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Effect of Enzymatic Deamidation on the Heat-induced Conformational Changes in Whey Protein Isolate and Its Relation to Gel Properties

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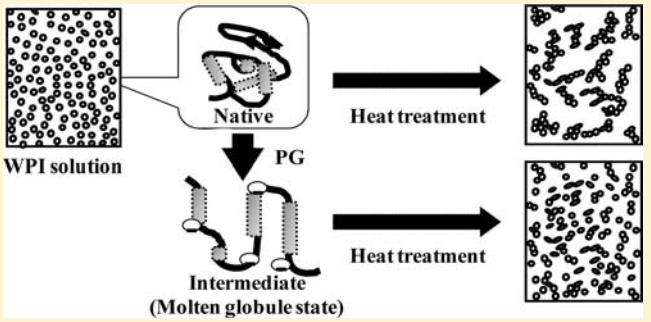
ABSTRACT: The effect of protein-glutaminase (PG) on the heat-induced conformational changes in whey protein isolate (WPI) and its relation to gel properties was investigated. The structural properties of WPI treated with PG were examined by several analytical methods. The analysis of the fluorescence spectrum and the binding capacity of a fluorescent probe demonstrated that deamidation prevented the increase in the fluorescence intensity caused by subsequent heat treatment. Measurements of the molecular weight distribution of WPI showed that PG-treated WPI was not likely to polymerize even after heating. This is thought to be due to an increase in electrostatic repulsion between carboxylic acid groups and a decrease in the formation of disulfide bonds, which results in the decrease in heat-induced aggregation. The properties of heat-induced WPI gels were modified by deamidation. PG-treated WPI gels had a soft texture and a high water-holding capacity in the presence of salts.



KEYWORDS: *deamidation, protein-glutaminase, whey protein isolate, structure, heat-induced gel*

INTRODUCTION

Protein deamidation can improve the solubility and other functional properties of food proteins by increasing the number of negatively charged carboxyl groups in the protein.[1,2] Extensive research has been conducted of the catalysis of the deamidation of various proteins by acids, bases and enzymes.[1] Enzymatic methods, especially, have been desired since they use mild reaction conditions and have high specificity. The deamidation of food proteins using some enzymes such as transglutaminase and protease is not easily done because of inevitable side reactions.[1] However, protein-glutaminase (PG; EC 3.5.1.44), first isolated in 2000, is thought to have significant potential for the enzymatic modification of food proteins. PG was discovered in culture supernatant from *Chryseobacterium proteolyticum*, and it catalyzes deamidation of glutamine residues in short peptides or proteins.[3,4] The action of PG has been investigated for several food proteins such as soy protein, wheat gluten, zein and milk proteins.[5−10]

Whey protein is a mixture of globular proteins isolated from whey, the liquid material created as a byproduct of cheese production. Today, whey proteins and their products, including whey protein isolate (WPI) are ingredients that are widely used in the food industry to obtain desired food products,[11] because of their high nutritional value and functional properties such as solubility, gelling and emulsifying. Whey proteins consist mainly of α-lactalbumin (α-La) and β-lactoglobulin (β-Lg), and other components include bovine serum albumin (BSA) and immunoglobulins.[12]

Numerous treatments have been investigated for improving whey protein functionality, such as chemical, physical and enzymatic methods.[13,14] Concerning enzymatic modification, for example, the functional properties of enzymatically hydrolyzed whey protein concentrate (WPC) have been reported.[15] Transglutaminase and laccase have been used to improve the functional properties of milk proteins through a cross-linking reaction.[16−18] However, for PG-treated whey protein, only limited published information is available. A report by Gu et al. demonstrated that PG treatment induced a structural change of native α-La, namely a molten globule-like form.[9] Nevertheless, the effect of PG on whey protein ingredients such as WPI and WPC has not been investigated.

Whey proteins are globular in structure and are heat sensitive. There have been many studies on heat-induced molecular structural changes in whey proteins.[19−21] It is generally recognized that in the heated whey proteins in solution at neutral pH, disruption of intra- and intermolecular bonds and exposure of previously buried hydrophobic residues

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to solvent take place, leading to denaturation or aggregation. The behavior of whey proteins upon heating is of interest because the formation of whey protein aggregates is often related to their food applications.[22,23] However, the effect of PG treatment on heat-induced conformational changes of whey proteins remains unclear.

Heat-induced gelation is an important property of proteins because it impacts the functionality and acceptability of foods.[24] Although the thermal gelation of WPI has been extensively studied,[25,26] to the best of our knowledge, there have been no reports concerning the effect of enzymatic deamidation by PG on the heat-induced whey protein gel. Since it is generally recognized that the balance of attractive and repulsive interactions affects the formation of heat-induced aggregation,[27] it is of interest to examine the physical properties of heat-induced whey protein gels pretreated with PG.

