated if most lysyl residues are masked by saccharides, as observed in the conjugates of proteins with monosaccharides and oligosaccharides. The folded proteins attach only one or two polysaccharides, while unfolded proteins attach several. Casein binds about four polysaccharides per mole in the conjugates (Kato *et al*., 1992a), because the lysyl residues of unfolded protein are exposed outside and react easily to the reducing-end carbonyl group in polysaccharide with a smaller steric hindrance than that of folded proteins.

On the other hand, the conjugation of proteins with small carbohydrate molecules such as glucose or lactose under controlled dry-heating is liable to react with most lysyl residues exposed outside and to result in insoluble aggregates due to the progressive side reaction, although mono- or oligosaccharide-protein conjugates can improve the solubility in an initial stage of the reaction. To improve the functional properties of the proteins, therefore, their conjugation with polysaccharides, but not with oligosaccharides, is desirable for industrial applications. The contaminant oligosaccharides should be removed from polysaccharide preparations.

3. Excellent Emulsifying Properties of Protein-Polysaccharide Conjugates

Both protein and polysaccharide have a role in the stabilization of oil in water emulsions. Proteins adsorb at the oil-water interface during emulsification to form a coherent viscoelastic layer, while polysaccharides confer colloid stability through their thickening and gelation behavior in the aqueous phase. Therefore, the protein-polysaccharide conjugates are expected to ex-

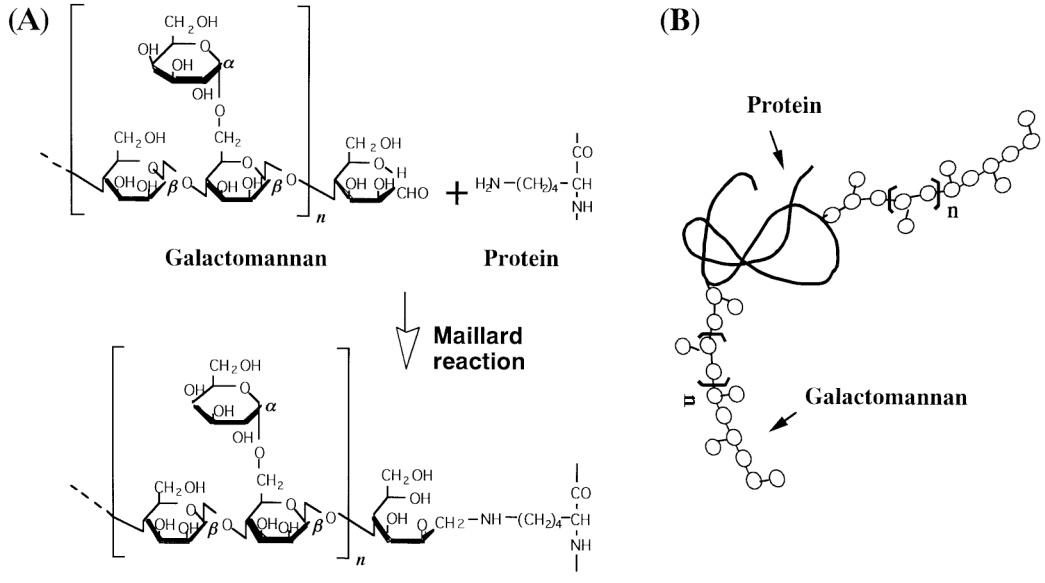

Fig. 1. Schematic presentation of the binding mode of polysaccharide with protein through Maillard reaction (A) and the resulting protein-polysaccharide conjugate (B).

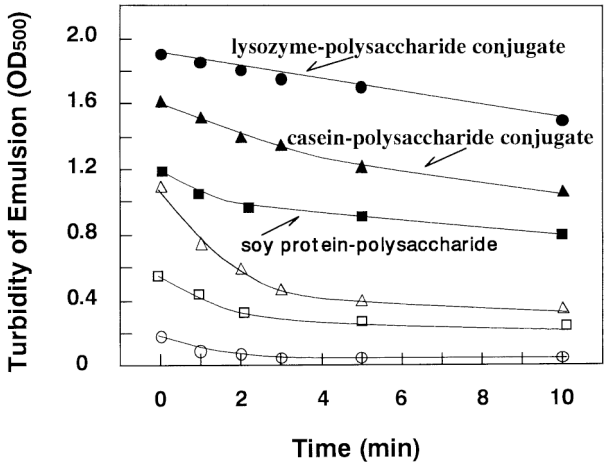

Fig. 2. Comparison of emulsifying properties of various protein-polysaccharide conjugates. ●, lysozyme-galactomannan conjugate (dry-heated for 2 weeks at 60°C under 65% relative humidity); ○, lysozyme-galactomannan mixture (no dry heating); ▲, αs1-casein-galactomannan conjugate (dry-heated for 1 day); △, αs1-casein-galactomannan mixture (no dry heating); ■, soy protein-galactomannan conjugate (dry-heated for 1 week); □, soy protein-galactomannan mixture (no dry heating).

