Effect of Transglutaminase Treatment on the Glass Transition of Soy Protein

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The effect of microbial transglutaminase (MTG) treatment on the glass transition temperature (T_g) of two fractions which were isolated from a soy protein sample was studied. The T_g of each fraction measured by differential scanning calorimetry was lowered by the MTG treatment, which generated cross-links in the samples, and this result agreed with the result of dynamic mechanical analysis. From the ^{1}H NMR measurement, the line width of the ^{1}H signal of the MTG-treated sample was observed to be greater than that of the MTG-nontreated sample at similar water content, which implied that there was relatively more immobilized water in the MTG-treated sample. The MTG treatment seemed to cause the increment in immobilized water, which might affect the T_g of the soy protein sample.

Keywords: *Glass transition; soy protein; transglutaminase; water mobility*

INTRODUCTION

The importance of the glass transition in low-moisture foods, such as cereal-based dry snacks and dry powders, has been widely recognized, because many mechanical changes in those foods during storage (loss of crispness, caking of powders, etc.) are known to be related to their glass transition (Levine and Slade, 1993; Peleg, 1993; Chuy and Labuza, 1994; Martinez and Chiralt, 1995). From this standpoint, a number of studies on the glass transition of food materials, especially saccharides, has been carried out (Roos and Karel, 1991; Noel and Ring, 1992; Ablett et al., 1993; Arvanitoyannis et al., 1993; Suzuki and Franks, 1993).

In the food industry, the utilization of soybeans is large as a dry material for tofu, seasonings, and many processed foods (Heinevetter et al., 1987; Utsumi et al., 1997). In addition, the utilization as an edible film (Ghorpade et al., 1995; Kunte et al., 1997; Stuchell and Krochta, 1994; Gennadios et al., 1993) and a dry biodegradable plastic (Jane et al., 1994; Wool et al., 1998) has also been studied. In that situation, the mechanical properties of dry soy protein are important, and the glass transition of soy protein related to mechanical changes during storage is important for the stability of dry soy protein. However, the number of studies on the glass transition of soy protein is relatively small (Morales and Kokini, 1997; Johari and Sartor, 1998), and there is little information on the effect of the manufacturing methods of soy protein on the glass transition of dry soy protein products.

In the manufacture of soy protein products, several enzymatic treatments are done for the improvement in the mechanical properties. Transglutaminase ((*R*)-glutaminyl peptide, amine γ-glutamyl transferase; EC 2.3.2.13) treatment is one of such enzymatic treatments. The enzyme catalyzes the acyl-transfer reaction, in which γ-carboxyamide groups of peptide-bound glutaminyl residues are the acyl donors (Sakamoto et al., 1994a). When the amino groups of peptide-bound lysine residues are the acyl acceptor, ϵ-(γ-Glu)Lys cross-links are generated. Many studies for the application of transglutaminase in the food industry have been conducted (Motoki et al., 1987; Nonaka et al., 1992; Chanyongvorakul et al., 1994), and in the manufacture of soy protein, the enzyme is used for improvement in the gel strength of the final products due to the generation of cross-links between proteins. However, we have little information on the changes in dry soy protein prepared by the transglutaminase treatment.

Therefore, in this study, we studied the change in the glass transition temperature (T_g) of low-moisture soy protein products caused by transglutaminase treatment. This information seems to be directly related to the stability of those soy protein products during storage and indirectly related to the properties of high-moisture final products made from soy protein. For this objective, we first measured the T_g of soy proteins by differential scanning calorimetry (DSC) and studied the effect of transglutaminase treatment on T_g. Furthermore, we measured the relative mobility of water molecules in protein samples by ^{1}H NMR and examined the relation between the change in T_g caused by transglutaminase treatment and the state of water in the protein samples.