The material used in this study was WPI because it is used more often than isolated proteins, due to cost and availability and it contains a high level of protein, that is, >90%. In addition, a better understanding of the effect of heat treatment subsequent to the PG reaction on WPI structural and gel properties would be of great importance to the food industry. Here we examined the degree of deamidation of WPI using PG at varying enzyme/substrate ratios, and we investigated the structural properties of the PG-treated WPI followed by heat treatment using a dilute WPI solution. And furthermore the effect of PG treatment on heat-induced WPI gel properties (fracture property and water holding capacity) was examined.

MATERIALS AND METHODS

Materials. The WPI used was Bipro from Davisco Foods (Le Sueur, MN). According to the manufacturer, the typical composition of this WPI on a dry basis was 93% protein, 0.2% fat, 2% ash, and 4.8% moisture, as determined by the supplier's standard proximate analysis procedures. Protein-glutaminase 'Amano 500' (Activity 500 U/g) was obtained from Amano Enzyme, Inc. (Nagoya, Japan). All chemicals were purchased from Sigma Chemicals (St. Louis, MO), Wako Pure Chemical Co. (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan) and were of analytical reagent grade unless indicated otherwise.

Deamidation of WPI and Heat Treatment. An aqueous solution of 1.5 wt % WPI was prepared by dissolving in 50 mM sodium phosphate buffer (pH 6.8). WPI solutions were preheated to 55 °C for 5 min, followed by incubating with 2, 4, 10, 50, and 100 U of PG per 1 g WPI (the unit is abbreviated as 'U/g') for 1 h at 55 °C. To investigate the effect of heat treatment subsequent to the PG reaction, both heated and unheated samples were prepared. After PG incubation, the samples were heated at 85 °C for 30 min using a dry heat block, and then cooled in ice. Unheated samples were transferred in an ice bath immediately after PG incubation and kept in the ice bath until further analysis. A control sample was similarly treated without PG.

Deamidation Degree of WPI. Amounts of ammonia released from deamidated glutamine residues were determined using F-Kit ammonia (Roche Diagnostics, Mannheim, Germany). The deamidation degree is expressed as the ratio (in percentage) of the amount of ammonia released by the PG reaction and the total released ammonia when WPI was treated with 2 N HCl at 100 °C for 4 h.[28]

Analysis of Circular Dichroism (CD). The reaction mixtures containing 1.5 wt % WPI were diluted with 50 mM sodium phosphate buffer (pH 6.8) to obtain the final concentration of 0.04 wt %. The measurements of far- and near- UV CD were conducted at room temperature using a Jasco J-720 spectropolarimeter (Jasco, Tokyo). The far-UV spectra were measured using a 0.1-cm light-path cell over the wavelength range of 190−260 nm. The near-UV spectra were measured using a 1.0-cm light-path cell over the wavelength range of 260−340 nm. The CD data are expressed as mean residue ellipticity (degree cm^2/dmol).

Fluorescence Measurement. The intrinsic fluorescence emission spectra of the WPI samples were determined using a fluorescence spectrophotometer (model F2000, Hitachi Ltd., Tokyo) using an excitation wavelength of 276 nm (10-nm bandwidth). WPI samples were diluted to a final concentration of 0.03 wt % with 50 mM sodium phosphate buffer (pH 6.8) prior to analysis.

Surface Hydrophobicity. The surface hydrophobicity of WPI samples was measured by the method of Kato and Nakai [29] using a fluorescence probe, 1-anilino-8-naphthalenesulfonate magnesium salt (ANS). The relative fluorescent intensity of each sample solution was measured using the fluorescence spectrophotometer mentioned above with excitation (390 nm) and emission (480 nm). WPI samples were diluted to a final concentration of 0.5 wt % with the buffer prior to analysis.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE was carried out following the method described by Laemmli [30] with 5%−20% polyacrylamide gradient gels. A diluted WPI solution (0.2 wt %) was mixed with a sample buffer solution (2×) for SDS-PAGE with or without 10% β-mercaptoethanol. All samples were heated for 5 min in boiling water before electrophoresis. Ten microliters of each sample were applied to each gel with a protein molecular weight marker (14.3−97.2 kDa) (Takara Bio, Shiga, Japan). The protein bands were fixed and stained using a solution of Coomassie Brilliant Blue R-250.

