Effect of Polysaccharide Conjugation or Transglutaminase Treatment on the Allergenicity and Functional Properties of Soy Protein

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The soy protein−galactomannan conjugate prepared by the Maillard reaction removed the allergenicity of the 34 kDa protein which is frequently recognized by the IgE antibody in the sera of soybean-sensitive patients as a major allergen. Monitoring of polyclonal antibody titers by an indirect enzyme-linked immunosorbent assay and immunoblotting of rabbit sera, monoclonal antibody, and human allergic sera showed that soy protein−galactomannan conjugation was more effective in reducing the allergenicity of the soy protein than transglutaminase treatments and/or chymotrypsin. The soy protein−galactomannan conjugate was highly soluble at all pHs, while untreated soy protein was sparingly soluble at pH 4−6. Heat stability and emulsifying properties were greatly improved by conjugation with galactomannan.

Keywords: *Galactomannan; transglutaminase; allergenicity; soy protein−polysaccharide conjugate; functionality*

INTRODUCTION

Soybeans have been recognized as one of the important protein sources in the field of food processing because of their nutritional and functional benefits. However, soybeans contain allergenic proteins. For patients allergic to soybeans, the safe choice of processed foods free from soy proteins may become more critical because soy proteins and their derivatives have been increasingly incorporated in a number of processed foods. Therefore, there is an urgent demand on food chemists to identify the protein components responsible for the human allergenicity. A study of crude soy protein, 7S and 11S fractions and whey fractions, revealed that both IgE and IgG levels were significantly increased in patients consuming crude soy protein compared to those in normal control patients and that there was an increase in IgE specific for 7S and IgG specific for 11S in the atopic patient (Burks et al., 1988). Ogawa et al. (1993) purified and characterized the 34 kDa protein in soybean as a major allergen. About 65% of patients atopic to soybeans suffered from the 34 kDa protein (Ogawa et al., 1993). Although trypsin inhibitor, a member of the 2S globulin fraction, was an allergen in one patient having severe allergenic reactions to soybean products, soybean-trypsin-inhibitor-specific radioallergosorbent tests (RASTs) were negative in two other patients with soybean allergy (Maroz et al., 1980). Fermentation was found to be effective in reducing the allergenicity of soybean protein (Yamanishi et al., 1995). The allergenicity of proteins interacting with oxidized lipids was examined by enzyme-linked immunosorbent assay (ELISA) using sera from soybean-sensitive individuals (Doke et al., 1989). The IgE titer of human sera from the soybean-sensitive patients was greatly increased when oxidized soybean oil was incubated with soybean 2S globulin. Treatment of soft and hard wheat flour with transglutaminase decreased the allergenicity of both salt-soluble and salt-insoluble proteins (Watanabe et al., 1994). This suggests that masking of the allergenic structure could be effective in decreasing allergenicity. A novel method of conjugating the ϵ-amino groups in the protein with the reducing end carbonyl group in the polysaccharide by a spontaneous Maillard reaction under controlled dry-heating conditions has been developed (Kato et al., 1990, 1991b; Nakamura et al., 1990, 1992). In this study, an attempt was made to mask the allergenic structure of soy protein using the Maillard-type polysaccharide conjugation.

MATERIALS AND METHODS

Materials. Chymotrypsin (52 units/mg), bovine serum albumin (BSA), Tween 20, goat anti-rabbit IgG−peroxidase conjugate, *O*-phenylenediamine (OPD), and Freund's complete and incomplete adjuvant were purchased from the Sigma Chemical Co. (St. Louis, MO). ^{125}I-labeled antihuman IgE (1.7×10^4 Bq/mL) was obtained from Pharmacia Co. (Uppsala, Sweden). Peroxidase-conjugated sheep anti-mouse IgG was from Organon Teknika (West Chester, PA). Unless otherwise stated, all reagents used in this study were reagent grade. New Zealand females white rabbits were purchased from Kyudo Co. (Tosu, Japan). BALB/c mice were obtained from SLC (Shizuoka, Japan). A galactomannan preparation (average molecular weight of 15 000) was obtained by dialyzing a mannase hydrolysate of guar gum from Taiyo Chemical Co. (Tokyo, Japan).

