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Effect of Sodium Bisulfite on Properties of Soybean Glycinin

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The objective of this work is to understand the function of glycinin in soy protein adhesive formation. Glycinin protein was treated with sodium bisulfite, and physicochemical, morphological, and adhesion properties of the modified soy glycinin were characterized. More disulfide bonds that associated acidic and basic polypeptides of glycinin broke as the sodium bisulfite concentration increased. The reduction of disulfide bonds did not decrease the thermal stability of glycinin. Instead, the denaturation temperature of modified glycinin increased as sodium bisulfite increased. Sodium bisulfite-induced disulfide bond cleavage increased the surface hydrophobicity of modified glycinin. Hydrophobic force is the main driving force for glycinin aggregation, and the balance between hydrophobic and electrostatic forces makes glycinin form chainlike aggregates. The adhesive strength and water resistance of glycinin dropped significantly at lower levels of sodium bisulfite and then increased as the amount of sodium bisulfite increased up to 24 g/L. The adhesive performance decreased again with further addition of sodium bisulfite. The adhesive strength of glycinin was not improved by sodium bisulfite modification in the studied range.

KEYWORDS: Soy glycinin protein; protein modification; sodium bisulfite; hydrophobic interaction; physicochemical property; morphology; adhesive strength

INTRODUCTION

Synthetic petrochemical polymers have been used extensively as adhesives in packaging, construction, and furniture industries. However, concerns about environmental pollution have created an interest in and a need for biobased alternatives (*1*). Soy protein, commonly used as a functional or nutritional component in food products, has the potential for use in biobased adhesives. Physical and chemical modifications such as heat, alkaline, urea, guanidine hydrochloride, sodium dodecyl sulfate, ethanol, and enzymes have been shown to enhance adhesive properties of soy protein (*1*−*5*).

Soy proteins are classified by sedimentation coefficients into 2S, 7S, 11S, and 15S fractions. Glycinin (11S) and conglycinin (7S) are the two major components (*6*). Glycinin is an oligomer with a molecular mass of about 350 kDa and consists of six subunits, each composed of an acidic and a basic polypeptide linked by a disulfide bond. Because of glycinin's high cysteine content, it has about 18−20 intra- and intermolecular disulfide bonds (*7*). Disulfide bonds contribute to the ordered structure and stability of proteins. Cleavage of disulfide bonds by a reducing agent can unfold a protein, causing loss of protein structure and changing physicochemical and functional properties (*7*). Cleavage of disulfide bonds increases glycinin's surface hydrophobicity, viscosity, and susceptibility to tryptic hydrolysis (*8*). Kim et al. found significant improvement in surface-active properties of reduced glycinin (*9*). Completely cleaved glycinin has a higher solubility in the neutral-to-alkaline pH range than native glycinin (*7*).

Soy protein modified with sodium bisulfite behaves like latex adhesives and has an adhesive strength comparable to formaldehyde-based adhesives (*10*). Previous studies have revealed that glycinin is a key ingredient for adhesion strength, especially water resistance. Soy protein-based adhesives with high glycinin contents have shown higher adhesion strength and water resistance than those with low glycinin contents (*11*). Because glycinin's structure and properties change in the presence of reducing agents, it might have unique characteristics that contribute to the adhesive. The objective of this study was to investigate the adhesion function of soy glycinin modified with sodium bisulfite and to characterize the solubility, surface hydrophobicity, and thermal properties of the modified soy glycinin.

MATERIALS AND METHODS

Materials. Defatted soy flour obtained from Cargill (Cedar Rapids, IA) was used for the isolation of soy glycinin. The soy flour contained 52.4% protein with a protein dispersion index of 90. Sodium bisulfite ($NaHSO_3$) was obtained from Fisher Scientific (Fair Lawn, NJ). Cherry wood veneers with dimensions of 50 mm × 127 mm × 4.8 mm (width × length × thickness) were provided by Veneer One (Oceanside, NY).

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Isolation of Glycinin. Glycinin was separated from soy flour using the method described by Thanh and Shibasaki (*12*). Flour was dissolved in a 30 mM Tris buffer at pH 8.0 containing 10 mM 2-mercaptoethanol. The slurry was centrifuged at 10000*g* for 20 min at 4 °C to remove fiber. The supernatant was adjusted to pH 6.4 and then centrifuged again at 10000*g* for 20 min at 4 °C. The precipitate was collected as glycinin. Isolated glycinin was washed twice with distilled water, redissolved in distilled water with the pH adjusted to 7.8, and lyophilized. Glycinin had about 97% purity, as evaluated with sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE).