Size Exclusion Chromatography. The 1.5 wt % WPI solution with or without PG treatment was eluted by size exclusion chromatography with an HPLC system (Waters, Milford, MA). The separations were performed on a TSK-GEL 4000 SWXL column (7.8 × 300 mm) with an SWXL guard column (Tosoh, Tokyo) at room temperature. Before the analysis, the samples were filtrated through a 0.45-μm filter (GL Science Co., Tokyo), and 10 μL of sample was injected on the column. The samples were eluted with 50 mM sodium phosphate buffer (pH 6.8) at a 0.6 mL/min flow rate. The components were detected at 280 nm.

Gel Preparation. WPI solutions were prepared by dissolving in deionized distilled water to obtain a final concentration of 12 wt % and stirring with a magnetic stirrer at 55 °C in a stainless beaker. The portion of the WPI solution was incubated with PG 10 U/g at 55 °C for 1 h and cooled to room temperature. A control sample was also prepared under the same conditions but without the addition of enzyme. Next, distilled water and NaCl were added to the WPI solutions and the desired concentrations of NaCl (0−400 mM) were adjusted. The portions of WPI solutions were poured into plastic cups and covered with aluminum foil to prevent evaporation. Subsequent heat treatment was carried out using a convection steam oven (SSC-04MSCNU, Maruzen, Japan) at 85 °C for 30 min. After heating, the samples were rapidly cooled in an ice bath and stored in a refrigerator overnight. Heat-induced gels of WPI were placed at room temperature 2 h before texture analysis. Three gel samples from each treatment were prepared.

Gel Properties. Mechanical properties of gels were determined by a TA XT-2 Texture Analyzer (Stable Microsystems, Godalming, UK). The gels were penetrated with a cylinder probe of 10-mm diameter to 20% of their original height. A force-time curve was obtained at a crosshead speed of 1.0 mm/s. Hardness at the fracture was determined by a texture analysis of the gels. The syneresis was determined by compressing the samples taken away from a plastic cup using a parallel plate. Samples were placed on a filter paper (Whatman No. 1) and compressed for 30 s to 30% strain. The difference in the weight of the filter paper before and after compression was used to calculate the percentage of syneresis.

RESULTS AND DISCUSSION

Deamidation of WPI by PG. The effect of the PG treatment of WPI on the deamidation degree was investigated. Table 1 shows the released ammonia and the deamidation degrees when WPI solutions were incubated with various amounts of PG. The released ammonia during the PG reaction at 55 °C for 1 h increased with the enzyme dosage and reached 4.5 mM at the dosage of 100 U/g of material. As the total amide content in 1.5 wt % WPI was 10.4 mM, the calculated deamidation degrees were approximately 13, 20, 30, 40, and 43%, respectively. The deamidation degree of the WPI samples increased in proportion to the enzyme dose up to 10 U/g. At more than 10 U/g, the deamidation degree increased gradually.

Table 1. Effect of PG on Released Ammonia[a] and the Deamidation Degree of WPI

abbreviated sample name	PG added (u/g)	released NH_3 (mM) average	released NH_3 (mM) SD	deamidation degree (%)
Native WPI	0	0.05	0.071	0.0
dWPI 13	2	1.42	0.026	12.9
dWPI 20	4	2.29	0.053	20.7
dWPI 30	10	3.16	0.138	30.4
dWPI 40	50	4.18	0.008	39.2
dWPI 43	100	4.54	0.053	42.5

[a]Average of triplicate measurements of standard deviation (SD). 1.5 wt % WPI solutions were incubated with different PG dosages at 55 °C for 1 h. A control sample was incubated without PG under the same conditions. The deamidation degree represents the ratio of the amount of released ammonia to the total released ammonia after amide hydrolysis with 2N HCl at 100 °C for 4 h.

Structural Analysis of WPI by using CD, Fluorescence Spectroscopy and ANS Binding Capacity. The CD spectrum in the near UV region (250−340 nm) reflects the environments of the aromatic amino acid side chains and thus gives information about the tertiary structure of the protein.[31] Figure 1 shows the near-UV CD spectra of native WPI, heat-treated WPI, and deamidated WPI samples (dWPI 13, 20, and 40). The main feature of the near-UV CD spectrum of the native WPI was the strong negative peak at 276 nm attributed to Tyr residues, confirming another recent finding.[17]

As Agyare and Damodaran [17] described, the peaks and shoulders between 284−305 nm and 255−270 nm suggested a fine structure around Trp and Phe residues, respectively.[17,32] The near-UV CD spectrum of heat-treated WPI exhibits an almost complete loss of tertiary structure. This result agrees with that of a previous report.[33] As for the deamidated WPI