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Preparation of Acid-Precipitated Soy Protein (APP). APP was prepared by the method of Iwabuchi et al. (1987). A sample of defatted meal (100 g) was extracted once with 2 L of 0.03 M Tris-HCl buffer (pH 8) containing 10 mM 2-mercaptoethanol (2-ME) at 20 °C. After centrifugation (8000*g*), the supernatant was acidified to pH 4.8 with 2 N HCl and then recentrifuged. The precipitates were dissolved with water at 4 °C, and the pH was adjusted to 8. After centrifugation (8000*g*), the clear supernatant was dialyzed against distilled water for 24 h at 4 °C and then freeze-dried.

APP−Galactomannan Conjugation. The APP−galactomannan powder mixture in the weight ratio of 1:5 was dissolved in water at 10% (w/v) and freeze-dried. Powdered APP−galactomannan mixtures were dry-heated at 60 °C under 79% relative humidity (RH) in a desiccator containing a saturated KBr solution in the bottom for a given time (0, 3, 7, 10, and 14 days).

Preparation of Chymotrypsin-Digested APP (APPC). A freeze-dried sample (4 g) of APP was suspended in 400 mL of 0.05 M Tris-HCl (pH 8.0) containing 0.05% sodium azide, and then 40 mg of chymotrypsin was added. The mixture was incubated at 37 °C for 6 h. After incubation, chymotrypsin was inactivated by heating at 100 °C for 3 min. The digested mixture was centrifuged (8000*g*) to precipitate a small amount of undigested protein; the supernatant was dialyzed against distilled water for 24 h at 4 °C and then freeze-dried.

Preparation of Microbial Transglutaminase (TGase). Microbial transglutaminase was purified from the culture medium of *Streptoverticillium cinnamoneum* sp. *cinnamoneum IFO12852* (Ando et al., 1989). The microorganism was inoculated in 200 mL of 0.2% polypeptone, 0.5% glucose, 0.2% dipotassium hydrogen phosphate, and 0.1% $MgSO_4$ for 48 h at 30 °C. The culture medium was added to 20 L of fresh medium (pH 7.0) composed of 2.0% polypeptone, 2.0% Lustergen, 0.2% dipotassium hydrogen phosphate, 0.1% $MgSO_4$, 0.2% yeast extract, and 0.05% Adekanol (Pharmacia Co.) and then cultured for 3 days. The culture medium (pH 6.5) was applied to a column of Amberlite CG-50 (Pharmacia Co.), and then the adsorbed fraction was eluted with 0.05 M phosphate buffer (pH 6.5) containing 0.5 M NaCl. The fraction having a high activity of TGase was collected and then adsorbed on Blue Sepharose CL-6B (Pharmacia Co.). The adsorbed sample was eluted with a gradient of 0−1 M NaCl. The peak of TGase was collected and dialyzed against deionized water.

Transglutaminase (TGase) Treatment. APP (10 mg/mL) in 0.1 M phosphate buffer (pH 7) was reacted with TGase (0.2 mg/mL). The mixture was incubated at 55 °C for 60 min. The enzyme was inactivated by *N*-ethylmaleimide (0.1 mL, 0.1%) (Kato et al., 1991a). The treated samples were dialyzed against distilled water and then freeze-dried.

SDS−Polyacrylamide Gel Electrophoresis. SDS−polyacrylamide gel electrophoresis (SDS−polyacrylamide gel) was carried out using the method of Laemmli (1970) with a 15% acrylamide separating gel and a 3% acrylamide stacking gel containing 0.1% SDS. Samples (20 μL, 0.2%) were prepared in a Tris-glycine buffer at pH 8.8 containing 1% SDS. Electrophoresis was carried out at a current of 10 mA for 5 h in electrophoretic Tris-glycine buffer containing 0.1% SDS. After electrophoresis, the gel sheets were stained for proteins and carbohydrate with 0.2% Coomassie brilliant blue-R250 and 0.5% periodate-fuchsin solution (Zacharius et al., 1969), respectively. Protein stain was destained with 10% acetic acid containing 20% methanol.