MATERIALS AND METHODS

Sample Preparation. Two types of soy protein products were used for this study; one was a soy protein isolate (heated for pasteurization), and the other was a defatted soybean flake (nonheated). The soy protein isolate (Ajipron-SU) was obtained from Ajinomoto Co., Inc. (Tokyo, Japan), and the defatted soybean flake was purchased from Asahi-Yushi Co. (Hokkaido, Japan). From each product, we isolated two fractions (7S fraction and 11S fraction) according to the method of Nagano et al. (1992), which involved extraction by pH adjustment. Those fractions seemed to be rich in subunits which constituted 7S globulin and 11S globulin, respectively. Each fraction was dialyzed and lyophilized after the isolation, and we obtained dry 7S and 11S fractions for the treatment and measurements described below.

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Transglutaminase Treatment. The transglutaminase treatment of each soy protein sample was carried out in the following manner. Each dry sample was first suspended in tris−HCl buffer (pH 7.5). The concentration of protein substrate in the suspension was 1%. To the suspension was added microbial transglutaminase (MTG) (about 3 units/mg) prepared from the culture of *Streptoverticillium* sp. by the method previously described (Ando et al., 1989) at a concentration of about 15 units/g of substrate, and the suspension was incubated at 37 °C for 1 h. After the reaction, the suspension was placed in boiling water for 15 min to inactivate the enzyme. We obtained a dry MTG-treated sample after dialysis and lyophilization of the suspension. Thus, we define the MTG treatment in this study as a series of processes from the reaction to the inactivation by heating.

Determination of the Glass Transition Temperature. Before each measurement, we adjusted the water content of the dry samples by blowing supersaturated water vapor over them for appropriate times and equilibrating them in a hermetic vessel for more than 24 h at ambient temperature. The water content (w/w, wet basis) of each sample was determined by heating at 105 °C for 18 h. After that, we basically determined the T_g of the samples by DSC in the following manner. We used a DSC 120 (Seiko Instruments) calibrated with In, Sn, and Ga for the calorimetric measurements. Each sample was placed in a silver pan (70 mL), and after the pan was sealed hermetically, the sample was weighed accurately (about 50 mg). An empty silver pan was used as the reference. The temperature range of the scan was from −20 to +120 °C, and the heating rate was about 6 °C/min. From the DSC scan, the specific heat (C_p) of the sample and the derivative of C_p were obtained. In this study, we defined T_g as the peak in the derivative of C_p accompanying the shift in C_p. For part of the samples, mechanical measurements with dynamic mechanical analysis (DMA) were also carried out by the same method previously described (Mizuno et al., 1998).

Measurement of the Relative Mobility of Water Molecules. The relative mobility of water molecules in protein samples was measured by using proton (^{1}H) NMR (α-400, JEOL, Tokyo, Japan) at a frequency of 400 MHz at 25 °C. Each sample, whose water content was previously adjusted from 16% to 26%, was placed in an NMR tube (5 mm o.d.) and then analyzed. The line width at half-height ($\delta_{1/2}$) of the ^{1}H signal was determined from the obtained NMR spectra. Each $\delta_{1/2}$ value was the average of two measurements. The ^{1}H signal may be affected by many factors, such as diffusional exchange and cross-relaxation, as well as the protons in the water molecules. Thus, we used the line width values as an indicator of the relative mobility of the water molecules in the protein samples by measuring them in the near range of water content.