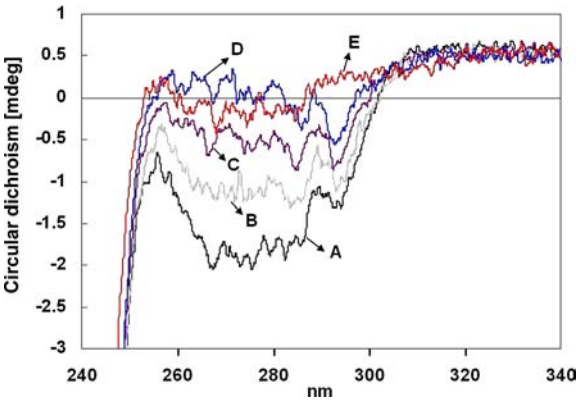

Figure 1. Near-UV circular dichroism spectra of WPI treated with PG. WPI samples were dissolved in 50 mM sodium phosphate (pH 6.8) and incubated with various amounts of PG at 55 °C for 1 h. (A) Native WPI, (B) dWPI 13, (C) dWPI 20, (D) dWPI 40, (E) heated WPI. Abbreviated sample names of PG-treated WPI are indicated in Table 1.

samples, there was a diminution in the peak intensity depending on the deamidation degree. The ellipticity at 276 nm increased with the increase in the deamidation degree, and it scarcely changed when the deamidation degree was more than 30%. These data suggest that Gln deamidation of WPI affects the environment of the aromatic amino acids side chain induced by a loss of tertiary structure. When deamidated WPI samples were heated at 85 °C for 30 min, the near-UV CD spectra were similar to those of the heat-treated WPI regardless of the deamidation degree (data not shown).

Although both heating and deamidation caused the tertiary structure denaturation, some differences were observed in the CD spectra between heat-treated WPI and the deamidated WPI sample, dWPI 40. Typical near-UV CD spectra of β-Lg show the bands at 294 and 285 nm ascribed to Trp vibrational fine structure and the bands at 277 and 265 nm to Tyr.[34] In the present study using WPI, the peaks corresponding to signals from Tyr and Trp side chains of β-Lg were observed, especially 294 nm due to Trp and 276 nm due to Tyr. In the heat-treated WPI, there was a complete loss of Trp band at 294 nm; dWPI 40 seems to have the residual tertiary structure in β-Lg. In contrast, the broad band around 276 nm in the heat-treated WPI probably arises from residual tertiary structure around the Tyr side chain in β-Lg.[34] Compared to the heat-treated WPI, the change in the corresponding signal in dWPI 40 was significant, suggesting that deamidation has more influence on the Tyr environment in β-Lg.

The intrinsic Trp fluorescence spectra of WPI samples were investigated to monitor structural changes of whey proteins treated with PG. The effect of heat treatment after the deamidation reaction was also examined. Figure 2 shows the intrinsic emission fluorescence spectra of native WPI and deamidated WPI samples. The maximum emission wavelength (λmax) of the fluorescence emission of native WPI was at 333 nm with excitation at 276 nm. Depending on the deamidation degree, a red shift was observed; for example, the dWPI 40 had a 6-nm red shift of λmax from 333 to 339 nm. The fluorescence intensity increased with the deamidation degree, and a 1.2-fold intensity increase was observed in the dWPI 40 sample compared to the native sample.

These results indicate that deamidation caused changes in the polarity of the Trp residues microenvironment of whey

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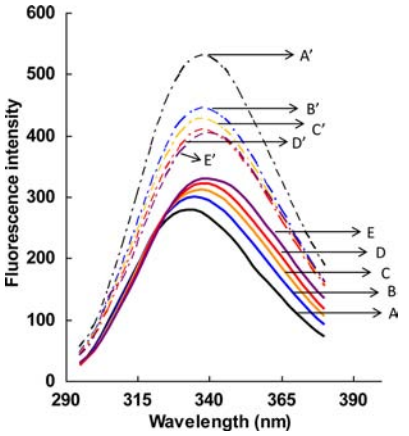

Figure 2. Fluorescence spectra of PG- and heat-treated WPI. The WPI samples were dissolved in 50 mM sodium phosphate buffer (pH 6.8) and incubated with various amounts of PG at 55 °C for 1 h (solid lines). After PG treatment, samples were heated at 85 °C for 30 min and cooled at room temperature (dotted lines). (A) Native WPI, (B) dWPI 13, (C) dWPI 20, (D) dWPI 30, (E) dWPI 43, (A′) heated WPI, (B′) heated dWPI 13, (C′) heated dWPI 20, (D′) heated dWPI 30, (E′) heated dWPI 43. Abbreviated sample names of PG-treated WPI are indicated in Table 1.

proteins from a less-polar to a more-polar environment by the electrical repulsion of the negative charge derived from carboxyl groups. This is in agreement with the previous result concerning the near-UV CD spectrum. When the native WPI sample was heated at 85 °C for 30 min, there was a significant (1.9-fold) increase in fluorescence emission intensity with a 4-nm red shift from 333 to 337 nm. A further shift of λmax after heat treatment was scarcely observed in the PG-treated WPI samples. The λmax values of the dWPI 30, 40, and 43 samples were 337 nm even after heat treatment. The intrinsic Trp fluorescence intensity of the PG-treated WPI did not increase as much as that of the heat-treated WPI. The PG treatment of WPI samples suppressed the major increase in the intrinsic Trp fluorescence intensity induced by heat treatment. This suppression was dependent on the deamidation degree, thereby reducing the difference of the fluorescence intensity between before and after heating.