Separation of APP Protein Bands by SDS−PAGE. Untreated APP protein bands in an SDS−polyacrylamide gel were separated by the method of Cleveland et al. (1977) with slight modifications. After electrophoresis, the first and last lanes of the gel were stained to visualize the bands of interest, and then the bands of the middle eight lanes were cut from the gel, extracted with water, and centrifuged. The supernatant was dialyzed against 0.01 M phosphate buffer (pH 7.5) and used as an antigen for the subsequent ELISA test.

Human Serum. Human serum samples were prepared from 361 patients with atopic dermatitis (mean age of 6.1 years old, ranging from 3 months to 23 years) who were asked to donate blood samples for testing at Tokushima Kensei Hospital and National Kagawa Children's Hospital.

Production of Polyclonal Antibodies in Rabbits. The immunization schedule and methods of immunization via the multiple-injection technique were according to Chu et al. (1979). Two rabbits were each injected with soy protein (2.0 mg/mL) in 0.01 M phosphate buffer (PB, pH 7.5) containing 0.85% NaCl (PBS), emulsified with 2.0 mL of Freund's complete adjuvant. For booster injections, immunogen (1.0 mg/mL) in PBS and 2.0 mL of Freund's incomplete adjuvant were used for each rabbit. The collected antisera were precipitated with $(NH_4)_2SO_4$ to a final saturation of 35% by mixing 2 mL of antisera with 1 mL of saturated $(NH_4)_2SO_4$ solution. The precipitates were redissolved in water and reprecipitated twice. Finally, the precipitates were reconstituted to half of the original volume with distilled water, dialyzed against distilled water for 1 h followed by 0.01 M PB overnight (all at 4 °C), and then stored at −20 °C until they were used.

Preparation of Monoclonal Antibody (mAb). The mAb against the 34 kDa protein was prepared using female 8-week-old BALB/c mice injected intraperitoneally with the allergenic protein (50 μg/mouse) by the method of Tsuji et al. (1993).

Monitoring of Polyclonal Antibody Titers by Indirect ELISA. The antibody titers were determined by an indirect ELISA (Huang and Chu, 1993). For the rabbit serum, APP, the separated proteins of APP, and the modified proteins were used separately as a solid-phase test antigen. One hundred microliters of each antigen (10 μg/mL) in 0.01 M PBS (pH 7.5) was added to each well of a 96-well ELISA microtiter plate (Nunc plate 2-69620, Nunc, Roskilde, Denmark). The plate was kept at 4 °C overnight. After the solution had been removed, the wells were washed four times (0.35 mL/well) with PBS−Tween buffer [0.01 M phosphate saline buffer (pH 7.5) with 0.5% Tween 20]. This was followed by incubation with 0.15 mL of 1.0% bovine serum albumin (BSA) in 0.01 M PBS at 37 °C for 1 h. The plate was washed four times with PBS−Tween to remove the excess BSA. To each well was added 0.1 mL of various dilutions of anti-APP serum; the plates were gently mixed and incubated at 37 °C for 2 h. After the plates were washed four times with PBS−Tween, 0.1 mL of goat anti-rabbit IgG−HRP at a 1:4000 dilution in 0.01 M PBS was added to each well, and the mixtures were incubated at 37 °C for 1.5 h. The plate was washed with PBS−Tween buffer, and 0.1 mL of a freshly prepared *O*-phenylenediamine (OPD) substrate solution (10 mg of OPD and 13 μL of 30% hydrogen peroxide in 25 mL of 0.05 M of citrate−phosphate buffer at pH 5.0) was added. Twenty minutes after incubation at room temperature in the dark, the reaction was terminated by adding 0.1 mL of 4 N H_2SO_4. The absorbance at 490 nm was determined using an automatic microplate reader (Bio-Rad, Hercules, CA).

