Improvement of Emulsifying Properties of Egg White Proteins by the Attachment of Polysaccharide through Maillard Reaction in a Dry State

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Dried egg white (DEW) was covalently attached to polysaccharide (galactomannan) in a controlled dry state (60 °C, 79% relative humidity) through Maillard reaction between the ϵ-amino groups in the protein and the reducing-end carbonyl residue in the polysaccharide. The resulting protein-polysaccharide conjugate had excellent emulsifying properties superior to those of commercial emulsifiers, especially in acidic pH and high salt concentration. The safety of the conjugate was confirmed by using mammalian cell. The growth-promoting activity of the CV-1 cell in DEW-galactomannan conjugate was the same as untreated egg white. Thus, DEW-polysaccharide conjugates may be fruitful products as novel macromolecular food ingredients.

INTRODUCTION

Egg white proteins are extensively utilized as functional food products in food processing. Because of the egg white's nutritional and wide range of functional properties, it is desirable to further improve their functional properties for industrial uses. Many attempts have been made to develop a rational molecular design using chemical and enzymatic modifications of proteins to improve the gelling, foaming, and emulsifying properties. For food applications, the use of many undesirable chemicals should be avoided and the safety of modified proteins should be ensured.

Proteins have unique surface properties due to their large molecular weight and tertiary structure, and their amphiphilic properties make them suitable as potent surfactants. Since proteins are generally unstable to heating for pasteurization, their industrial application has been limited. However, proteins can be partially denatured without loss of their solubility or they can be modified to stably form for heat-induced coagulation. Our goal was to use proteins as new functional emulsifying, foaming, or gelling agents. To achieve this goal, our strategy was directed to heating the proteins in the absence of water molecules. Thus, we have reported that the functional properties of spray-dried egg white proteins were greatly improved by controlled dry-heating without loss of solubility (Kato et al., 1989). Further, this technique was employed to prepare the conjugates of proteins with polysaccharides through naturally occurring Maillard reaction in a dry state (Kato et al., 1990; Nakamura et al., 1991). As expected, the covalent cross-linkage was formed between ϵ-amino groups in proteins and the reducing-end carbonyl group in polysaccharide. The proteins were converted into stable and soluble forms by binding with polysaccharides. In addition to these properties, the resulting protein-polysaccharide conjugates revealed excellent emulsifying properties. Among many chemical and enzymatic modifications of proteins to improve their functionality, this method is one of the most promising for food applications, if the safety is ensured and the conjugates can be prepared economically. We used dextran as a polysaccharide, which is expensive for a food ingredient. Thus, for further utilization of dried egg white proteins, the conjugation was tried with guar gum, which is available as a thickener, binder, and stabilizing agent in food systems. Favorably, the mannase hydrolysate of guar gum (galactomannan) was developed as a soluble dietary fiber (Yamamoto et al., 1990) and was easily obtained as a commercial product. We have already reported that lysozyme-galactomannan conjugate had excellent emulsifying properties (Nakamura et al., 1992). Since egg white contains various bioactive components other than lysozyme, the egg white protein-galactomannan conjugate might be a good candidate for food application rather than the lysozyme-galactomannan conjugate, because of cheaper and more practical materials.

The present paper describes the characterization of dried egg white protein-galactomannan conjugate prepared through naturally occurring Maillard reaction in a dry-heating state.

MATERIALS AND METHODS

Materials. Egg white spray-dried at 60–70 °C after decarbohydrate treatment was provided by Q.P. Corp., Tokyo. Galactomannan preparation (MW 15 000–20 000) was obtained by dialyzing the mannase hydrolysate of guar gum supplied from Taiyo Chemicals Co. Commercial emulsifiers, Sunsoft SE 11 and Q 18S, were supplied from Taiyo Chemicals Co. The CV-1 cell line, kidney fibroblast cell from *Cercopithecus aethiops*, was provided by Dr. K. Takimoto, Yamaguchi University.

Preparation of DEW-Galactomannan Conjugates. DEW (moisture 7.5‰) and galactomannan were mixed in water at the weight ratio of 1:1 and then lyophilized. The powder mixture spread over Petri dish (10 × 1.5 cm) was incubated at 60 °C under a relative humidity of 79% for 2 weeks in a desiccator containing saturated KBr solution in the bottom. To accelerate the Maillard reaction, the powder mixture was mixed over a Petri dish once a day.