In previous studies, it was found that heat treatment of β-Lg causes a red shift on the fluorescent emission spectrum in addition to the increase in the fluorescence intensity at λ_{max}, indicating the exposition of the Trp to aqueous solvent.[35,36] Also, α-La has four tryptophans, even though it is present at lower concentrations than β-Lg. It was reported that the emission spectrum of α-La in molten globule state also exhibits a red shift and the increase in the fluorescence intensity.[37] The modification of whey protein ingredients (WPI or WPC) by other treatments such as pulsed electric field and high hydrostatic pressure treatments also increased the intrinsic Trp fluorescence intensity of WPI and led to red shifts in the emission wavelength.[38,39] In the present study, we confirmed that PG treatment induced a gradual increase in the fluorescence intensity with the deamidation degree, despite a greater red shift than that produced by heat treatment. This suggests that deamidation causes the mild denaturation differently from heat denaturation, which results in the partial disruption of the tertiary structures of proteins, mainly β-Lg and α-La in WPI, perhaps molten globular forms. Moreover, PG treatment reduced the increase in the fluorescence intensity caused by heat treatment. This result implies that PG-treated whey proteins tend to be more resistant to heat denaturation.

The conformational modifications that lead to the denaturation of proteins include the exposure of hydrophobic sites previously buried inside the native structure of protein molecules. Therefore, measurements of the surface hydrophobicity may be useful in predicting the extent of whey protein denaturation.[35] The fluorescence dye ANS has been extensively used as a probe of hydrophobic binding sites.[29] In the present study, we evaluated the surface hydrophobicity of WPI treated with PG using ANS, and we analyzed the effect of the heat treatment of deamidated WPI samples on the surface hydrophobicity. Figure 3 shows the relative fluorescence

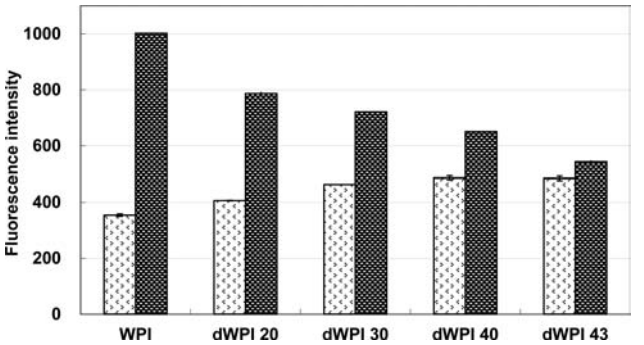

Figure 3. Surface hydrophobicity measured by ANS binding of native WPI and deamidated WPI samples before (open bar) and after (closed bar) heat treatment at 85 °C for 30 min. Each point represents the average of duplicate measurements with standard deviation.

intensity of native WPI and deamidated WPI samples (deamidation degree 20 to 43%). There was a 2.8-fold increase in the ANS extrinsic fluorescence in the native sample after heat treatment at 85 °C for 30 min. All proteins in WPI were presumably completely unfolded or denatured by this treatment. The fluorescence also increased with the increase in the deamidation degree, and a 1.4-fold increase was observed in the dWPI 43 sample compared to the native sample. The noteworthy feature in Figure 3 was that the relative fluorescence intensity of the PG-treated WPI samples after heat treatment was reduced in accord with the deamidation degree and that the minimum value was obtained in the dWPI 43 sample. The heat treatment of dWPI 43 resulted in only a 1.1-fold increase in the relative fluorescence intensity compared to the unheated sample. These results agree with previous findings concerning intrinsic fluorescence. Heat denaturation of proteins causes a marked increase of surface hydrophobicity due to the exposure of hydrophobic groups that are folded inside the intact native protein molecule.