Immunoblotting with Polyclonal Antibody. Immunoblotting was done according to the method of Ventling and Hurley (1988) with slight modifications. Samples were electrophoresed on 15% SDS−polyacrylamide slab gels (8 × 7 cm, 0.5 mm thick) as described by Laemmli (1970). Proteins were transferred to a nitrocellulose membrane by electroblotting in a semidry system (AE-6675, ATTO Co., Tokyo, Japan) at 40 mA for 2 h at 22 °C, in 24 mM Tris (pH 8.3), 192 mM glycine, and 20% methanol (v/v). Protein blots were blocked with 3% bovine serum albumin (BSA) in TTBS [0.1% Tween 20, 100 mM Tris-HCl (pH 7.5), and 0.9% NaCl] for 1.0 h at room temperature with agitation. The allergen on the membrane was reacted at 37 °C for 2 h with rabbit sera. After blots had been washed three times (10 min in each wash) in TTBS, the immunocomplex of the allergen was incubated with a purified goat anti-rabbit IgG (H & L) diluted (1:800) in 5 mL of 1% BSA TTBS for 1 h at room temperature with constant agitation. Blots were washed as described before and detected using DAB/$NiCl_2$ visualization solution freshly prepared as follows: 5 mL of 100 mM Tris-HCl (pH 7.5), 100 μL of DAB (40 mg/mL in H_2O), 25 μL of $NiCl_2$ (80 mg/mL in H_2O), and 15 μL of 3% H_2O_2 mixed just before use.

Dot Immunoblotting with mAb. Dot immunoblotting was done by the method of Tsuji et al. (1993) with slight

modification. The 34 kDa protein (0.5 or 1.0 μg) and the galactomannan conjugate (0−30 μg) were spotted on the membrane. After drying out, the membrane was blocked with 1% BSA in 20 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl and 0.05% Tween 20 (buffer A), and then the membrane was incubated at 37 °C for 1 h with a mAb against the allergen. As a control, nonimmune IgG was used. The bound mAb was reacted with peroxidase-conjugated sheep anti-mouse IgG, diluted 1:2000 with buffer A containing 1% BSA.

The immunocomplexes on the membrane were detected by a 20 min incubation at room temperature with 4-chloro-1-naphthol (0.3 mg/mL) and 0.03% H_2O_2 as a substrate in 50 mM Tris-HCl buffer (pH 7.3).

Dot Immunoblotting with Human Sera of Allergenic Patient. Dot immunoblotting was done by the method of Ogawa et al. (1991) with slight modification. The 34 kDa protein (0.5 or 1.0 μg) and the galactomannan conjugate (0−30 μg) were spotted on the membrane. After drying out, the membrane was blocked with 3% BSA in 10 mM Tris-HCl buffer (pH 8) containing 0.9% NaCl (TBS) at 25 °C for 30 min. The membrane was washed with TBS and then incubated with 1 mL of a 5-fold diluted solution of patient's serum at 4 °C for 20 h with gentle stirring. The membrane was washed three times with TBS, twice with TBS containing 0.5% Nonidet P-40, and finally with TBS. The membrane was incubated with 0.6 mL of ^{125}I-labeled anti-human IgE (1.7×10^4 Bq/mL) for 20 h at 20 °C. After being washed with TBS, the membrane was wrapped with polyethylene film. Autoradiography of the IgE-binding proteins was taken on Fuji AIF New RX medical film (Fuji Photo Film Co., Kanagawa, Japan) in a cassette equipped with Kodak intensifying screen by exposure for several days at −80 °C. Nonspecific binding of IgE antibodies or antihuman IgE antibodies to the antigen was determined without patient's serum.

Measurement of Solubility. Protein solubility was determined by the method of Aoki et al. (1980) with slight modifications. Sample solutions (0.2%) at various pH values (pH 2−3, 0.05 M citrate buffer; pH 4−5, 0.05 M acetate buffer; pH 6−8, 0.05 M phosphate buffer; pH 9−11, 0.05 M carbonate buffer; and pH 12, 0.05 M NaOH slightly adjusted with 0.05 M HCl) were centrifuged at 8000*g* for 20 min. Protein solubility at various pHs was indicated by the ratio of the protein concentration in the supernatant to that in the original protein solution at pH 12.

Heat Stability. Heat stability was determined by the method described by Kato et al. (1995). The samples were dissolved at a protein concentration of 2 mg/mL in 50 mM Tris-HCl buffer (pH 7.0) and heated at 50−90 °C for 20 min. Aggregates were precipitated by centrifugation at 8000*g* for 20 min. Soluble protein was determined and expressed as a percentage of total protein in the solution.