hibit good emulsifying properties. As expected, dramatic enhancement of the emulsifying properties of protein-polysaccharide conjugates were observed in common for lysozyme-polysaccharide, casein-polysaccharide and soy protein-polysaccharide conjugates (Fig. 2). The conjugates of proteins with polysaccharide revealed much better emulsifying activity and emulsion stability than the control mixtures of proteins with polysaccharides. The emulsifying property of the conjugate of lysozyme with galactomannan was the best among various proteins. Similar excellent emulsifying properties were obtained in the conjugates of lysozyme with dextran. The use of galactomannan is desirable for food ingredients, because it is not as expensive as dextran and is already utilized as a thickener, binder and stabilizing agent in food. To evaluate their potential to industrial applications, the emulsifying properties of dried egg white (DEW)-polysaccharide conjugates were compared with commercial emulsifiers (Kato *et al*., 1993). DEW is not expensive and contains the egg white proteins ovalbumin, ovotransferrin and lysozyme etc. The DEW-galactomannan conjugate showed much better emulsifying properties than conventional emulsifiers (sucrose-fatty acid ester and glycerin-fatty acid ester). In addition, the emulsifying properties of the conjugates were not affected under acidic conditions, in the presence of 0.2 M NaCl or by heating of the conjugates. Since high salt conditions, acidic pH, and/or heating process are commonly encountered in industrial application, the protein-galactomannan conjugate may be an ingredient suitable for use in food processing.

3.1. Effect of the Molecular Size of Polysaccharide Chains on the Emulsifying Properties of Protein-Polysaccharide Conjugates

The effect of the molecular size of conjugates is shown in Fig. 3 (Shu *et al*., 1996). The emulsifying properties of the lysozyme-galactomannan conjugates increased in proportion to the length of their polysaccharide chains. The emulsion stability of the lysozyme-GM (3.5–6 kDa) conjugate was very low, suggesting that at least the conjugation of protein with galactomannan having a molecular size of more than 6–12 kDa is essential for improvement of the emulsifying properties. Xyloglucan was used to further elucidate the effect of the oligosaccharide chain on the emulsifying properties of the protein-saccharide conjugate, (data not shown). Xyloglucan is a well-documented oligosaccharide composed of hepta-, octa- and nona-saccharides at a weight ratio of 10.4, 33.3 and 53.2%, respectively. The emulsifying properties of the lysozyme-xyloglucan conjugate were almost the same as those of the lysozyme-GM (3.5–6 kDa) conjugate. Thus, it was confirmed that lysozyme-oligosaccharide conjugates did not improve the emulsifying properties. A similar attempt was made using soy protein-saccharide conjugates, and, as shown in Fig. 4, the emulsifying activity and emulsion stability were improved in proportion to the molecular size of attached saccharides. It seems likely that saccharides having Mw 10 kDa are effective for getting better emulsifying properties.

3.2. Effect of the Kinds of Polysaccharide on the Emulsifying Properties of Protein-Polysaccharide Conjugates

What kinds of polysaccharides are preferable to obtain opti-

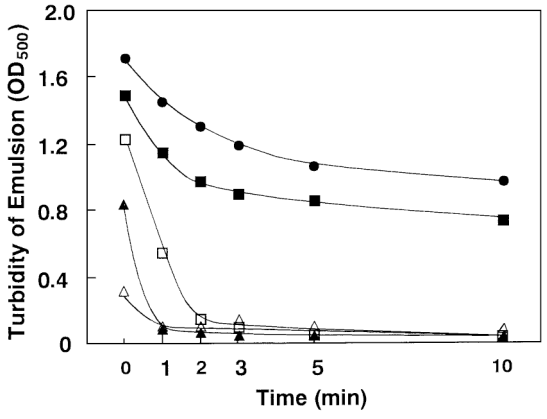

Fig. 3. Effect of the size of saccharides on the emulsifying properties of lysozyme saccharide conjugates. △, lysozyme; ▲, lysozyme-glucose conjugate; □, lysozyme-galactomannan (3.5–6.0 kD) conjugate; ■, lysozyme-galactomannan (6.0–12 kD) conjugate; ●, lysozyme-galactomannan (12–24 kD) conjugate.