Molecular Size Analysis of WPI by Size Exclusion Chromatography and SDS-PAGE. The major whey proteins (β-Lg and α-La) and BSA exist as monomers or dimers at neutral pH.[40] Figure 4a shows the size exclusion chromatography profile of the native WPI solution in 50 mM sodium phosphate buffer (pH 6.8). Two major peaks were observed, corresponding to the mixtures of β-Lg and α-La. After heating at 85 °C for 30 min, the peak areas of β-Lg and α-La decreased while there were increased peaks corresponding to high-molecular-weight proteins (Figure 4c). These trends agree with previous studies[21,41,42] and indicate that native whey proteins are converted to aggregates by heat denaturation. Figure 4b shows the elution profile of the deamidated WPI sample (dWPI

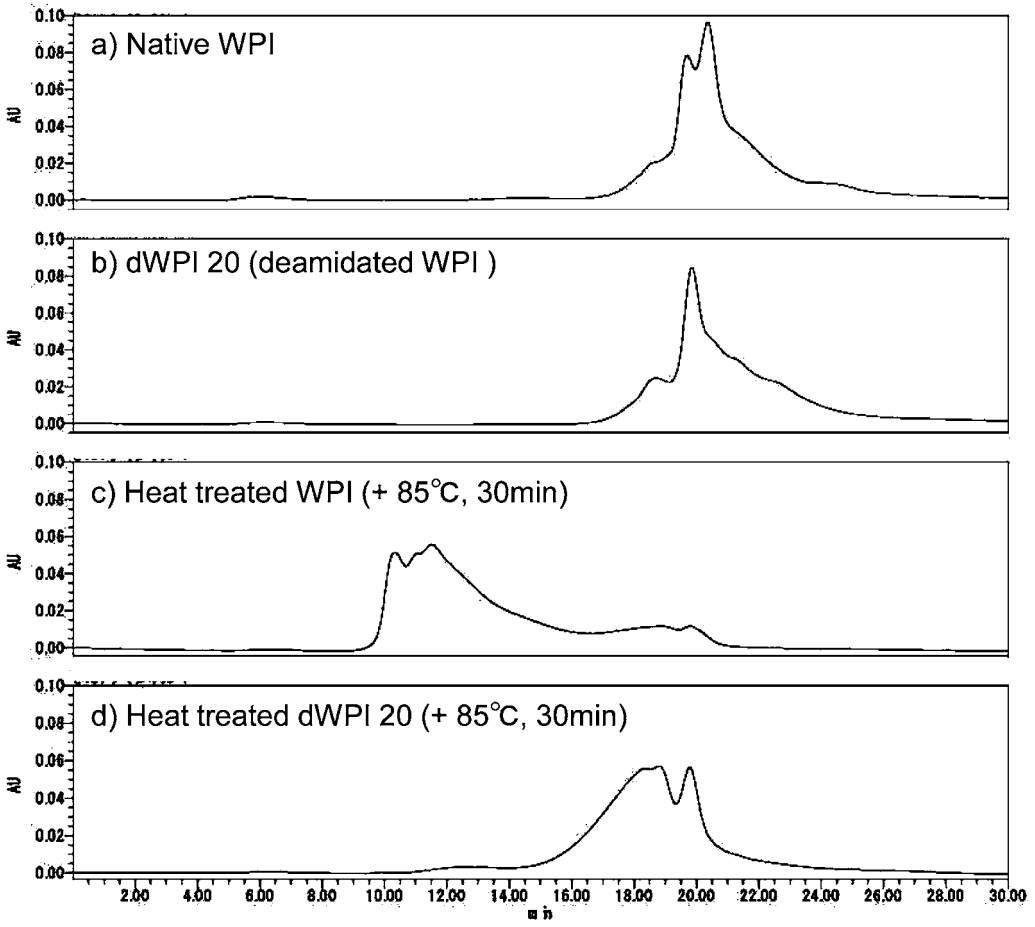

Figure 4. Size exclusion chromatography profiles of 1.5 wt % WPI solutions without heat treatment (a) and after heat treatment at at 85 °C for 30 min (c). Size exclusion chromatography profiles of dWPI 30 (1.5 wt % WPI solution treated with PG 10 U/g) without heat treatment (b) and after heat treatment at at 85 °C for 30 min (d). All samples were dissolved in 50 mM sodium phosphate buffer, pH 6.8.

30). There were no major changes in the elution profile compared to native WPI. However, it seems that PG treatment caused the two main peaks to merge. As protein deamidation accompanies a molecular weight change of only 1 Da, this minor change was caused by the conformational change of whey proteins rather than the molecular weight change. It is probable that a partial unfolding of whey proteins increases the apparent molecular size, resulting in a slightly earlier shift of the elution time. Figure 4d shows the elution profile of dWPI 30 heated at 85 °C for 30 min. The formation of heat-induced aggregates decreased very markedly. These results suggest that protein deamidation prevents the formation of larger aggregates caused by heat treatment. It has been reported that heat treatment causes the exposure of nonpolar amino acids and the increase in the hydrophobic interactions, resulting in the formation of larger aggregates. Especially in the presence of salts, this phenomenon promotes due to the extinguishment of the electrostatic repulsion between the charged molecules.[27] Our results show that even in the presence of 50 mM phosphate buffer (pH 6.8), PG-treated deamidated whey proteins do not undergo extensive aggregation because of the relatively strong electrostatic repulsion after heating.