$NaHSO_3$ Treatment. Glycinin was dispersed in deionized water at 6.25% solid content. The solution was adjusted to pH 9.5 with 1 N NaOH and stirred at room temperature for 1 h. $NaHSO_3$ was added to the dispersion at 0, 48, 96, 192, 384, and 576 mg/g glycinin, equivalent to 0, 3, 6, 12, 24, and 36 g/L (gram $NaHSO_3$ per liter of solution). The resulting solution was maintained at pH 9.5 by adding 1 N NaOH, and the reaction was implemented with mild stirring at room temperature for 2 h.

SDS-PAGE. SDS-PAGE was performed on a 4% stacking gel and a 12% separating gel with a discontinuous buffer system according to the method described by Laemmli (*13*). Protein samples were mixed with a sample buffer containing 2% SDS, 25% glycerol, and 0.01% bromphenol blue. To avoid disulfide bond breakage not induced by $NaHSO_3$, SDS-PAGE was performed in the absence of 2-mercaptoethanol for $NaHSO_3$-modifed glycinin samples. Unmodified glycinin was used as a control. To estimate the purity of the glycinin, 2-mercaptoethanol was added to the sample buffer to perform the reducing SDS-PAGE. Molecular weight standards were run with the samples. The gel was stained in 0.25% Coomassie brilliant blue R-250 and destained in solution containing 10% acetic acid and 40% methanol. Densitometry was obtained by analyzing the gel image using the Kodak 1D Image Analysis software, version 4.6 (Kodak, Rochester, NY).

Determination of Solubility. The pH-dependent solubility profile was determined by measuring the absorbance of the supernatant of the centrifuged glycinin sample solutions with various pH values at 280 nm based on aromatic groups of amino acids in glycinin, as described by German et al. (*14*). Modified glycinin samples were diluted to 0.1% with deionized water. Diluted glycinin solutions were adjusted to desired pH values ranging from 3 to 10, stirred for 1 h, and centrifuged at 10000*g* for 20 min. The absorbance of the supernatant was measured at 280 nm by a spectrophotometer (Biomate 3, Thermo Electron Corp., Madison, WI). All measurements were done in duplicate, and means were reported.

Differential Scanning Calorimetry (DSC). Thermal properties of modified glycinin samples were assessed with a DSC (DSC7, Perkin-Elmer, Norwalk, CT) calibrated with indium and zinc. Protein solutions (about 50 μL) were hermetically sealed in the large-volume stainless steel DSC pan. Each sample was held at 20 °C for 1 min and then scanned from 20 to 150 °C at a heating rate of 10 °C/min. Peak temperatures and denaturation enthalpies were calculated from thermograms. Two replicates were made for each sample, and average values were reported.

Surface Hydrophobicity. The surface hydrophobicity of modified glycinin samples was evaluated by measuring SDS-binding capacity (*3, 15*). The modified glycinin samples were diluted to 0.1% with deionized water. Then, 0.07 mM SDS was added to the glycinin dispersion, and the solution was stirred for 30 min. The SDS−glycinin solution was dialyzed against a 0.02 M bicarbonate buffer (pH 9.5) at 4 °C for 48 h. One milliliter of the dialyzed glycinin solution was mixed with 10 mL of chloroform. The mixture was blended with 2.5 mL of a 0.0024% methylene blue solution and centrifuged at 800*g* for 10 min. The absorbance of the SDS-methylene blue mixture in the lower layer was measured at 655 nm. A standard calibration curve was obtained using the above-described method with different defined amounts of SDS without glycinin to determine the amount of unbound SDS. The SDS-binding capacity was calculated based on the amount of unbound SDS and initial SDS quantity (0.07 mM) and represented as micrograms of SDS bound to 1 mg of glycinin as a measure of glycinin hydrophobicity.

Morphology Properties. A Philips CM 100 (FEI Co., Hillsboro, OR) transmission electron microscope (TEM) was used to investigate the microstructure of glycinin samples. To determine the nature of the interaction between modified glycinin molecules, modified glycinin samples were further treated in two ways: reacted with 0.1% SDS to interrupt hydrophobic interaction and dialysis against 0.01 M bicarbonate buffer (pH 9.5) to remove excess salt. All glycinin samples were diluted to 1% with deionized water for imaging.