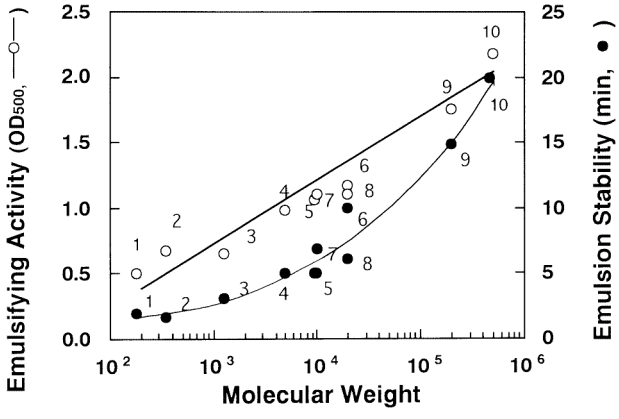

Fig. 4. Relationships between emulsifying properties and molecular weight of saccharide in the conjugation with soy protein. The numbers 1–10 indicate the saccharides conjugated with soy protein. 1. glucose; 2. lactose; 3. xyloglucan (Mw 1400); 4, galactomannan (Mw 3500–6000); 5, galactomannan (Mw 10,000); 6, galactomannan (Mw 20,000); 7, dextran (Mw 9300); 8, dextran (Mw 19,600); 9, dextran (Mw 200,000–300,000); 10, Xyloglucan (Mw 470,000).

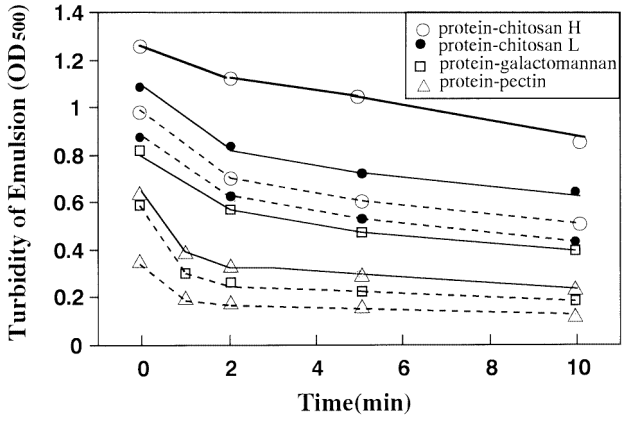

Fig. 5. Comparison of emulsifying properties of various types of protein-polysaccharide conjugates prepared by dry-heating at 60°C under 65% relative humidity for 1 week. Solid line indicates lysozyme-polysaccharide conjugates and dotted line indicates ovalbumin-polysaccharide conjugates. △, protein-pectin conjugate; □, protein-galactomannan conjugate; ●, protein-chitosan (L) conjugate; ○, protein-chitosan (H) conjugate.

mal functional properties? The optimal polysaccharide was investigated using acidic, neutral and basic polysaccharides for the optimal emulsifying properties of protein-polysaccharide conjugates (Song *et al*., 2002). Pectin, galactomannan and chitosan were used as acidic, neutral and basic polysaccharides, respectively, and ovalbumin and lysozyme as model proteins. The chitosan-protein conjugates showed the best emulsifying properties (Fig. 5), while those of the pectin-protein conjugate were poor. The emulsifying activity and emulsion stability calculated from Fig. 5 are shown in Table 1. The emulsion stability was especially high in chitosan-protein conjugates, and the positive charge of polysaccharide may contribute to stabilizing the emulsion. This is also supported by the result that the emulsifying properties of lysozyme-galactomannan conjugate are close to those of chitosan-protein conjugates. The positive charges in lysozyme must contribute to stabilizing the emulsion. Since the high molecular chitosan (H) is soluble only in acidic pH and the low molecular chitosan (L) is expensive to use for industrial applications, the lysozme-galactomannan conjugate can be substituted for chitosan-protein conjugates.

4. Heat Stability of Protein-Polysaccharide Conjugates

The effect of the polysaccharide chains on the heat stability of the lysozyme-polysaccharide conjugates is shown in Fig. 6 (Shu *et al*., 1996). The apparent heat stability of the native and conjugated lysozymes was examined at a pH of 7.4 from 50 to 95°C. Turbidity of the native lysozyme gradually increased with a transition point at 85°C and reached a maximum turbidity of 1.7 (OD_{500}) at 95°C; in contrast, the lysozyme-galactomannan conjugates showed no aggregation up to 95°C. This result suggests that the lysozyme is converted into the heat stable form by its conjugation with galactomannan. Xyloglucan (1.4 kDa) was used in the experiment to elucidate the critical size of the saccharide chain that stabilizes the protein-saccharide conjugates. As shown in the figure, the lysozyme-xyloglucan conjugate was not as heat-stable as the lysozyme-polysaccharide conjugates, although it was more stable than the native lysozyme. Therefore, the molecular size of several kDa of saccharide may be necessary for the lysozyme to stabilize to heating. This suggests that the polysaccharide attached to lysozyme may stabilize the protein molecule depending to the manner in which the unfolded protein during heating is sterically protected to aggregate by polysaccharide.

Thus, the heat stability of lysozyme was also dramatically increased by the conjugation with polysaccharide but not with oligosaccharide. The attachment of polysaccharide may cause proteins to form a stable structure against heating. It seems likely that the protein denaturation process may be reversible in the protein-polysaccharide conjugates, because of the inhibition of the unfolded protein-protein interaction due to the attached polysaccharide. The attached polysaccharide may function like a molecular chaperone that protects the aggregation of unfolded protein.