This finding was confirmed by the SDS-PAGE analysis. To determine the significance of intermolecular disulfide bonds, SDS-PAGE of the WPI samples was performed under reducing (β-mercaptoethanol) and nonreducing conditions, as shown in Figure 5. The profile of the native WPI under reducing

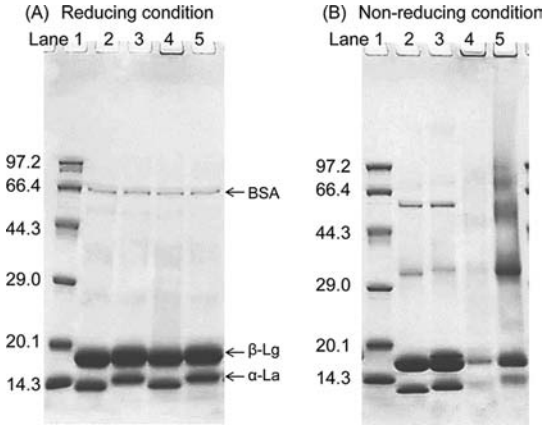

Figure 5. SDS-PAGE electrophoretic profiles of native WPI and dWPI 30 (1.5 wt % WPI solution treated with PG 10 U/g) with or without heat treatment (85 °C, 30 min) under (a) reducing and (b) nonreducing conditions. Lane 1: Low molecular weight marker. Lane 2: native WPI. Lane 3: dWPI 30. Lane 4: heated WPI. Lane 5: heated dWPI 30.

conditions showed three main bands attributed to α-La, β-Lg, and BSA monomers, respectively (Figure 5A). The bands of β-Lg and α-La were almost identical irrespective of heat treatment when separated under reducing conditions. The band of α-La was shifted upward slightly in the PG-treated

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sample regardless of the heat treatment. This small change in the migration rate is thought to be due to the increase in the negative charge of α-La by deamidation, affecting the SDS-binding to the protein. A similar result was reported for whey proteins in 10 wt % skim milk treated with PG.[10] Under nonreducing conditions, very weak bands of β-Lg and α-La were observed in the heat-treated WPI samples, and bands of high-molecular-weight polymers were visible on top of the gel (Figure 5B, lane 4). This indicates that the majority of the monomers of α-La and β-Lg were involved in the formation of aggregates induced by heat treatment, which is in agreement with previous results.[21,41,42] Following the initiation of aggregation by hydrophobic forces, disulfide bonding is known to play an important role in the strengthening of the aggregates.[27] Our result also suggests that disulfide bonds are largely responsible for the formation of the high-molecular-weight polymers by heating, because these polymers disappeared under reducing conditions. In contrast, the electrophoretic profile of the nonreduced PG-treated sample shows that the bands of β-Lg and α-La remained even after heating at 85 °C for 30 min. Moreover, there seems to be an increase in the intermediate aggregates, including protein bands corresponding to molecular weights of approximately 33−35, 50−60 and 70−80 kDa. These proteins were not observed in the WPI sample that was only heated at 85 °C for 30 min, implying that the intermediate aggregates were polymerized via sulfhydryl-disulfide interchange reactions to higher-molecular-weight polymers that could not enter the stacking gel.[42]

Conformational Change of Deamidated-WPI Dispersion. The understanding of WPI aggregation behavior upon heat treatment is of great significance to the food industry because of the widespread use of WPI as functional food ingredients. As whey proteins are heated, they unfold and form soluble aggregates.[43] Our results also revealed that when a WPI solution (pH 6.8) with a relatively low ionic strength was heated at 85 °C for 30 min, protein aggregates were formed. This means that the soluble aggregation occurred via covalent intermolecular disulfide bonds and noncovalent interactions, in particular hydrophobic interactions. TOC graphic illustrates the image of the mechanism of enzymatic deamidation of WPI and the effect of heat treatment. One of the important findings in our study is that deamidation by PG leads to the partial denaturation of whey proteins depending on the deamidation degree. However, PG-induced denaturation appears to be much milder than heat treatment.