Samples were absorbed onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Science, Fort Washington, PA) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, VT). The microstructure of the glycinin was observed with operation conditions at an accelerating voltage of 100 kV.

Wood Specimen Preparation. Cherry wood veneers were preconditioned in a chamber (Eletro-Tech System, Inc., Glenside, PA) for at least 7 days at 23 °C and 50% relative humidity. The 350 μL glycinin solution was applied with a brush to a marked area of 127 mm × 20 mm (length × width). Two brushed wood pieces were left at room conditions for 15 min, then assembled, and pressed using a hot press (model 3890 Auto M; Carver, Inc., Wabash, IN) at 4.9 MPa and 170 °C for 10 min. Pressed specimens were cooled and stored in the same chamber at 23 °C and 50% relative humidity for 3 days. The glued wood assemblies were cut into five pieces with dimensions of 80 mm × 20 mm (glued area of 20 mm × 20 mm), and cut wood specimens were conditioned for another 4 days before measurement.

Shear Strength. Wood specimens were tested using an Instron (model 4465, Canton, MA) according to ASTM Standard Method D2339-98 (*16*). The crosshead speed was 1.6 mm/min, and the stress at maximum load was recorded as shear strength. Reported results are the averages of five replicates.

Water Resistance. Water resistance was measured according to ASTM Standard Methods D1183-96 and D1151-00 (*17, 18*). Wood specimens were soaked in tap water at 23 °C for 48 h and tested for wet strength immediately after soaking. The soaked strength was assessed after soaked specimens were dried and conditioned at 23 °C and 50% humidity for another 7 days. The shear strength was tested as described previously.

RESULTS AND DISCUSSION

SDS-PAGE Analysis. Unmodified glycinin gave main bands corresponding to acidic and basic subunits on SDS-PAGE (**Figure 1**, lane A). Glycinin with reducing agent $NaHSO_3$ at 0 g/L exhibited a major band at about 56 kDa corresponding to the glycinin subunit composed of an acidic (A) and a basic (B) polypeptide linked by a disulfide bond. The band that appeared at 30 kDa should be A_5B_3 complex, which is the disulfide bond-linked part of glycinin subunit G_4 ($A_5A_4B_3$) (*19, 20*). Another band that occurred at 32 kDa might be due to the noncovalently bonded acidic polypeptides (A_4) in the G_4 glycinin subunit. The intensity of the bands at 38 and 23 kDa increased as $NaHSO_3$ increased. Those two bands, corresponding to acidic and basic polypeptides, resulted from reduction of the disulfide bond-linked AB complex (**Figure 1**, lanes C−G). Some small bands around 100 kDa (**Figure 1**, lane B) faded with the addition of $NaHSO_3$ (**Figure 1**, lanes C−G). Those bands could be due to the formation of disulfide bond-linked polymers caused by freeze drying or thiol-disulfide exchange during modification (*20*). These results indicate that intermolecular disulfide bonds that link acidic and basic polypeptides of glycinin subunits were cleaved by $NaHSO_3$. Although the degree of cleavage increased as the $NaHSO_3$ concentration increased, complete reduction of disulfide bonds was not achieved. Even at the highest $NaHSO_3$ concentration (36 g/L), a considerable amount of AB complexes was still presented.

Effect of $NaHSO_3$ Concentration on Surface Hydrophobicity. The surface hydrophobicity of glycinin was evaluated using SDS-binding capacity, which is proportional to the protein's surface hydrophobicity (*15*). Glycinin treated with 3 g/L $NaHSO_3$ did not show an obvious increase in surface