5. Polymannosyl Lysozyme Constructed by Genetic Modification

To further elucidate the molecular mechanism of the improvement of functional properties of proteins by attachment with polysaccharide, genetic glycosylation of lysozyme was successfully attempted using the yeast expression system (Nakamura *et al*., 1993a, 1993b; Kato *et al*., 1994; Shu *et al*., 1998; Kato *et al*., 1996). In yeast cells, the proteins having an Asn-X-Thr/Ser sequence are *N*-glycosylated in the endoplasmic reticulum and the attached oligosaccharide chain can be elongated with further extension of a large polymannose chain in the Golgi apparatus. Therefore, contruction was attempted of a yeast expression plas-

Table 1. Emulsifying properties of protein-polysaccharide conjugates.

Conjugates	Emulsifying activity (OD 500 nm)	Emulsion stability (Half time, min)
OVA-Pectin	0.370	1.0
OVA-Galactomannan	0.605	1.0
OVA-Chitosan (L)	0.857	10.0
OVA-Chitosan (H)	0.990	12.0
LYS-Pectin	0.620	5.0
LYS-Galactomannan	0.805	11.0
LYS-Chitosan (L)	1.100	15.0
LYS-Chitosan (H)	1.227	20.0

Protein-polysaccharide conjugates were prepared by dry-heating at 60°C under 65% relative humidity for 1 week.
OVA, ovalbumin; LYS, lysozyme.

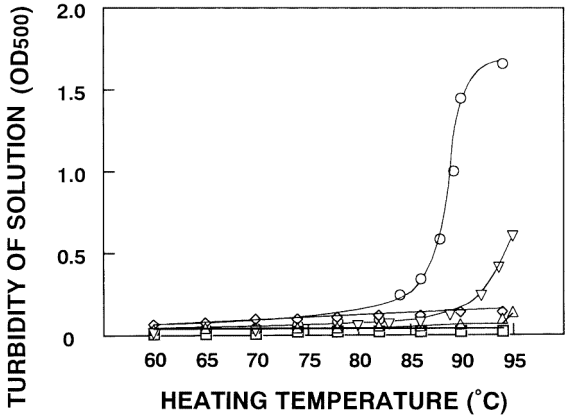

Fig. 6. Heat stability of lysozyme-polysaccharide conjugates. ○, lysozyme; ▽, lysozyme-xyloglucan (1.4 kDa) conjugate; □, lysozyme-galactomannan (24 kDa) conjugate; ◇, lysozyme-galactomannan (6–12 kDa) conjugate; △, lysozyme-galactomannan (3.5–6 kDa) conjugate.

mid carrying the mutant cDNA of hen egg white lysozyme having an N-glycosylation signal sequence (Asn-X-Thr/Ser) at the molecular surface. The expression plasmid vector was introduced into *Saccharomyces cerevisiae*. As expected, a large amount of polymannosyl lysozyme was predominantly secreted in the yeast medium (Nakamura et al., 1993a). We were successful in constructing a single polyannosyl lysozyme at positions 19 and 49, and double polymannosyl lysozyme at both positions 19 and 49 (Kato *et al*., 1994). As shown in Fig. 7, these polymannosyl lysozymes showed remarkable heat stability and excellent emulsifying properties like the Maillard-type lysozyme-polysaccharide conjugate described above. Furthermore, the molecular mechanism of the functional properties of polymannosyl lysozyme was elucidated in detail. The oligomannosyl and polymannosyl chains were attached to lysozyme, NAG_2-$Man_{14–18}$ and NAG_2-$Man_{300–330}$, respectively. Oligomannosyl lysozyme is not effective in improving the emulsifying properties and heat stability. The advantage of genetic modification is to be able to design the binding site as wanted. The single polyannosyl lysozyme at positions 19 and 49, and the double polymannosyl lysozyme at both positions were constructed and their emulsifying properties were compared; the latter showed much higher emulsifying properties than the former lysozymes (Shu *et al*., 1998). This suggests that the formation of the thick steric stabilizing adsorbed layer around the emulsion is further enhanced and the coalescence of oil droplets was more effectively inhibited by increasing the number of glycosylation sites.