Measurement of Emulsifying Properties. Emulsifying properties were determined according to the method of Pearce and Kinsella (1978). To form emulsion, 1.0 mL of corn oil and 3.0 mL of 0.1% sample solution in 100 mM sodium phosphate buffer, pH 7.4, were shaken together and homogenized in a Polytron (Kinematica, Switzerland) 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 different times and diluted with 5 mL of 0.1% sodium dodecyl sulfate solution. The absorbance of diluted emulsion was then determined at 500 nm. The emulsifying activity was determined from the absorbance measured immediately after emulsion formation. The emulsion stability was estimated by measuring the half-time of the turbidity measured immediately after emulsion formation.

SDS-PAGE Analysis. SDS-slab polyacrylamide gel electrophoresis (SDS-PAGE) was done according to the method of

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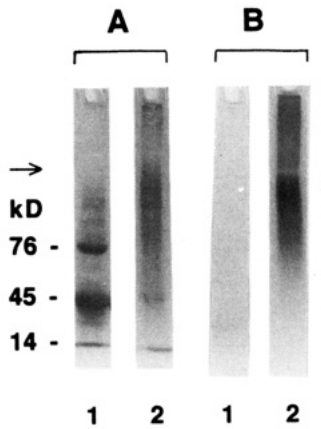

Figure 1. SDS–polyacrylamide gel electrophoresis patterns of DEW–galactomannan conjugate. Lane 1, untreated DEW–galactomannan mixture; lane 2, DEW–galactomannan conjugate obtained by dry-heating at 60 °C for 2 weeks; panel A, protein stain; panel B, carbohydrate stain. Horizontal arrow shows the boundary between stacking and separating gels. Ovotransferrin (76 000), ovalbumin (45 000), and lysozyme (14 000) are used as molecular weight markers.

Laemmli (1970) using 15% acrylamide separating gel and 5% acrylamide stacking gel, containing 0.1% SDS. Samples (20 μL, 0.1%) were prepared in Tris–glycine buffer, pH 8.8, containing 1% SDS and 1% mercaptoethanol. Electrophoresis was done at constant current of 10 mA for 5 h in Tris–glycine buffer containing 0.1% SDS. The gel sheets were stained for protein and carbohydrate with Coomassie brilliant blue G-250 and Fuchsin, respectively.

Cell Proliferation Assay. Confluent cells were twice washed with phosphate-buffered saline (PBS) and trypsinized with 0.5% trypsin solution containing 0.5% EDTA for 1 min. After removal of the trypsin solution, cells were suspended in serum-free Eagle's medium (MEM) or in 1% serum and seeded into plastic tissue culture dishes at a seeding density of 10^5 cells/35-mm dish or 0.5×10^5 cells/35-mm dish and then incubated in 5% CO_2/95% air for a given day. The number of cells in each dish was counted after incubation for various periods of time.

RESULTS

Figure 1 shows the SDS–polyacrylamide gel electrophoretic patterns of DEW–galactomannan conjugate prepared through Maillard reaction in a dry state (relative humidity of 79%) at 60 °C for 2 weeks. The broader and higher molecular weight bands appeared in protein stain (A) and carbohydrate stain (B) in the DEW–galactomannan conjugate (lane 2), although they were not observed in the DEW–galactomannan mixture without dry-heating. This suggests that the covalent attachment of galactomannan to DEW was formed through Maillard reaction with dry-heating. Since dried egg white contains various molecular sizes of proteins, the broader bands may be observed in the separating and stacking gels. Most peaks of ovalbumin (45 000) and ovotransferrin (76 000) in native egg white proteins disappeared in the conjugate with galactomannan, although a small amount of lysozyme (14 000) remained. This suggests that the ratio of polysaccharide to protein used (2:1) was adequate to form the conjugate. The mole ratio used was greater than 2 assuming the average molecular weight of egg white proteins was about 45 000.