RESULTS AND DISCUSSION

Characterization of Prepared Samples. In Figure 1, we show the SDS−polyacrylamide gel electrophoresis patterns of two fractions (7S and 11S fractions) isolated from soy protein isolate and their MTG-treated fractions. We can see that two fractions which appear to constitute 7S globulin and 11S globulin were isolated from the soy protein isolate (Ajipron-SU) fairly well. Especially, the 7S fraction seemed to consist mainly of three subunits, α and α′ subunits (MW about 70 000) and a β subunit (MW about 50 000). As a result of this isolation, about 2 g of the 7S fraction and 4 g of the 11S fraction were obtained from 150 g of soy protein isolate. The bands of the MTG-treated samples appeared in a higher molecular region, including the region outside the gel, compared to those of the nontreated samples. The formation of cross-links by the MTG treatment for soy protein or 11S globulin has been so far proved by the analysis of the ϵ-(γ-Glu)Lys bond (Kang et al., 1994; Sakamoto et al., 1994b; Nonaka et al., 1994). Since the observed change in SDS−polyacrylamide gel electrophoresis patterns is similar to the results shown in those studies, it is certain that the polymerization of the MTG-treated 7S and 11S fractions occurred by cross-links. In the case of fractions isolated from defatted soybean flake, the patterns were similar to those of the fractions from soy protein isolate, and polymerization was also confirmed. However, a larger amount of the 7S and 11S fractions was obtained from defatted soybean flake (about 6 g of the 7S fraction and 24 g of the 11S fraction from 150 g of defatted soybean flake), compared to the amount from soy protein isolate. The low yield of the fractions from soy protein isolate appears to be caused by preheating for pasteurization (130 °C, about 10 s) of the soy protein isolate. The preheating is generally considered to cause insolubility of the protein sample by changing the higher-order structure and influencing the extraction by pH adjustment, that is, decreasing the amount of the extract. In this study, we measured the T_g of samples prepared from soy protein isolate and defatted soybean flake, which might differ in higher-order structure.

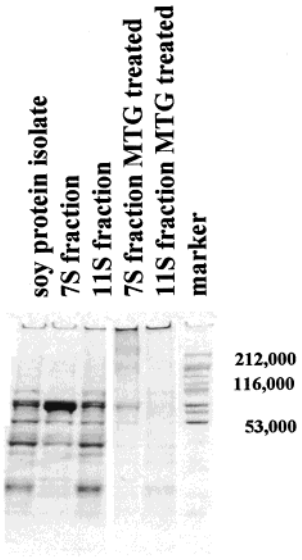

Figure 1. SDS−polyacrylamide gel electrophoresis patterns of 7S and 11S fractions isolated from soy protein isolate.

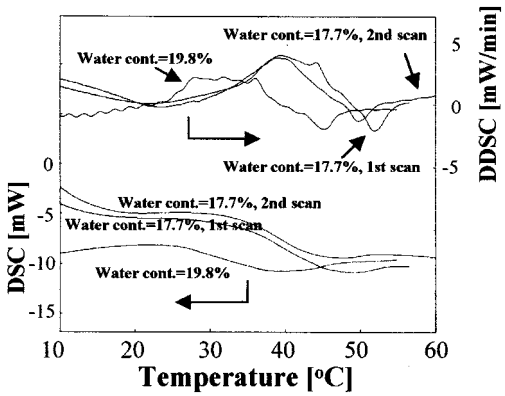

Figure 2. DSC thermogram of the MTG-treated 7S fraction isolated from soy protein isolate. The data are for the low water content (17.7% w/w water) and high water content (19.8% w/w water) samples.

***T_g* Measurement.** In Figure 2, we show the DSC chart of the MTG-treated 7S fraction isolated from soy protein isolate, showing the heat flow (DSC) curve and the derivative of the heat flow (DDSC) curve. In the figure, we show data for the soy fractions containing

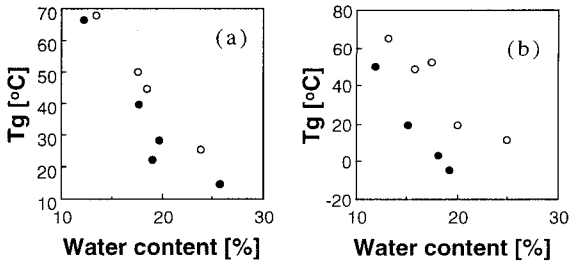

Figure 3. Dependency of the T_g of 7S fractions (a) and 11S fractions (b) isolated from soy protein isolate on the water content: (○) MTG nontreated; (●) MTG treated.