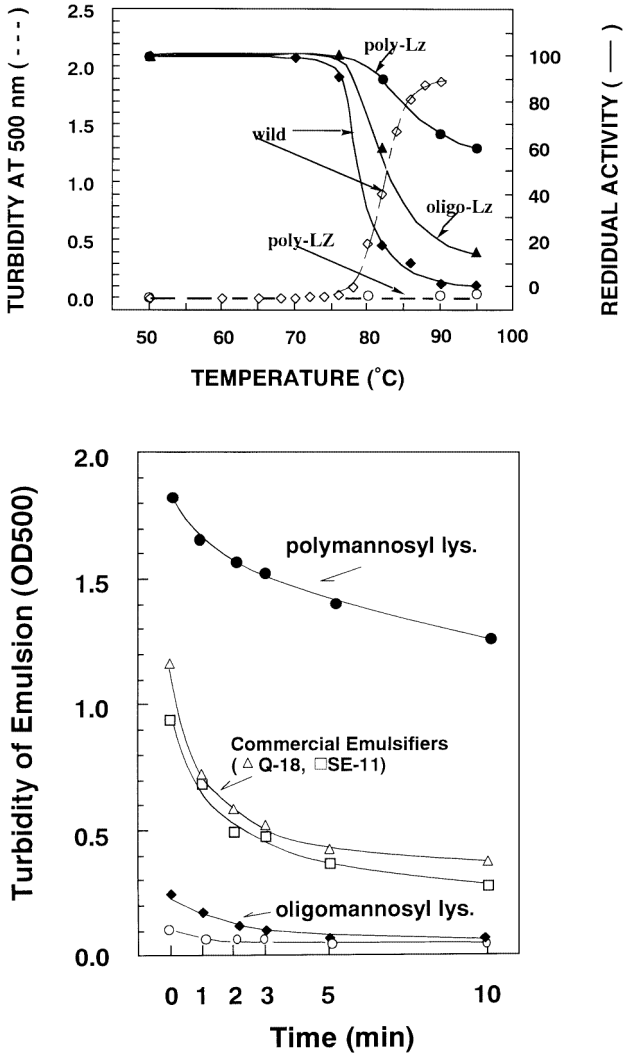

Fig. 7. Thermal denaturation curves (upper) and emulsifying properties (bottom) of polymannosyl and oligomannosyl lysozymes constructed by genetic engineering. Changes in the residual activity and turbidity of mannosyl lysozymes are shown by solid and dotted lines, respectively, in denaturation curves (upper). Changes in the turbidity of emulsion of mannosyl lysozymes are shown at the bottom (●, polymannosyl lysozyme; ◆, oligomannosyl lysozyme; ○, lysozyme; △, commercial emulsifier Q-18, polyglycerin-fatty acid ester; □, commercial emulsifier SE-11, sucrose-fatty acid ester).

6. Antimicrobial Action of Lysozyme-Polysaccharide Conjugates

Many attempts have been made to develop food preservatives having a superior antimicrobial effect without toxicities; Hen egg white lysozyme may be one of the most promising ingredients for this purpose. It is well known that 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 material such as lipopolysaccharide (LPS) covering the thin peptidoglycan layer, lysozyme fails to lyse Gram-negative bacteria when it is simply added to the cell suspension in its native form. As discussed in a previous paper (Nakamura & Mizushima, 1975), synergistic factors such as detergents and heat treatment destabilize and consequently solubilize the outer membranes that are mainly composed of LPS. Therefore, the excellent surfactant activity of lysozyme-polysaccharide conjugate seems to cause destruction of the outer membrane of Gram-negative bacterial cells synergistically along with thermal stress. As shown in Fig. 8, the antimicrobial action of lysozyme-polysaccharide conjugates was observed in both Gram-positive and Gram-negative bacteria (Nakamura *et al*., 1991). The living cells were dramatically decreased with heating time at 50˚C in the presence of lysozyme-polysaccharide conjugate. Thus, it was concluded that the lethal effect was effectively induced by exposing the cells to lysozyme-polysaccharide conjugate.

The antimicrobial action of chitosan is well known in a wide range of bacteria including Gram-negative bacteria and fungi. Therefore, chitosan-lysozyme conjugates must be one of the most promising antimicrobial ingredients; as expected, these conjugates showed potent antimicrobial action at room temperature (Song *et al*., 2002). The antimicrobial action of high molecular chitosan-lysozyme conjugate was much higher than that of low molecular chitosan-lysozyme conjugate, as shown in Fig. 9. The HMC-lysozyme conjugates were found to be very effective in inhibiting the growth of bacterium cells at all temperatures. In contrast, LMC conjugates were especially effective in reducing the number of surviving cells when the temperature was increased. It has been reported (Tsai & Su, 1999) that *Escherichia coli* cells in the mid-exponential phase at pH 7.0 are less susceptible to chitosan at lower temperature because chitosan is less soluble at pH 7.0; lower temperature may cause changes in the number of available binding sites at the cell surface, accordingly reducing the interaction between chitosan and the cells. However, our results demonstrate that low temperature stress had little influence on the bactericidal activity of HMC conjugated with lysozyme. This may be due to increases in the solubility of chitosan after conjugation with lysozyme. Our results confirmed that

the number of cationic amino groups or the degree of polymerization greatly influenced the bactericidal activity of chitosan. As the number of cationic amino groups of HMC increases, the number of inhibited cells increases. The data indicate that the functional groups for the growth inhibition are the cationic amino groups of chitosan.