It is presumed that the stability of protein structure is enhanced by the conjugation with polysaccharide. This was confirmed for lysozyme–dextran conjugate (Kato and Kobayashi, 1991). Besides the stabilization of protein structure, the most interesting finding is the dramatic enhancement of emulsifying properties for protein–polysaccharide conjugates, as shown in Figure 2. The

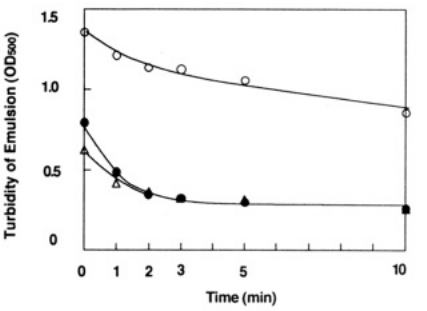

Figure 2. Emulsifying properties of DEW, untreated DEW–galactomannan mixture, and DEW–galactomannan conjugates in water. ●, DEW obtained by dry-heating at 60 °C for 2 weeks; △, untreated DEW–galactomannan mixture; ○, DEW–galactomannan conjugate obtained by dry-heating at 60 °C for 2 weeks.

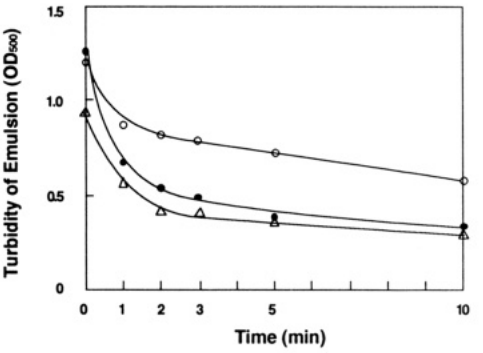

Figure 3. Comparison of emulsifying properties between DEW–galactomannan conjugate and commercial emulsifiers in 1/15 M sodium phosphate buffer, pH 7.4. ○, DEW–galactomannan conjugate; △, commercial emulsifier (Sunsoft SE11, sucrose–fatty acid ester); ●, commercial emulsifier (Sunsoft Q18S, decaglyceryl monostearate).

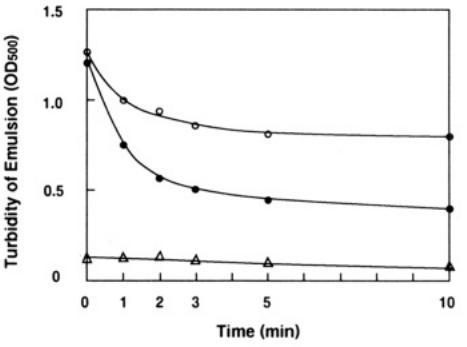

Figure 4. Comparison of emulsifying properties between DEW–galactomannan conjugate and commercial emulsifier in acidic pH (1/15 M sodium citrate buffer, pH 3.0). ○, DEW–galactomannan conjugate; △, commercial emulsifier (Sunsoft SE11, sucrose–fatty acid ester); ●, commercial emulsifier (Sunsoft Q18S, decaglyceryl monostearate).

turbidity of emulsion is plotted as ordinate and standing time after emulsion formation as abscissa according to the method of Pearce and Kinsella (1978). The value of the ordinate at 0 time is relative emulsifying activity, and the half-life of initial turbidity reflects the stability of the emulsion. The conjugates of egg white protein with galactomannan revealed much better emulsifying activity and emulsion stability than these untreated mixtures without Maillard reaction. As shown in Figure 3, the emulsifying property of egg white protein–galactomannan conjugate was much better than those of commercial emulsifiers (sucrose–fatty acid ester and glycerin–fatty acid ester). In addition, the emulsifying properties of the conjugates were still excellent in acidic pH (Figure 4) as well as in the presence of 0.2 M NaCl (Figure 5), although

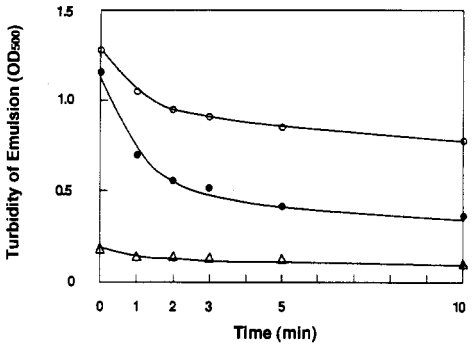

Figure 5. Comparison of emulsifying properties between DEW–galactomannan conjugate and commercial emulsifier in high salt concentration (1/15 M sodium phosphate buffer, pH 7.0, containing 0.2 M NaCl). ○, DEW–galactomannan conjugate; △, commercial emulsifier (Sunsoft SE11, sucrose–fatty acid ester); ●, commercial emulsifier (Sunsoft Q18S, decaglyceryl monostearate).