Measurement of Emulsifying Properties. The emulsifying properties of the samples were determined by the method of Pearce and Kinsella (1978). To prepare emulsions, 1.0 mL of corn oil and 3.0 mL of protein solution (0.2%) in 0.1 M phosphate buffer (pH 7.0) were shaken together and homogenized in an Ultra Turrax instrument (Hansen & Co., West Germany) at 12000*g* for 1 min at 20 °C. A 50 μL sample of the emulsion was taken from the bottom of the container at different times and diluted with 5 mL of a 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted emulsion was then determined at 500 nm. The emulsifying activity was determined from the absorbance measured immediately after the emulsion formation. The emulsion stability was estimated by measuring the half-time of the initial turbidity of the emulsion.

RESULTS AND DISCUSSION

Effect of Various Modifications on the Molecular Mass of Soy Protein. Regardless of any heating time (3, 7, 10, or 14 days), soy protein conjugated with galactomannan showed high-molecular mass bands in the top of the separating and stacking gel in both protein

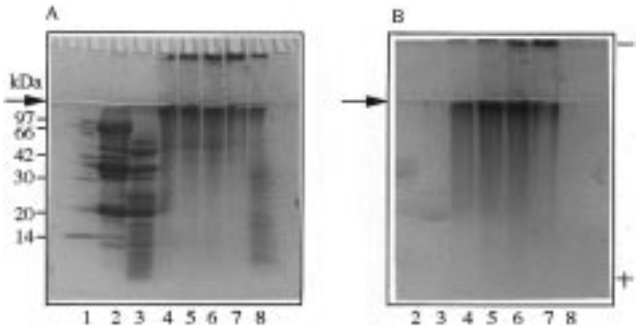

Figure 1. SDS−PAGE patterns of acid-precipitated soy protein (APP), chymotrypsin digest (APPC), TGase treatment, and galactomannan conjugate. (A) Protein stain and (B) carbohydrate stain: lane 1, molecular mass markers; lane 2, APP; lane 3, APPC; lanes 4−7, galactomannan conjugates obtained by dry-heating for 3, 7, 10, and 14 days, respectively; and lane 8, TGase-treated APP. Arrows indicate the boundary between the stacking (upper) and separating (lower) gels.

(lanes 4−7, Figure 1A) and carbohydrate staining (Figure 1B) gels. The results indicated that APP was covalently attached to galactomannan through the Maillard reaction between the ε-amino groups in the proteins and the reducing end carbonyl groups in galactomannan during dry-heating at 60 °C and 79% relative humidity. Similar results were reported for an ovalbumin−dextran mixture (Kato et al., 1990), a Pronase-treated gluten−dextran mixture (Kato et al., 1991b), and a lysozyme−dextran conjugate (Nakamura et al., 1990). TGase-treated APP (lane 8, Figure 1A) also showed a higher-molecular mass band in the top of the stacking gel. The result indicated that TGase may have catalyzed the transfer reaction between an amide group in a protein-bound glutamine and an ε-amino group in a protein-bound lysine side chain, resulting in cross-links between the protein molecules (Nonaka et al., 1989; Kato et al., 1991a; Sakamoto et al., 1994, 1995; Sergo et al., 1995).

Identification and Modification of the Allergenic Protein in Soybean. To identify the protein response to the antibody produced, soy proteins were separated by gel cutting and subsequent extraction from the bands of SDS−polyacrylamide gel (Figure 2A) into different fractions. Polyclonal antibodies against soy protein were obtained from rabbits after they had been immunized with the same protein. Monitoring of antibody titers (1:10000) by enzyme-linked immunosorbent assay (ELISA) (Figure 2B) against the separated proteins (10 μg/mL) showed that only the 34 kDa protein (lane 7, Figure 2B) had a higher value ($OD_{490} = 0.822$), while other proteins showed lower values (less than 0.037). To reduce the allergenicity, APP was digested by chymotrypsin, cross-linked by transglutaminase, or conjugated with galactomannan. To determine the effect of these modifications on the allergenicity of soy protein, antibody titers (1:10000) were monitored by ELISA against APP, chymotrypsin digest (APPC), TGase polymer, and APP−galactomannan conjugates (10 μg/mL) (Figure 3). The chymotrypsin digest was still allergenic ($OD_{490} = 0.522$), while the TGase treatment ($OD_{490} = 0.279$) considerably reduced the allergenicity of APP ($OD_{490} = 0.792$) and, regardless of any dry-heating time, greatly reduced that of galactomannan conjugates ($OD_{490} = 0.003$). To further confirm the allergenicity of this protein, immunoblotting was done using rabbit sera (Figure 4). The immunoblotting pattern for the separated proteins showed that only the