These results indicate that chitosan-lysozyme conjugates are very effective in inhibiting the growth of the bacterium and are promising for use in industrial applications, because the conjugates had excellent emulsifying properties and solubility, especially at neutral pH, in addition to antimicrobial action. Chitosan-lysozyme conjugate can be used as a new functional ingredient having excellent emulsifying properties and bactericidal action.

7. Masking of Allergen Structure of Proteins

The allergen structure of proteins can be masked by conjugation with polysaccharide. Soy protein is a well-known allergenic protein, and the 34 kDa protein is a well identified allergenic protein in soy protein (Ogawa *et al*., 1993). Most patients who can not take soy protein are sensitive to 34 kDa protein. It has been reported that the allergenic protein of soybean reacts easily with polysaccharide by Maillard reaction. Therefore, it was expected that the allergen structure might be masked by the attachment of polysaccharide. The masking of the allergen structure of soy protein was evaluated using the solid phase ELISA method (Babiker *et al*., 1998). As shown in Fig. 10, the binding affinities of antibody with soy protein were greatly decreased by polysaccharide

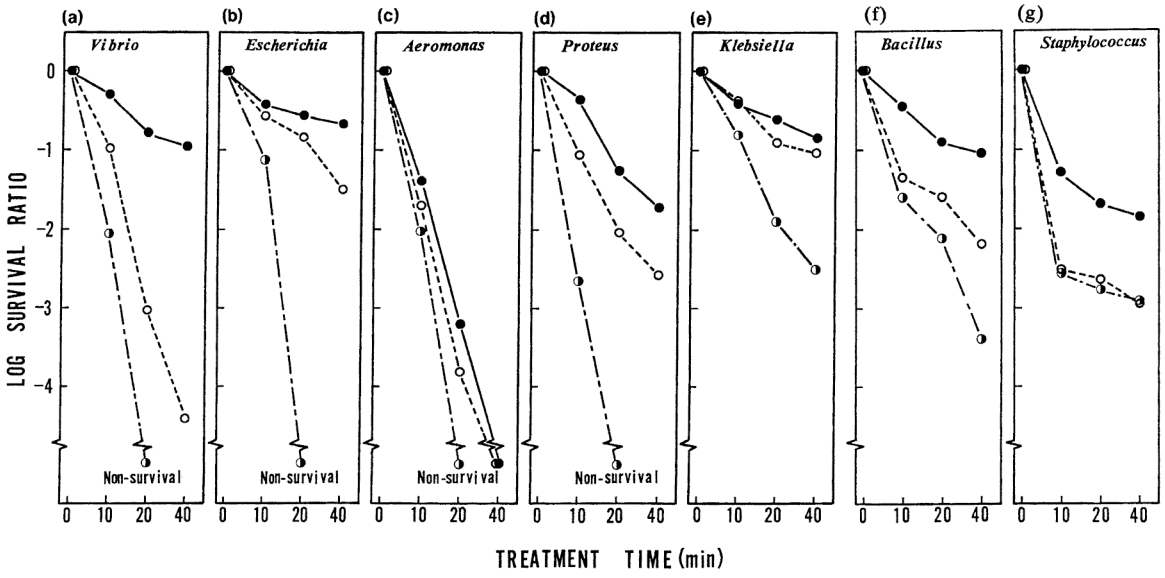

Fig. 8. Antimicrobial activity of lysozyme-dextran conjugate for Gram-negative (a–e) and Gram positive (f, g) bacteria. ●, control medium without the addition of lysozyme or conjugate; ○, lysozyme (50 μg/ml); ○, lysozyme-dextran conjugate (50 μg/ml). The number of surviving cells were counted after incubation at different temperatures for 30 min.