both low water content (17.7%) and high water content (19.8%). For low water content, the first and second scans obtained from repetitive scans are also shown. The denaturation temperature of soy protein is said to be about 70−90 °C (Damodaran, 1988). Therefore, in the first scan of the low water content sample, we stopped heating below 60 °C, because the protein sample might denature at a temperature above that temperature and the comparison of the first and second scans might become difficult. For the low water content sample, a shift in the DSC curve and a peak in the DDSC curve were observed in the first scan (35−45 °C), and in the second scan the behavior was also observed in the same temperature range. This result implies that the thermal behavior observed in the scan is reversible heat flow. This reversibility agrees with the tendency generally observed in the T_g (Gallagher, 1997). Moreover, for the high water content sample, the characteristic heat flow (a shift in the DSC curve and a peak in the DDSC curve) was observed at a lower temperature (25−35 °C), compared to the case of the low water content sample. This result implies that the temperature where the thermal behavior occurs decreases with increasing water content and that this dependency on water content also agrees with that of T_g; that is, water acts as a strong plasticizer for biopolymers (Roos, 1995). In addition, the high water content sample was observed to be sticky during equilibration at ambient temperature before the DSC measurement. The stickiness of a dry material is considered to be related to its glass transition (Peleg, 1993); therefore, this visual information implies that the T_g of the sample exists near the ambient temperature. On those bases, we judged that the behavior observed here was caused by glass transition, and we determined the T_g of these samples from the peak in the DDSC curves of the first scan, following the definition of T_g in this study. That is, the T_g of the low water content sample (17.7%) is 39.5 °C, and that of the high water content sample (19.8%) is 28.1 °C.

We show the dependency of the T_g of the 7S fractions isolated from soy protein isolate on water content in Figure 3a and the T_g of the 11S fractions isolated from soy protein isolate in Figure 3b. In each figure, the closed symbols are data for MTG-treated samples. From these figures, we can see that the T_g values of the MTG-treated samples are lower than those of the MTG-nontreated samples in the cases of both 7S and 11S fractions at the same water content. This result implies that the MTG treatment of soy protein isolate causes a lowering of its T_g. In Figure 4, we show plots of G' and tan δ against temperature, measured by DMA. The closed symbols are data for the MTG-treated 11S fraction, and the open symbols are data for the MTG-nontreated 11S fraction. The water contents of the

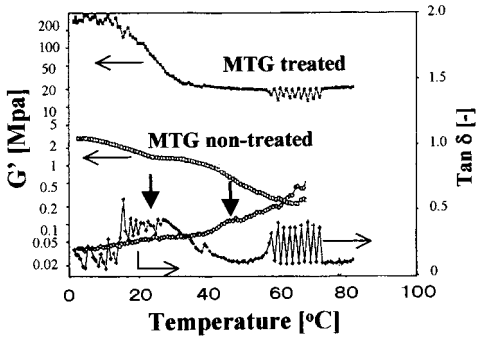

Figure 4. Storage modulus, G', and loss tangent, tan δ, against temperature for 11S fractions: (○) MTG-nontreated sample (26.3% w/w water); (●) MTG-treated sample (23.1% w/w water).

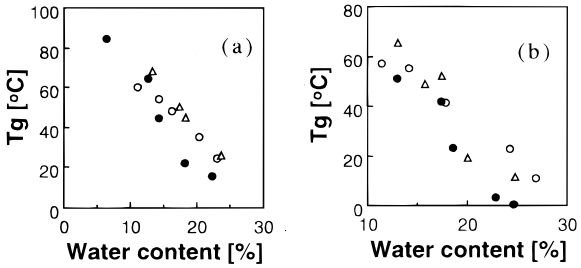

Figure 5. Dependency of the T_g of 7S fractions (a) and 11S fractions (b) isolated from defatted soybean flake and soy protein isolate on the water content: (△) MTG-nontreated sample from soy protein isolate; (○) MTG-nontreated sample from defatted soybean flake; (●) MTG-treated sample from defatted soybean flake.