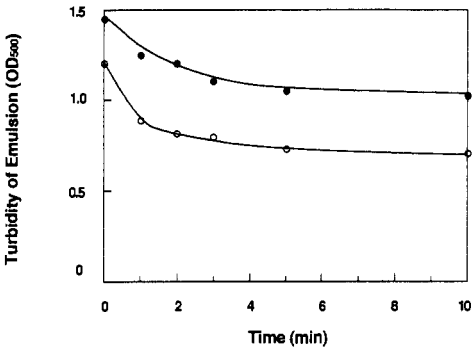

Figure 6. Effect of heat treatment of DEW–galactomannan conjugate on the emulsifying properties. ○, Unheated conjugate; ●, heated conjugate. DEW–galactomannan conjugate was heated in water at 100 °C for 3 min, and then the emulsifying properties were measured in 1/15 M phosphate buffer, pH 7.4, at room temperature.

those of the commercial emulsifiers were greatly lowered. Since high-salt condition, acidic pH condition, and heating process are commonly used in industrial application, the egg white protein–galactomannan conjugate is a suitable ingredient for food application. Figure 6 shows the effect of heat treatment of the DEW–galactomannan conjugate on the emulsifying properties. The conjugate was stable to heating at 100 °C for 3 min, and no coagulates were observed during heat treatment. Interestingly, the emulsifying properties were greatly increased by heating the DEW–galactomannan conjugate. This suggests that the resulting unfolded form of DEW was kept by the attached galactomannan and better amphiphilic balance was formed to have better emulsifying properties. This heat stability is favorable to the pasteurization for food application.

Since the conjugates were prepared by Maillard reaction, the products may contain some toxic compounds. Therefore, the toxicity was examined by using mammalian cell (CV-1). Figure 7 shows the effect of DEW–galactomannan conjugate on the proliferation of mammalian cell (CV-1 cell). We reported that the growth-promoting components of mammalian cells might exist in egg white (Zou et al., 1991). Interestingly, the conjugate also revealed the growth-promoting effect on CV-1 cells in serum-free medium (panel B). The growth-promoting effect was almost the same as that of untreated egg white (Zou et al., 1991). On the other hand, in the presence of 1% fetal bovine serum, where the CV-1 cells can proliferate, no detrimental effect of DEW–galactomannan on the growth of mammalian cell was observed (panel A). This suggests that there is no cell toxicity in the conjugate and that the

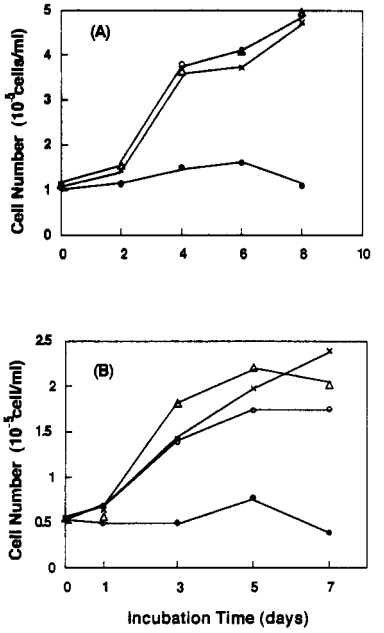

Figure 7. Effects of DEW–galactomannan conjugate on the proliferation of mammalian cell (CV-1) in 1% serum (A) and in serum-free culture (B). Panel A: ●, MEM (Eagle's medium); ○, MEM + 1% FBS (fetal bovine serum); ×, MEM + 1% FBS + 0.01% DEW–galactomannan conjugate; △, MEM + 1% FBS + 0.01% DEW. Panel B: ●, MEM; ×, MEM + 0.01% DEW–galactomannan conjugate; △, MEM + 0.005% DEW–galactomannan conjugate; ○, MEM + 0.0025% DEW–galactomannan conjugate.