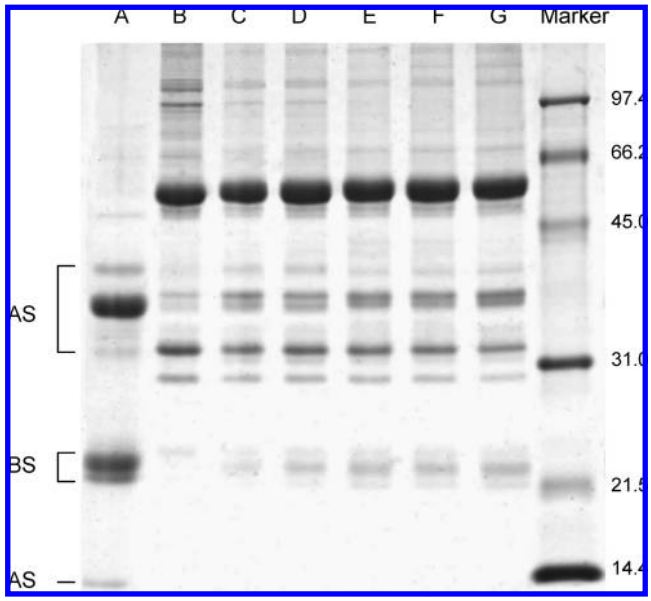

Figure 1. SDS-PAGE pattern of unmodified glycinin in the presence of 2-mercaptoethanol: acidic subunits (AS) and basic subunits (BS) (lane A). In the absence of 2-mercaptoethanol: unmodified glycinin (lane B), glycinin modified by 3 g/L $NaHSO_3$ (lane C), glycinin modified by 6 g/L $NaHSO_3$ (lane D), glycinin modified by 12 g/L $NaHSO_3$ (lane E), glycinin modified by 24 g/L $NaHSO_3$ (lane F), and glycinin modified by 36 g/L $NaHSO_3$ (lane G).

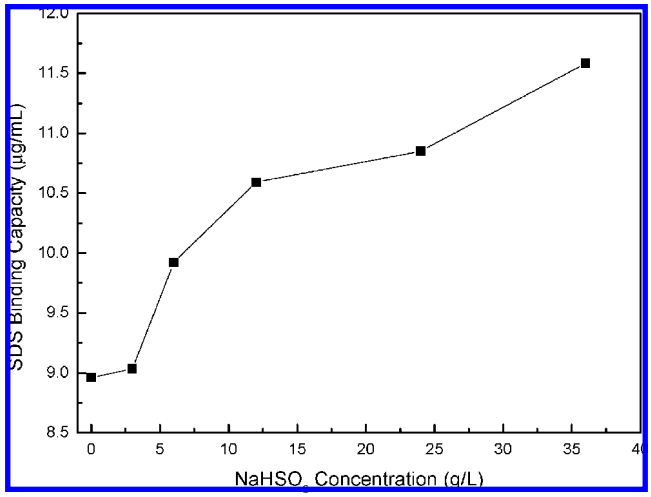

Figure 2. Effect of $NaHSO_3$ concentration on SDS-binding capacity of glycinin.

hydrophobicity. A sharp upsurge in hydrophobicity occurred when $NaHSO_3$ was beyond 3 g/L, but hydrophobicity leveled off at 12 g/L $NaHSO_3$ and increased slightly after that (**Figure 2**).

Cleavage of disulfide bonds induced by $NaHSO_3$ modification involves separation of acidic and basic polypeptides as well as structural conformation changes, leading to exposure of some nonpolar groups previously buried inside the protein interior. Hettiarachchy reported that soy protein had a higher surface hydrophobicity in alkali media (pH 8−10) than in a neutral environment, implying that hydrophobic groups might be exposed by alkali treatment (*3*). In this study, because glycinin during preparation was first dispersed in an alkali environment at pH 9.5, NaOH could break some internal hydrogen bonds of the glycinin molecules and elevate hydrophobicity of untreated glycinin. Although low $NaHSO_3$ (3 g/L) probably was not enough to bring a considerable quantity of hydrophobic groups

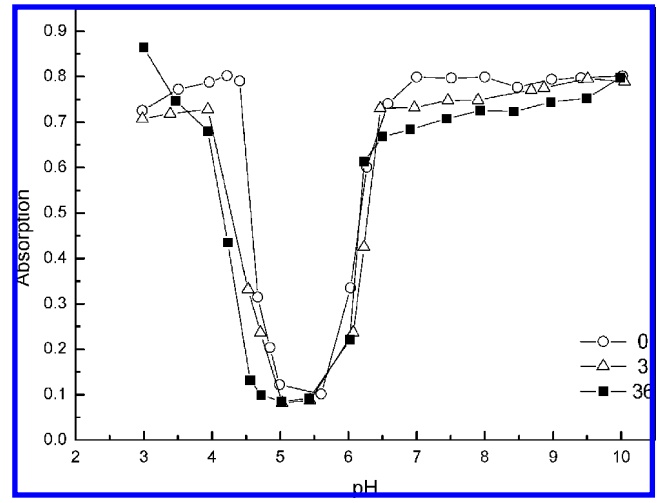

Figure 3. pH solubility profile of glycinin, glycinin modified with 3 g/L $NaHSO_3$, and glycinin modified with 36 g/L $NaHSO_3$.

to the surface, as the level of $NaHSO_3$ increased, more disulfide bonds were broken and more hydrophobic groups were brought out to the protein surface, increasing the surface hydrophobicity. As $NaHSO_3$ continued to increase, the surface hydrophobicity was limited by inadequate disulfide bond breakage, as shown by SDS-PAGE results.