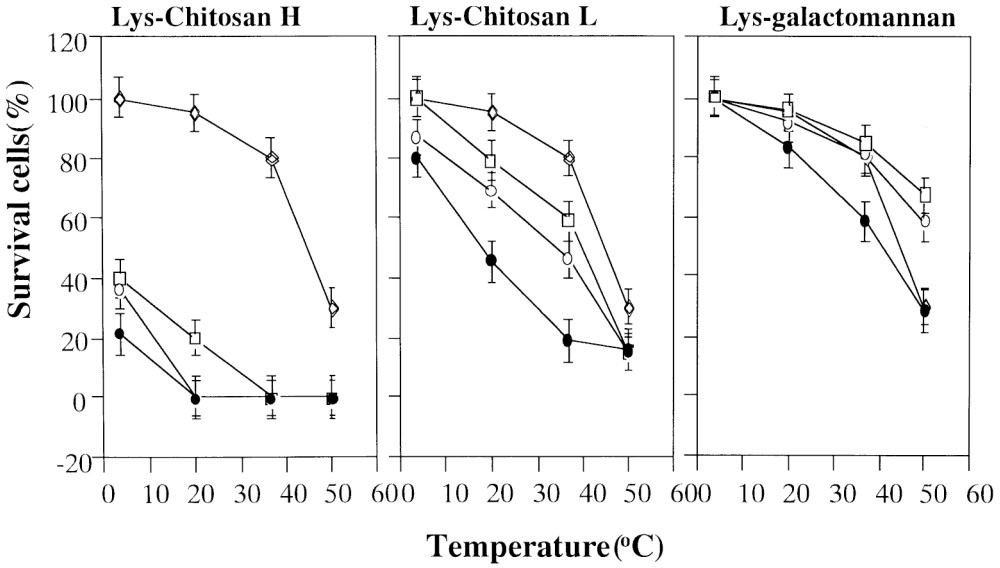

Fig. 9. Effect of polysaccharide conjugation on the bactericidal action of lysozyme against *E. coli* at different temperatures. Left, high molecular weight chitosan (HMC) conjugate; Middle, low molecular chitosan (LMC) conjugate; Right, galactomannan conjugate. ◇, lysozyme; ○, HMC, LMC or galactomannan; □, lysozyme-polysaccharide mixture; ●, lysozyme-polysaccharide conjugates dry-heated for 10 days.

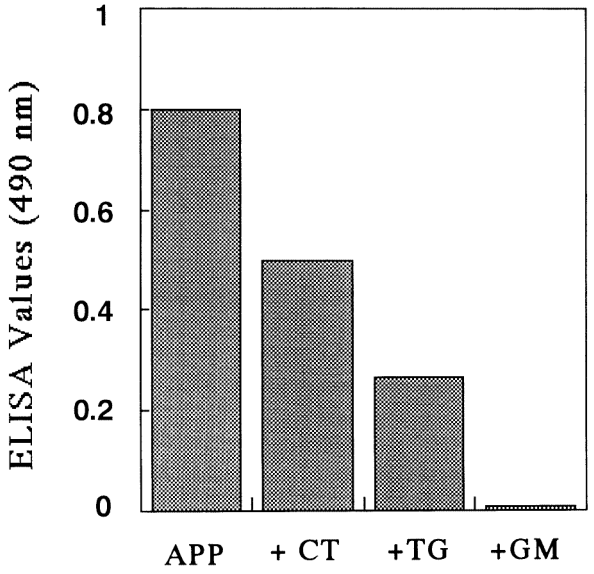

Fig. 10. Masking of antigen structure of soy protein by conjugation with polysaccharide. APP, acid precipitate of soy protein; +CT, chymotrypsin treated APP; +TG, transglutaminase treated APP; +GM, galactomannan-APP conjugate.

attachment through Maillard reaction in the dry state. It has been reported that the protease digestion and transglutaminase treatment are also effective in decreasing the allergenicity of food proteins. Therefore, the effect of polysaccharide attachment, the protease digestion and transglutaminase treatments on the reduction of allergenicity were compared, and the polysaccharide attachment was found to be the best method to reduce the allergenicity of soy protein, as shown. The similar effect of polysaccharide conjugation on the allergenicity of proteins was observed for other antigen proteins such as lysozyme. These results were obtained from *In vitro* experiments. The effect of polysaccharide attachment on the reduction of allergenicity should be elucidated by the *in vivo* system. Thus, we confirmed that polysaccharide conjugation is effective in lowering the allergenicity of these proteins in an *in vivo* system (Arita *et al*., 2001). When lysozyme-polysaccharide or P34-polysaccharide conjugates were administered intraperitoneally in mouse, the production of IgG was not suppressed, while the production of IgE was significantly suppressed compared to untreated antigen proteins. This finding suggests that the attachment of polysaccharide to allergen proteins is a promising therapeutical approach.

As described here, Maillard-type protein-polysaccharide conjugates showed excellent emulsifying properties which were superior to conventional commercial emulsifiers, heat stability, and antimicrobial activity. Therefore, the conjugates can be useful for industrial applications as natural emulsifiers and antimicrobial agents devoid of toxicity. It has also been proposed that conjugation of the allergen protein with polysaccharides may be effective in reducing the allergenicity.

References

Arita, K., Babiker, E.E., Azakami, H. and Kato, A. (2001).Effect of chemical and genetic attachment of polysaccharides to proteins on the production of IgG and IgE. *J. Agric. Food Chem.*, **49**, 2030–2036.

Babiker, E.E., Azakami, H., Matsudomi, N., Iwata, H., Ogawa, T., Bando, N. and Kato, A. (1998). Effect of polysaccharide conjugation or transglutaminase treatment on the allergenicity and functional properties of soy protein. *J. Agric. Food Chem.*, **46**, 866–871.

Kato, A. and Kobayashi, K. (1991). Excellent emulsifying properties of protein-dextran conjugates. In "Microemulsions and Emulsions in Foods," ed. by M. El-Nokaly and D. Cornell. ACS Symposium Series 448, pp. 213–229.

