

PHYSICOCHEMICAL, MORPHOLOGICAL, AND ADHESION PROPERTIES OF SODIUM
BISULFITE MODIFIED SOY PROTEIN COMPONENTS

by

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Abstract

Soybean protein modified with sodium bisulfite behaves like latex adhesives, with adhesive strength comparable to formaldehyde-based adhesives. β -conglycinin and glycinin are two major protein components of the adhesive system. The objective of this research was to investigate the effect of sodium bisulfite on the physicochemical, morphological, and adhesion properties of glycinin and β -conglycinin in order to better understand the function of glycinin and β -conglycinin in the formation of the soy latex adhesive.

Sodium bisulfite broke the disulfide bonds that linked acidic and basic polypeptides of glycinin, and the reducing effect was enhanced with increasing sodium bisulfite concentration. Although cleavage of disulfide bonds was expected to destabilize proteins, the thermal stability of glycinin increased as the sodium bisulfite concentration increased. Sodium bisulfite modified glycinin had higher surface hydrophobicity, which facilitated hydrophobic interactions between molecules and aggregation of glycinin. The balance between hydrophobic interactions and electrostatic forces makes glycinin form unique chain-like structures. Adhesive performance of glycinin dropped significantly at lower sodium bisulfite concentration and then increased as sodium bisulfite concentration increased up to 24 g/L. Excess sodium bisulfite was detrimental to adhesive strength and water resistance.

High-molecular-weight aggregates were observed in unmodified β -conglycinin, but these aggregates were dissociated by sodium bisulfite treatment. Similar to glycinin, the thermal stability of β -conglycinin was improved by the modification. However, the denaturation enthalpy of β -conglycinin decreased significantly at high level of sodium bisulfite (36 g/L). The turbidity at pH 4.8 also dropped extensively at the concentration of 36 g/L. The contact angle of β -conglycinin reached its minimum at 6 g/L sodium bisulfite on cherry wood and 24 g/L on glass. Morphology study proved that sodium bisulfite modification made the β -conglycinin solution more dispersed. At pH 9.5, water resistance of β -conglycinin was improved to a small extent by 6 g/L sodium bisulfite. At pH 4.8, adhesive performance was enhanced by 3 g/L and 6 g/L sodium bisulfite. High level of sodium bisulfite at 36 g/L reduced the adhesive performance of β -conglycinin drastically.

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CHAPTER 1 - INTRODUCTION

Literature Review

Soy Protein

Soybean, *Glycine Max*, is a legume native to eastern Asia, where it has been cultivated as a principal crop for at least 5,000 years (Soybean, the columbia encyclopedia, sixth edition. 2001-07). Nowadays, more soybeans are planted in the United States than in any other country in the world. In 2007, soybeans were planted on 63.6 million acres, accounting for about 25% of the US crop area planted. About 2.6 billion bushels were harvested in the US, which is about 32% of the world's soybean production (Soystats, 2008). Soybeans consist mainly of 40% protein, 35% carbohydrate, 20% oil and 5% ash on a dry basis. In the US, 71% of the edible consumption of fats and oils are derived from soybean (Soybean, the columbia encyclopedia, sixth edition. 2001-07). Soybeans are normally processed following the procedure: cracking, dehulling, flaking, extracting oil, redrying, and milling. After oil removal, the remaining flakes are processed into various edible soy protein products, or used to produce soy meal for animal feeds. Besides food and feed industry, soy protein is also used in many industrial applications including adhesives, emulsions, cleansing materials, cosmetics, inks, plastics, particle boards, pharmaceuticals, pesticides and textile fibers.

Soybean proteins are classified into two types based on biological functions in plants: metabolic proteins and storage proteins. Storage proteins make up the majority of soybean protein and are synthesized during soybean seed development. Classically, the Osborne scheme has been used to categorize soybean proteins. In soybean, 90% of the seed proteins are salt-soluble globulins, and the remainder is water-soluble albumins (Nielsen 1974).

Soybean proteins consist of various groups of polypeptides that span a broad range of molecular sizes. On the basis of the sedimentation coefficient, a typical ultracentrifuge pattern of soybean proteins has four major fractions: 2S, 7S, 11S, and 15S (Hou and Chang 2004) (Table 1.1). The 2S fraction, which accounts for 20% of the extractable protein, contains low molecular weight polypeptides and is mainly composed of metabolic proteins like trypsin inhibitor. The 7S account for about 37% of the extractable protein and is designated conglycinin. The 7S fraction

is separated into three major immunologically distinct components: β -conglycinin, γ -conglycinin, and basic 7S globulin (Hirano et al. 1987). β -conglycinin is the major component of 7S globulin and accounts for 30-50% of the total seed proteins (Lampart-Szczapa 2001). The 11S fraction, corresponding to glycinin, accounts for 30% of the total soybean proteins. The 15S fraction accounts for about 10% of the total soybean proteins, and is believed to be a dimer of glycinin (Nielsen 1974). Among all the fractions, the predominant structural proteins in soybean are glycinin and β -conglycinin. Their amino acid compositions are shown in Table 1.2.