Effect of $NaHSO_3$ Concentration on Solubility. The U-shaped solubility−pH distribution was observed for all glycinin modified with various levels of $NaHSO_3$ (**Figure 3**). The overall solubility of glycinin was not significantly affected by $NaHSO_3$, but minimum solubility gradually shifted to lower pH values as $NaHSO_3$ increased. For example, unmodified glycinin had an insoluble region between pH 4.5 and pH 6.0 with a minimum solubility at around pH 5.6, but the minimum solubility of glycinin treated at 3 g/L $NaHSO_3$ moved to around pH 5.0−5.5. At 36 g/L $NaHSO_3$, the pH range for the minimum solubility was pH 4.7−5.5. Also, the solubility in the alkaline region (pH > 6.5) decreased as $NaHSO_3$ increased.

A protein usually reveals minimum solubility at its isoelectric point. At the isoelectric point, electrostatic repulsions between protein molecules reach minimum because of the zero net charge on the surface of protein molecules. During disulfide bond cleavage by $NaHSO_3$, some sulfhydryls resulting from deoxidization are blocked as a sulfonate group ($RS\text{-}SO_3^-$) (*21*). Extra negative charges induced by $NaHSO_3$ increased the surface charge of glycinin and shifted the minimum solubility range to a lower pH. The protein solubility depends not only on electrostatic forces but also on hydrophobic interactions. The SDS-binding capacity results indicated that the surface hydrophobicity increased as $NaHSO_3$ concentration increased.

An increase in surface hydrophobicity facilitated hydrophobic interactions, stimulating protein−protein association. Basic polypeptides, which are highly hydrophobic and released via reduction of disulfide bonds, are believed to be involved in these protein−protein interactions (*8*). Reinforced hydrophobic interactions offset electrostatic repulsions, resulting in lower solubility for modified glycinin in the alkaline pH range.

Morphology of $NaHSO_3$ Modified Glycinin. Unmodified glycinin existed mainly in the form of globular aggregates of various sizes, some of which formed into irregular-shaped clumps (**Figure 4A**). In glycinin modified by 3 g/L $NaHSO_3$, the number of small globular aggregates increased, and clumps became larger than those in unmodified glycinin (**Figure 4B**). Increasing the concentration of $NaHSO_3$ to 6 g/L caused significant changes in the appearance of glycinin molecules.