Kato, A., Mifuru, R., Matsudomi, N. and Kobayashi, K. (1992a). Functional casein-polysaccharide conjugates prepared by controlled dry heating. *Biosci. Biotechnol Biochem.*, **56**, 567–571

Kato, A., Kameyama, K. and Takagi, T. (1992b). Molecular weight determination and compositional analysis of dextran-protein conjugate using low-angle laser light scattering technique combined with high-performance gel chromatography. *Biochim. Biophys. Acta*, **1159**, 22–28.

Kato, A., Minaki, K. and Kobayashi, K. (1993). Improvement of emulsifying properties of egg white proteins by attachment of polysaccharide through Maillard reaction in a dry state. *J. Agric. Food Chem.,* **41**, 540–543.

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

Kato, A., Nakamura, S, Takasaki, H. and Maki, S. (1996). New functional properties of glycosylated lysozymes constructed by chemical and genetic modifications. In "Macromolecular Interactions in Food Technology," ed. by N.Parris, A.Kato, L.Creamer and J.Pearce, ACS Symposium Series 650; American Chemical Society, pp.243–256.

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

Kato, A., Shimokawa, K. and Kobayashi, K. (1991). Improvement of functional properties of insoluble gluten by pronase digestion followed by dextran conjugation. *J. Agric. Food Chem.*, **39**, 1053–1056.

Kato, A., Takasaki, H. and Ban, M. (1994). Polymannosylation to asparagines-19 in hen egg white lysozyme in yeast. *FEBS Lett.*, **355**, 76–80.

Kato, A., Tanaka, A., Lee, Y., Matsudomi, N. and Kobayashi, K. (1987). Effects of deamidation with chymotrypsin at pH 10 on the functional properties of proteins. *J. Agric. Food. Chem.*, **35**, 285–288.

Marshall, J.J. and Rabinowitz, M.L. (1976). Preparation and characterization of dextran-trypsin conjugate. *J. Biol. Chem.*, **251**, 1081–1087.

Motoki, M., Seguro, K., Nio, N. and Takinami, K. (1986). Glutamine-specific deamidation of αs1-casein by transglutaminse. *Agric. Biol. Chem.*, **50**, 3025–3030.

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

Nakamura, S., Kato, A. and Kobayashi, K. (1992a). Enhanced antioxidant effect of ovalbumin due to covalent binding of polysaccharides. *J. Agric. Food Chem.*, **40**, 2033–2037.

Nakamura, S., Kato, A. and Kobayashi, K. (1992b). Bifunctional lysozyme-galactomannan conjugate having excellent emulsifying properties and bactericidal effect. *J. Agric. Food Chem.*, **40**, 735–739.

Nakamura, K. and Mizushima, S. (1975). *In vitro* reassembly of the membranous vesicle from *Escherichia coli* outer membrane components. Role of individual components and magnesium ions in reassembly. *Biochim. Biophys. Acta*, **413**, 371–393.

Nakamura, S., Takasaki, H., Kobayashi, K. and Kato, A. (1993a). Hyperglycosylation of hen egg white lysozyme in yeast. *J. Biol. Chem.*, **268**, 12706–12712.

Nakamura, S., Kobayashi, K. and Kato, A. (1993b). Novel surface functional properties of polymannosyl lysozyme constructed by genetic modification. *FEBS Lett.*, **328**, 259–262.

Nio, N., Motoki, M. and Takinami, K. (1985). Gelation of casein and soybean globulins by transglutaminase. *Agric. Biol. Chem.*, **49**, 2283–2286.

Ogawa, T., Tsuji, H., Bando, N., Kitamura, K., Zhu,Y, Hirano, H. and Nishikawa, H. (1993). Identification of the soybean allergenic protein gly m Bd 30K, with the soybean seed 34 kDa oilbody-associated protein. *Biosci. Biotechnol. Biochem.*, **57**, 1030–1033.

Shu, Y.W., Maki, S., Nakamura, S. and Kato, A. (1998). Double-glycosylated lysozyme at positions 19 and 49 constructed by genetic modification and its surface functional properties. *J. Agric. Food Chem.*, **46**, 2433–2438.

Shu, Y.W., Sahara, S., Nakamura, S. and Kato, A. (1996). Effects of the length of polysaccharide chains on the functional properties of the Maillard-type lysozyme-polysaccharide conjugate. *J. Agric. Food Chem.*, **44**, 2544–2548.

Song, Y., Babiker, E.E., Usui, M., Saito, A. and Kato, A. (2002). Emulsifying properties and bactericidal action of chitosan-lysozyme conjugates. *Food Res. Int.*, **35**, 459–466.

Tsai, G.J. and Su, W.H. (1999). Antibacterial activity of shrimp chitosan against *Escherichia coli*. *J. Food Protect.*, **62**, 239–243.