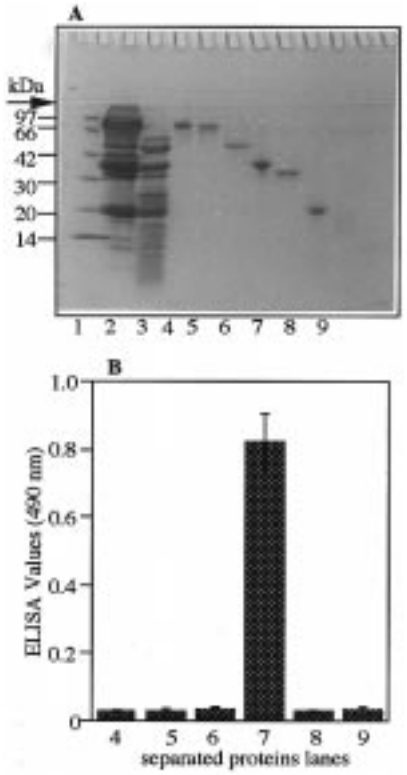

Figure 2. (A) SDS−PAGE patterns of acid-precipitated soy protein (APP), chymotrypsin digest (APPC), and the separated protein bands of APP: lane 1, molecular mass markers; lane 2, APP; lane 3, APPC; and lanes 4−9, proteins separated by gel cutting. The horizontal arrow indicates the boundary between the stacking (upper) and separating (lower) gels. The vertical arrow indicates the allergic protein. (B) ELISA analysis of the separated proteins. The lane number of the separated proteins is the same as that in panel A. The antibody was diluted 10 000 times and reacted with 10 μg/mL antigen.

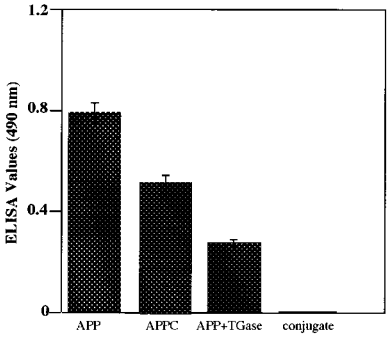

Figure 3. Determination of antibody titers by indirect ELISA of acid-precipitated soy protein (APP), chymotrypsin digest (APPC), TGase-treated APP, and galactomannan conjugate dry-heated for 7 days. Antibody diluted 1:10000 was reacted with 10 μg/mL antigen. Error bars indicate the standard deviations ($n = 6$).

34 kDa protein was strongly cross-reacted with the antibody, while other proteins did not. The pattern also showed that the 34 kDa proteins of both APP and chymotrypsin digest were strongly cross-reacted with the antibody, while TGase-treated samples and galactomannan conjugates did not (Figure 4). Further studies were done by dot immunoblotting with monoclonal antibody or human sera of the soybean-sensitive patients against the 34 kDa protein and galactomannan conjugates of different concentrations. As shown in Figure 5, the cross-reactivity of 0.5 μg of the 34 kDa protein was very strong against mAb, while that of high

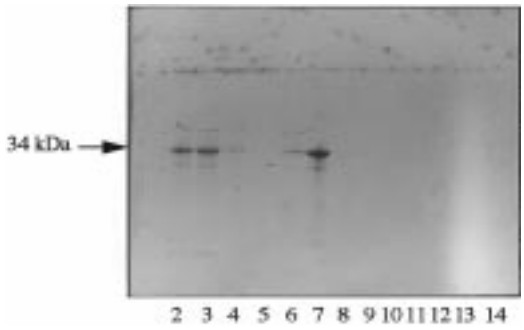

Figure 4. Immunoblotting of acid-precipitated soy protein (APP), chymotrypsin digest (APPC), TGase-treated APP, galactomannan conjugates, and protein bands separated from APP by SDS−PAGE: lane 2, APP; lane 3, APPC; lanes 4−9, proteins separated as indicated in Figure 2A, respectively; lane 10, TGase-treated APP; and lanes 11−14, APP−galactomannan conjugates dry-heated for 3, 7, 10, and 14 days at 60 °C and 79% relative humidity, respectively.