MTG-treated and -nontreated samples were close, that is, 23.1% and 26.3%, respectively. In both samples, remarkable decreases in G' and the peak or shoulder in tan δ (indicated by arrows) were observed with increasing temperature. Generally, the decrease in G' accompanied by the peak in tan δ occurs in the glass transition, and the quantity of the decrease is considered to be more than 1 order of magnitude in the case of biopolymers (Kalichevsky et al., 1993). Moreover, if DMA measurement is done at 1 Hz, the peak temperature in tan δ will be approximately 15 °C higher than the T_g measured by DSC at low heating rates (Kalichevsky et al., 1993). By comparing the DMA data with the DSC data (Figure 3b) on this information, we can see that the decrease of G' occurs about 20 °C higher than T_g measured by DSC at the close water content. Therefore, the decrease of G' accompanied by the peak in tan δ was considered to result from glass transition of the 11S fractions. The temperature where the decrease of G' occurred in the MTG-treated sample was 0−20 °C, while it was 30−60 °C in the MTG-nontreated sample. This result means that glass transition of the MTG-treated sample occurs in a lower range of temperature than that of the MTG-nontreated sample, and agrees with the result obtained from the DSC measurements. From the comparison of DSC and DMA data, we can confirm the lowering effect of the MTG treatment on the T_g of the soy protein samples.

In Figure 5a, we show the dependency of the T_g of the 7S fraction isolated from defatted soybean flake (circles) and the 7S fraction isolated from soy protein isolate (triangles) on water content. Closed symbols are for the data of the MTG-treated sample from defatted soybean flake. It can be seen that T_g values of the MTG-treated sample are lower than those of the nontreated

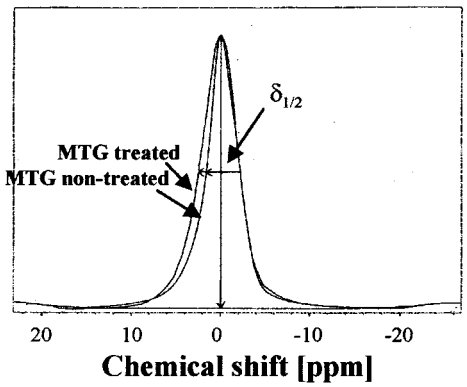

Figure 6. ^{1}H NMR spectra for MTG-treated and -nontreated 7S fractions, with water contents of 23.6% and 25.0%, respectively.

sample. This result is similar to the case of samples isolated from soy protein isolate. In addition, it can be seen that T_g values of two samples (open circles and triangles), which are non-MTG-treated, were almost the same at the same water content. In Figure 5b, we show the dependency of the T_g of 11S fractions isolated from defatted soybean flake and soy protein isolate. In this plot, the values varied slightly, compared with the previous plot of 7S fractions. The variety is considered to be caused by the heterogeneity of the 11S fraction sample. As we can see from the SDS−polyacrylamide gel electrophoresis patterns (Figure 1), the 11S fraction is composed of many subunits, and is more heterogeneous than the 7S fraction. Even if we consider the variety, it can be seen that the difference in T_g values between two MTG-non-treated samples is not so wide, and T_g values of MTG-treated samples are slightly lower. As mentioned previously, soy protein isolate used in this study was preheated for pasteurization (130 °C, about 10 s). This heating condition seems to be enough for thermal denaturation, because the denaturation temperature of soy protein is about 70−90 °C (Damodaran, 1988). The actual low yield of 7S and 11S fractions from soy protein isolate appears to be caused by the denaturation (changing of the higher-order structure). From this result concerning the T_g of MTG-nontreated samples, it is suggested that the effect of the higher-order structure on the T_g of soy protein is not as great as that of the MTG treatment.

Relationship between the State of Water and T_g. The line width of the ^{1}H signal determined from ^{1}H NMR spectra can be used as a practical indicator of the mobility of water molecules (Li et al., 1998; Mitsuiki et al., 1998). Thus, we used ^{1}H NMR for evaluating the state of water in the protein samples. However, we must note that the evaluation is a relative method comparing several samples in a near range of water content, because the ^{1}H signal may be affected by many factors, such as solid protons, diffusional exchange and cross-relaxation, and the protons in the water molecules.