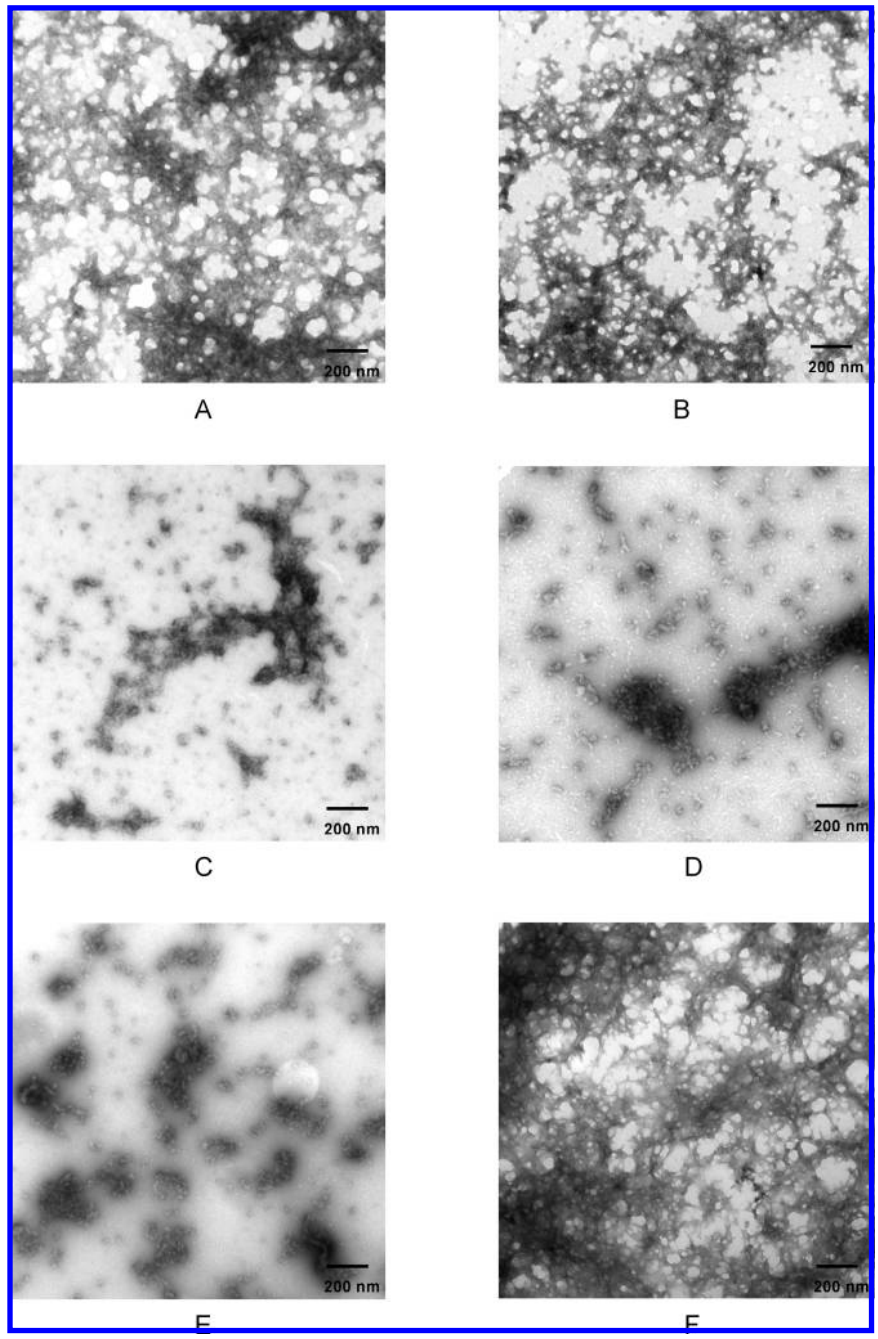

Figure 4. TEM images of soy glycinin: unmodified glycinin (**A**), glycinin modified by 3 g/L $NaHSO_3$ (**B**), glycinin modified by 6 g/L $NaHSO_3$ (**C**), glycinin modified by 36 g/L $NaHSO_3$ (**D**), glycinin modified by 6 g/L $NaHSO_3$ dialyzed against 0.01 M bicarbonate buffer (**E**), and glycinin modified by 6 g/L $NaHSO_3$ treated with 0.1% SDS (**F**).

Globular aggregates were broken into smaller clusters, which built chainlike structures (**Figure 4C**). At 36 g/L $NaHSO_3$, the number of short chains and rods increased as dimensions of large, chainlike aggregates decreased (**Figure 4D**). The formation of disulfide bonds through thiol−disulfide interchange has been shown to be involved in cross-linking of protein molecules to form aggregates (*22*). However, $NaHSO_3$ is not only a reducing agent but also a sulfhydryl-blocking reagent, which hinders thiol−disulfide exchange during protein interaction. As shown in the nonreducing SDS-PAGE, bands (around 100 kDa) that might be caused by disulfide cross-linking were small and vague. The intermolecular disulfide exchange played a limited role in glycinin aggregation after $NaHSO_3$ modification.

In addition to disulfide cross-linking, hydrophobic interactions and electrostatic forces are crucial variables for protein cluster formation (*23*). SDS, which can bind to exposed hydrophobic groups on the protein surface, is capable of disrupting hydrophobic bonds. After adding 0.1% SDS, glycinin modified by 6 g/L $NaHSO_3$ no longer had chainlike structures but appeared similar to unmodified glycinin (**Figure 4F**). This suggested that hydrophobic bonds between nonpolar groups of proteins were the main driving force for the formation of chainlike structured aggregates in the 6 g/L $NaHSO_3$-modified glycinin. After being dialyzed against 0.01 M bicarbonate buffer, the large, chainlike structure in glycinin modified by 6 g/L $NaHSO_3$ fragmented into relatively small chains and clumps (**Figure 4E**). After dialysis, the ionic strength of modified glycinin decreased, which could disrupt the large, chainlike aggregates into small pieces. Although the hydrophobic force facilitates protein aggregation, electrostatic repulsion between protein molecules inhibits ag-

Table 1. Denaturation Temperature (T_d) and Enthalpy of Denaturation (ΔH) of Glycinin Modified with Various $NaHSO_3$ Concentrations

$NaHSO_3$ (g/L)	T_d (°C)	ΔH (J/g)
0	70.0/81.6	7.8
3	82.6	14.5
6	83.4	14.7
12	88.8	15.5
24	92.2	18.3
36	97.3	16.4

gregate growth. These results indicate that the increase in ionic strength could shield electrostatic charges and favor protein aggregation (*24*).