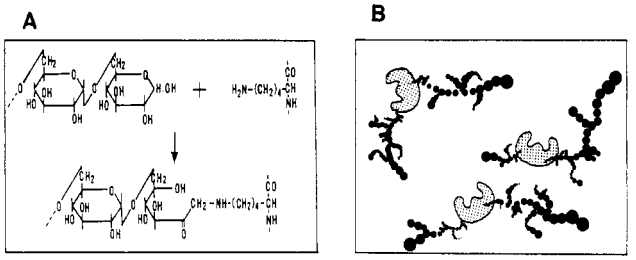

Figure 8. Scheme for the binding of protein with polysaccharide through Maillard reaction (A) and the binding mode (B). Dotted areas indicate protein molecules; branched solid circles represent polysaccharide molecules.

protein structure in the conjugate is kept in the native form. In addition, egg white contains antimicrobial components such as lysozyme, ovotransferrin, ovoinhibitor, and avidin. Therefore, it is expected that the antimicrobial activity in egg white protein–galactomannan is also kept. As shown in a previous paper (Nakamura et al., 1992), the antimicrobial property of lysozyme was enhanced in the conjugate with galactomannan, suggesting that the other bioactive proteins in egg white preserved their functions in the conjugation with galactomannan.

DISCUSSION

We have reported that ovalbumin or lysozyme binds 1 or 2 mol of polysaccharide, when the protein–polysaccharide conjugates were prepared with dextran or galactomannan (Nakamura et al., 1992; Kato et al., 1992). The possible binding reaction and the binding mode are shown in Figure 8. The covalent cross-linkage is formed between the ϵ-amino groups in proteins and the reducing-end carbonyl group in polysaccharide, which is only one active functional group per molecule. The covalent attachment was already confirmed by the results that 2 mol of lysine in protein were reduced and 2 mol of polysaccharide were

attached to the protein in the lysozyme–polysaccharide conjugates through Maillard reaction (Nakamura et al., 1992; Kato et al., 1992). In addition, we have recently identified the attachment sites of polysaccharides in lysozyme. This reaction is accelerated in the dry-heating used here, where the moisture of DEW was 7.5% and the relative humidity was 79%. The subsequent reactions may be suppressed in a protein–polysaccharide system, because side products and color development were not observed as in the protein–glucose system. The limited number of bound polysaccharide may be attributed to the steric hindrance of attached polysaccharide. This is suitable for designing the functional properties of proteins. When glucose was attached to proteins in a similar way, the function of proteins was unfavorably lowered and detrimental effects (browning color development, etc.) were observed in the product. However, when polysaccharides were bound to protein, the thermal stability was enhanced and the function was conserved without browning color development. By screening of various polysaccharides, galactomannan (MW 15 000–20 000), mannase hydrolysate of guar gum, was also found to be a suitable polysaccharide for new functional properties described above. Since the commercial mannase hydrolysate of guar gum is contaminated with a considerable amount of free small molecular weight carbohydrates, the preferential conjugation of proteins with these oligomeric saccharides may produce less satisfactory emulsifying properties and bioactive properties. We 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 protein–polysaccharide conjugate, the low molecular weight galactomannan should be removed. This should be also noted for conjugation with other polysaccharides. The most striking function of egg white–polysaccharide conjugates is the enhanced emulsifying properties superior to those of commercial emulsifiers. One can support the assumption that the properties of protein–polysaccharide conjugate as an emulsifier must be attributed to the amphiphilic nature of the conjugated molecule. The hydrophobic residues of protein denatured at the oil–water interface may be anchored to the surface of oil droplets in emulsion, while the hydrophilic residues of polysaccharide oriented to water may cover oil droplets to inhibit the coalescence of oil droplets. Thus, the stable emulsion was formed in the presence of the protein–polysaccharide conjugate.

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 ovalbumin–galactomannan conjugate is nontoxic for oral administration using rats and negative for Ames test and Rec assay (Nakamura et al., 1992). In addition, a 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, egg white protein–galactomannan conjugate can be used as a food preservative and emulsifier.

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