In Figure 6, we show the NMR spectra for MTG-treated (water content 23.6%) and -nontreated (water content 25.0%) 7S fractions. The ^{1}H signal for the MTG-treated sample is seen to be broadened toward the low field, and its $\delta_{1/2}$ was wider than that for the MTG-nontreated sample. The water contents of these two samples were almost equal; therefore, we can consider that the difference in $\delta_{1/2}$ was attributable to the difference in the state of water in the protein samples; that is, there was relatively more immobilized water in the MTG-treated sample than the MTG-nontreated sample in the measured water content.

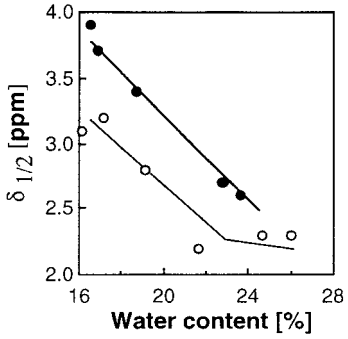

Figure 7. Change in the line width at half-height ($\delta_{1/2}$) values with water content: (●) MTG-treated samples; (○) MTG-nontreated samples.

In Figure 7, we show the change in $\delta_{1/2}$ values for both samples with water content. We can observe that the $\delta_{1/2}$ values of the MTG-treated 7S fraction were greater than those of the MTG-nontreated 7S fraction for the measured water content (16−24%). From this result, we can say that there is relatively more immobilized water in the MTG-treated sample than in the MTG-nontreated sample at the same water content, within the range of measured water content. That is to say, the MTG treatment seemed to cause the increment in immobilized water, which might affect the T_g of the soy protein sample. In general, water molecules immobilized by a bioopolymer act as a strong plasticizer, which increases the molecular mobility of the polymer and lowers its T_g (Slade and Levine, 1991; Arvanitoyannis and Biliaderis, 1998; Arvanitoyannis et al., 1998). In this case, the increment in immobilized water by the MTG treatment may result in the lowering of T_g. However, it is necessary for further investigation to clarify the effect of the immobilized water on the protein. For that study, one approach is to measure directly the molecular mobility of protein, as well as water.

Changes in Protein Samples by MTG Treatment. Through the MTG treatment, we could confirm the polymerization of soy protein samples by ϵ-(γ-Glu)Lys cross-links. The T_g of synthetic polymers is generally considered to be elevated by generation of cross-links in them (Brinke et al., 1983). Concerning the T_g of biopolymers, Zeleznak and Hoseney (1987) reported that the T_g of gelatinized starch was lower than that of native starch, and Mizuno et al. (1998) reported that the T_g of retrograded starch was higher than that of gelatinized starch. These studies on starch suggest that the increase in crystallinity of starch is connected with the elevation of the T_g and that the crystalline regions in starch are believed to act as physical cross-links formed by hydrogen bonding. Therefore, the T_g of starches is also considered to be elevated by cross-links. In this study, we obtained the opposite effect of cross-links on the T_g of soy protein samples, compared with studies on other polymers. Because there are complicating intra- and interactions to maintain the appropriate molecular structure of soy protein, it is possible that generating ϵ-(γ-Glu)Lys cross-links by the MTG treatment causes severe structural changes by cleavage of noncovalent bonds. Motoki and Seguro (1994) reported that soy protein acquired a high water-holding capacity with the generation of covalent cross-links by the MTG treatment. Though this result was obtained under experimental conditions different from those of our

studies, it suggests that MTG treatment of soy protein increases the hydrophilic property of the surface of the protein. Water acts as a strong plasticizer for soy protein as mentioned above; therefore, this change in hydrophilicity may have a much greater effect on the T_g of soy protein than cross-links by the MTG treatment. From this point of view, the study on the changes in soy protein caused by the MTG treatment is important for understanding the effect of the MTG treatment on the T_g of soy protein. This type of study may be useful for understanding the T_g of proteins in general, as well as soy protein.

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