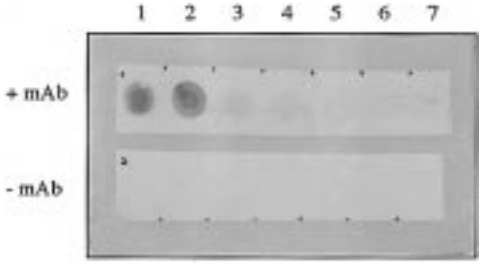

Figure 5. Dot immunoblotting of the separated 34 kDa protein and the galactomannan conjugate (7 days) against monoclonal antibody: lanes 1 and 2, 1.0 and 0.5 μg of 34 kDa protein, respectively; and lanes 3−7, 30.0, 15.0, 7.5, 3.75, and 0.0 μg of galactomannan conjugate, respectively.

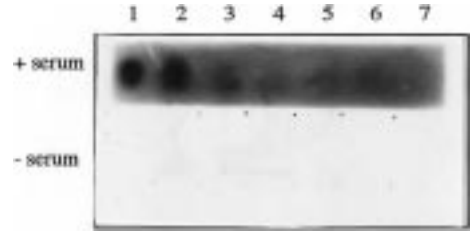

Figure 6. Dot immunoblotting of the separated 34 kDa protein and the galactomannan conjugate (7 days) against patient serum: lanes 1 and 2, 1.0 and 0.5 μg 34 kDa protein, respectively; lanes 3−7, 30.0, 15.0, 7.5, 3.75, and 0.0 μg of galactomannan conjugate, respectively.

concentrations (30 μg) of the conjugate was not observed. The common cross-reactivity between rabbit and mouse shows the strong antigen structure of the 34 kDa protein which has been reported as a major allergenic protein in soybean (Ogawa et al., 1991) with epitopes recognized by mouse monoclonal antibodies localized in peptide ^{31}QGGCGRGWAFSATGAIEA^{48} (Hosoyama et al., 1996). This antigen structure seems to be masked completely by the conjugation with galactomannan. The effect was investigated by human sera of soybean-sensitive patients who have IgE of soy protein. As shown in Figure 6, the cross-reactivity of the 34 kDa protein against human sera was very strong, while it was completely diminished by the galactomannan conjugation. It has been reported that ELISA values of less than 0.05 represent hypoallergenicity (Doke et al., 1989). This means that chymotrypsin digestion and TGase treatment did not remove allergenicity of soy protein. On the other hand, galactomannan conjugation removed the

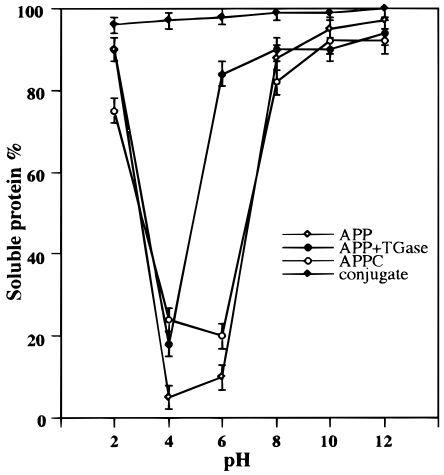

Figure 7. Effect of pH on solubility of acid-precipitated soy protein (APP), chymotrypsin digest (APPC), TGase-treated APP, and galactomannan conjugate dry-heated for 7 days. Error bars indicate the standard deviations ($n = 3$).