Gu et al. showed that PG-induced deamidation causes the change in the tertiary structure of α-La, whereas the secondary structure was not affected by deamidation, suggesting that the highly deamidated α-La takes on the molten globule-like form.[9] Our results also showed that PG treatment of WPI causes the change in the near-UV CD ellipticity with the deamidation degree, although far-UV circular dichroism was barely affected by PG (data not shown). This suggests that a mixture containing globular proteins, mainly β-Lg and α-La, adopt a molten globule-like conformation after PG treatment. Another important finding is that PG treatment before heating may be effective to prevent the formation of heat-induced protein aggregates. The present SDS-PAGE and size-exclusion chromatography results demonstrated that heat-induced polymerization of proteins was clearly restrained in the deamidated WPI sample. It is certain that deamidated WPI tends not to form a protein−protein interaction, including the disulfide bond interchange reaction, due to the increase in electrostatic repulsion between negatively charged groups.

Heat-induced Gel Properties. Gelation is an important functional property of whey proteins. Their ability to form heat induced gels and provide appropriate texture is determined by its molecular structure and interactions with other components such as salts.[22] We investigated the effect of PG on the properties of heat-induced gels with different levels of NaCl. The gel firmness of heat-induced WPI gels treated with/without PG (Figure 6a) and the syneresis of the gels (Figure 6b) are plotted as a function of NaCl concentration of 0 to 400 mM. The gel firmness of heat-treated WPI (control) increased with the NaCl concentration up to 100 mM to a maximum value, followed by a sharp decrease in the gel firmness. This result agrees with previous data.[44] Such variation in the firmness of heat-induced WPI gels has been attributed to a shift in gel structure from fine-stranded to particulate.[45] This means that the addition of NaCl leads to a decrease in electrostatic repulsions allowing protein aggregation prior to gel formation, resulting in particulate gels. It is known that this type of gel has a poor water-holding capacity because the size of the pores within the gel network increases.[46] As shown in Figure 6b, we observed that the syneresis of the WPI gels of heat-treated WPI (control) increased significantly at higher NaCl concentrations, although it was inhibited at low levels of added NaCl (<100 mM).

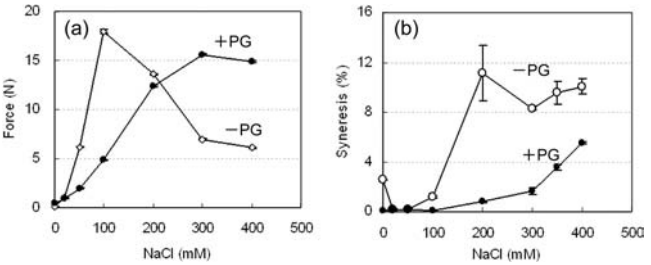

Figure 6. (a) Strength of heat-induced WPI gels prepared from 12 wt % WPI solution treated with or without PG 10 U/g at 55 °C for 1 h. (b) Syneresis of WPI gels treated with or without PG. Open symbols represent WPI gels without PG, and closed symbols represent WPI gels treated with PG. Each point represents the average of triplicate samples with standard deviation.

As for the heat-induced WPI gel pretreated with PG, increasing the concentration of NaCl caused a gradual increase in the gel firmness to a maximum with 300 mM NaCl. PG treatment improved the water-holding capacity of the heat-induced gels of WPI. Although the syneresis increased gradually with the increase in NaCl concentration, the value was much lower than that of the control at all NaCl concentrations. These results suggest that heat-induced gels of PG-treated WPI are more resistant to syneresis. Even when concentrations of a counterion such as NaCl increase, the gel network of deamidated WPI maintains a fine-stranded structure because of the increase in glutamic acid residues, which causes a simultaneous increase in electrostatic repulsions and hydrophilic regions.

As described above, we found that the heat-induced gels of WPI were significantly modified by PG-induced deamidation. The gels pretreated with PG tended to have a soft texture with a high water-binding capacity even in the presence of high salt concentration. Regarding whey protein gels, there have been many reports of cold-setting whey protein gels.[27,42,47] This

property was ascribed to the formation of soluble aggregates after the preheating of the protein solution, followed by the addition of salts.[27] Our results imply that PG also affects the formation of cold-setting whey protein gels. Further research is needed to evaluate the effect of enzymatic deamidation on other functionalities of whey protein and related food products, in order to develop their industrial applications.

We have shown that the PG-treatment caused a conformational change in proteins of dilute WPI solutions and that the change seemed different from heat-treatment. Also, we found that heat-induced WPI gels were modified by PG pretreatment. The evaluation of gel properties was conducted using WPI solutions at a much higher concentration. This was unavoidable experimentally, however, the observed data about the WPI gels can be explained to some extent by the results of the structural analysis and would be helpful information for practical use.

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Notes

The authors declare no competing financial interest.

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