Therefore, the balance between hydrophobic force and electrostatic force was the major factor determining shape and size of glycinin aggregates. When the balance was reached, glycinin molecules turned into large, chainlike aggregates, which also were observed by Sun et al. in soy protein (*23*). As in glycinin modified by 3 g/L $NaHSO_3$, the hydrophobic interaction was relatively weak. Although an increase in ionic strength could weaken electrostatic repulsion, glycinin molecules were not able to grow into large aggregates. In glycinin modified by 36 g/L $NaHSO_3$, surface negative charges induced by blocking sulfhydryls as sulfonate groups ($RS\text{-}SO_3^-$) could increase greatly due to high $NaHSO_3$ concentration, and the large increase in electrostatic repulsion could break the balance between hydrophobic and electrostatic interaction, resulting in smaller chainlike aggregates.

Effect of $NaHSO_3$ Concentration on Thermal Properties. The denaturation temperature (T_d) and enthalpy (ΔH) of glycinin were affected significantly by $NaHSO_3$ (**Table 1**). Unmodified glycinin showed two peaks during heat denaturation (**Figure 5**); usually, glycinin shows only one endothermic peak. Because the insignificant presence of β-conglycinin in glycinin is not able to cause a peak in the thermogram, the extra peak might have been caused by an alternative form of glycinin. Under low ionic strength and slightly alkaline pH values, glycinin (11S) partially dissociates to a half molecule with the size of 7S (*25*). The so-called $\bar{7}$S component exhibited an endothermic peak at 69.8 °C at low ionic strength ($\mu = 0.01$) and pH 7.6 (*26*). Thus, the additional peak at 70.0 °C was due to the occurrence of the $\bar{7}$S component. As the $NaHSO_3$ concentration increased from 3 to 36 g/L, the peak corresponding to $\bar{7}$S disappeared, and the 11S peak gradually shifted to a higher temperature.

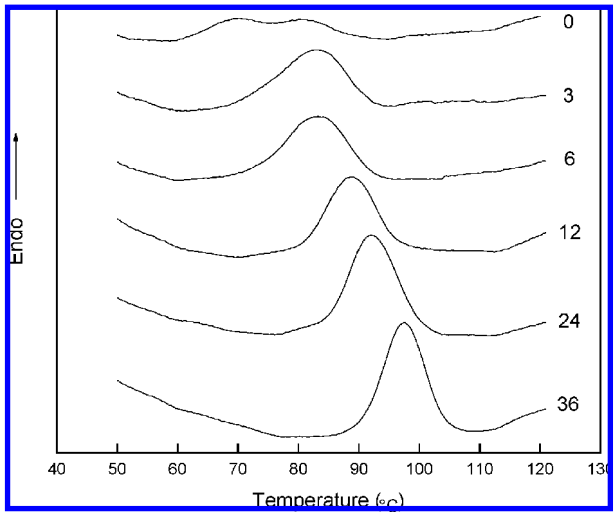

Figure 5. DSC theromogram of glycinin modified with $NaHSO_3$ concentrations of 0, 3, 6, 12, 24, and 36 g/L.

Table 2. Effect of $NaHSO_3$ Concentration on Adhesive Strength of Glycinin[a]

	$NaHSO_3$ concentration (g/L)					
shear strength (MPa)	0	3	6	12	24	36
dry strength	6.65 b	3.80 d	5.62 bc	4.74 cd	5.16 cd	5.08 cd
wet strength	1.67 b		0.73 cd	1.19 bc	1.15 c	0.52 d
soaked strength	4.71 b	2.30 d	3.84 bc	4.07 bc	4.00 bc	3.13 cd

[a] ANOVA and LSD tests were performed using SAS. Means with the same letters in the same row are not significantly different at $P = 0.05$.