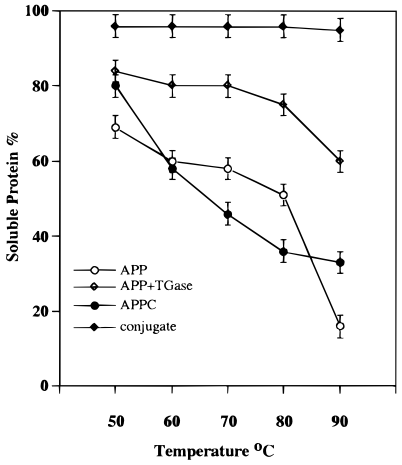

Figure 8. Heat stability of acid-precipitated soy protein (APP), chymotrypsin digest (APPC), TGase-treated APP, and galactomannan conjugate dry-heated for 7 days. Error bars indicate the standard deviations ($n = 3$).

allergenicity of soy protein. Therefore, the protein−polysaccharide conjugation is more effective than TGase treatment and protease digestion in masking the allergenic structure inside the conjugated molecules.

Effect of Various Modifications on the Functional Properties of Soy Protein. APP and APPC were less soluble at pH 4−6; TGase-treated APP was least soluble at pH 4, while APP−galactomannan conjugates were highly soluble at all pHs (Figure 7). Improvement in the solubility of TGase-treated APP at various pHs was likely due to an increase in the electrostatic repulsion caused by partial deamidation of glutamine (Motoki et al., 1986). Therefore, it seems likely that both polymerization and deamidation of the soy protein may occur in the TGase treatment as reported in α_{s1}-casein (Motoki et al., 1986). The solubility of APP−galactomannan conjugates was greatly improved at acidic pH compared to that of APP. The improvement in the solubility at various pH levels is apparently due to the attachment of galactomannan to APP through the Maillard reaction between amino groups in APP and the reducing end carbonyl group in galactomannan. It seems likely that the hydrophilicity of galactomannan may be attributted to the increase in solubility. Similar results were reported when pronase-treated gluten (PTG) was conjugated with dextran (Kato et al., 1991b). The solubility of both APP and APPC decreased as the heating temperature increased, whereas APP treated with TGase substantially resists heat-induced aggregation up to 80 °C, after which its solubility slightly decreased (Figure 8). On the other hand, APP−galactomannan conjugates were highly soluble even after being heated at 90 °C for 20 min. Similar results were reported for a lysozyme−galactomannan conjugate (Kato et al., 1996). The effect of various modifications of APP on the emulsifying properties is shown in Figure 9. Although the emulsifying properties of APP were improved by chymotrypsin digestion and TGase treatment, further improvement was observed when APP was conjugated with galactomannan. The emulsifying activity of APP, which is estimated by the turbidity of the emulsion measured immediately after emulsion formation, was increased in the range of 0.64−0.94 after various modifications. The emulsion stability (the half-time of the initial turbidity) was found to be 1.5, 2.5, 8.5, and 15 min for APP, APPC, TGase-treated APP, and APP−galactomannan conjugate, respectively. The results revealed that, regardless of any heating time, the emulsion stability of APP was greatly improved by galactomannan conjugation. It has been reported that the hydrophobic residues of soy protein may be anchored to the surface of oil droplets in an emulsion and the hydrophilic parts of polysaccharide may be oriented to the water phase, thereby inhibiting the coalescence of the oil droplets. Thus, a stable emulsion may be formed in the presence of APP−galactomannan conjugate (Kato et al., 1991b).

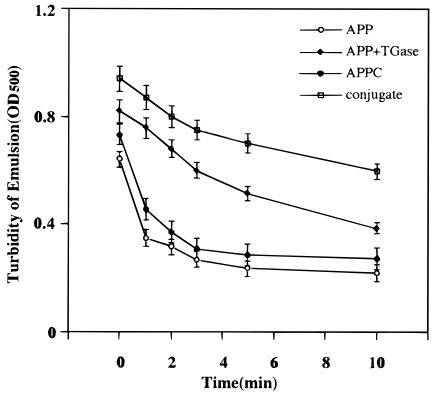

Figure 9. Emulsifying properties of acid-precipitated soy protein (APP), chymotrypsin digest (APPC), TGase-treated APP, and galactomannan conjugate dry-heated for 7 days. Error bars indicate the standard deviations ($n = 3$).

In conclusion, polysaccharide conjugation of soy protein was found to be effective in masking the allergenic structure of soy protein and in improving the solubility, heat stability, and emulsifying properties. In addition, this conjugation is one of the most promising methods for food applications, because of the safety due to the naturally occurring reaction.

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