The increase in thermal stability of 11S protein treated with a reducing agent (i.e., sodium sulfite) also was observed by Petruccelli and Anon (*27*). Disulfide bonds in proteins contribute to the ordered structure and stability of proteins. Although cleavage of disulfide bonds should destabilize glycinin and potentially lower the T_d, the T_d of glycinin increased as $NaHSO_3$ increased. This could be due to the screening effects of salts on the protein's electrostatic forces and to the greater strengthening of hydrophobic interactions (*28*). In addition, the ΔH of glycinin increased at $NaHSO_3$ concentrations up to 24 g/L. The ΔH of glycinin modified by 36 g/L $NaHSO_3$ dropped almost 2 J/g as compared with that modified by 24 g/L $NaHSO_3$ (**Table 1**); this might have been caused by a less-ordered structure that could not be stabilized by the effect of the salt.

Effect of $NaHSO_3$ on Adhesive Shear Strength. Glycinin without $NaHSO_3$ modification showed greater adhesive strength than modified glycinin (**Table 2**). The adhesive strength decreased extensively with the addition of 3 g/L $NaHSO_3$. However, the strength began to recover at 6 g/L $NaHSO_3$ to 5.62 MPa, which is not significantly different from the strength of unmodified glycinin. The adhesive strength leveled off at $NaHSO_3$ concentrations greater than 6 g/L.

Water resistance, reflected by wet and soaked adhesive strength, is an important property that determines adhesive bond durability. Similar to dry strength, the wet strength and soaked strength of glycinin dropped dramatically at 3 g/L $NaHSO_3$ (**Table 2**). Although wood specimens glued with glycinin modified with 3 g/L $NaHSO_3$ did not delaminate during soaking, the wet strength was too low to be accurately determined and reported. Wet and soaked strengths were regained as $NaHSO_3$ increased to 12 g/L and then decreased slightly at a $NaHSO_3$ concentration of 24 g/L. Further addition of $NaHSO_3$ to a concentration of 36 g/L resulted in considerable decreases in water resistance.

The strength of a protein adhesive depends on its structure, composition, and conformation as well as interaction of its hydrophobic and hydrophilic groups with the wood surface (*29*). As previously discussed, part of the R-SH groups was converted to $RS\text{-}SO_3^-$, resulting in extra negative charges in the glycinin due to $NaHSO_3$. The presence of $RS\text{-}SO_3^-$ can decrease the effective interfacial area and enhance the electrostatic repulsion between protein and wood, subsequently decreasing the adhesive strength (*30*). In addition, the higher salt concentration can weaken the interaction between polar groups of the protein and polar groups of the wood (*30*). Conversely, significant increases in surface hydrophobicity after moderate $NaHSO_3$ modification prompted hydrophobic interaction. Enhanced protein interaction is favorable for entanglement of proteins in the curing process, which is beneficial for adhesive strength, especially water resistance. The increase in surface hydrophobity of glycinin modified at 3 g/L $NaHSO_3$ is too small to offset the loss of interfacial area and the weakening of the protein−wood polar interaction. As the $NaHSO_3$ concentration increased to more than 3 g/L, escalation of hydrophobicity overwhelmed the

negative effect of $NaHSO_3$ and increased adhesive strength and water resistance.

Glycinin modified at 36 g/L $NaHSO_3$ lost a substantial amount of strength after soaking and exhibited depressed water resistance (**Table 2**). During water soaking, water molecules penetrate the glue area and weaken the interaction between protein and wood (*11*). Although hydrophobic interactions in glycinin were induced and strengthened by $NaHSO_3$ modification, salt present in the adhesive might have attracted more water into the glue area and destroyed adhesion bonds extensively. As a result, glycinin modified at 36 g/L $NaHSO_3$ showed average dry strength but inferior water resistance.

Modification of glycinic protein with $NaHSO_3$ increased surface hydrophobicity and introduced extra charges and ionic strength. Hydrophobic interaction, electrostatic force, and presence of salt ions affect the protein's physicochemical properties including solubility, surface hydrophobicity, morphological properties, thermal stability, and adhesion performance. The balance between hydrophobic interaction and electrostatic force determined the extent of glycinin aggregation, which affected solubility, thermal stability and adhesion performance. Although modification with a high concentration of $NaHSO_3$ could enhance hydrophobicity interaction by increasing surface hydrophobicity, the resulting excessive salt is detrimental to adhesive strength, especially water resistance. $NaHSO_3$ modification does not have much effect on improving the adhesive performance of glycinin, so further researches on modification of soy protein components are need to comprehend the properties and superior adhesive performance of $NaHSO_3$-modified soy protein.

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