β -conglycinin

β -conglycinin is a heterogeneous class of glycoproteins composed of varying combinations of three subunits designed α' (MW 57,000-72,000), α (MW 57,000-68,000), and β (42,000-52,000) and gives 7 heterogeneities (B_0 - B_6) (Yamauchi et al. 1991). These subunits are highly negatively charged and compactly folded glycopeptides with significant hydrophobic regions (Kinsella et al. 1985). The isoelectric points of α' , α , and β are 5.15, 4.9, and 5.66-6.00, respectively. All these three subunits are rich in aspartate/asparagin, glutamate/glutamine, arginin and leucine. α' and α subunits are very similar in amino acid composition and they are immunologically related (Thanh and Shibasaki 1977). The β -conglycinin is a trimeric protein in which subunits are noncovalently associated by hydrophobic interaction and hydrogen bonding without any disulfide bonds (Thanh and Shibasaki 1978).

β -conglycinin undergoes a complicated association-dissociation phenomenon in response to changes in ionic strength and pH. When the ionic strength is less than 0.2, β -conglycinin exists as a 9.8S dimer between pH 4.8 and 11. It has a trimer structure as 7S at neutral pH or less than 4.8, when ionic strength is greater than 0.5. Both forms exist at intermediate ionic strengths (Thanh and Shibasaki 1979). At higher ionic strength of > 0.8 , β -conglycinin dissociated to 5.6S and 2S (Ibuchi and Imahori 1978). However, as the ionic strength decreases, dissociation of the 9.8S dimer into individual polypeptides occurs at pH less than 3.0 (Nielsen 1974). The forces responsible for the association-dissociation are presumed to be electrostatic (Kinsella et al. 1985).

Glycinin

Glycinin is a large hexamer with a molecular weight of 300-380 KDa. Each subunit is composed of an acidic polypeptide (34-44 KDa) and a basic polypeptide (20 KDa) linked together by a disulfide bond (Staswick et al. 1984). Five subunits are identified in glycinin:

A_{1a}B_{1b}, A₂B_{1a}, A_{1b}B₂, A₅A₄B₃, and A₃B₄. A glycinin molecule is composed of two identical hexagonal layers. The acidic and basic polypeptides alternate in the same layer and are held together by hydrophobic and disulfide bonds, forming three subunits. The two layers are held together by electronic interaction and hydrogen bonding (Peng et al. 1984). Acid peptides have higher amount of glutamic acid, proline, and cysteine than basic peptides. But basic polypeptides have significantly more hydrophobic amino acid content.

Like β -conglycinin, glycinin also has association-dissociation behavior under different environmental conditions such as pH, ionic strength, and temperature. When the ionic strength decreases from 0.5 to 0.1, glycinin (11S form) dimerizes to 15S aggregates. At pH=7.6 under low ionic strength (0.01), glycinin partially dissociates to 3S (subunits) and 7S (half-molecules), and the half-molecules associate reversibly to 11S (Wolf and Briggs 1958).

Protein Structure Stabilization Force

The forces involved in stabilizing the protein structure include hydrogen bonding, electrostatic interactions, van der Waals forces, and hydrophobic interactions. Covalent disulfide bonds are also important in soy protein. The properties of the stabilizing forces of protein structure are shown in Table 1.3.

Hydrogen bonds are the attraction force between the hydrogen atom attached to an electronegative atom (oxygen, nitrogen or sulfur) and another electronegative atom. The strength of a hydrogen bond is between 2 and 10 kcal/mol. The stability of hydrogen bonds depends on the difference in free energy between protein-protein and protein-solvent hydrogen bonds. The net energy gain for formation of a buried hydrogen bond is only 0.6 kcal/mol. In hydrophobic domains, hydrogen bonding may have significant stabilization effect on proteins (Kinsella et al. 1985).

Electrostatic interactions occur between molecules with opposite charges. At neutral pH, aspartic acid and glutamic acid are negatively charged. Cysteine and tyrosine are negatively charged at alkaline environment. Arginine, lysine and histidine usually are positively charged. Thus, electrostatic interactions are pH dependent. The surface charge density of glycinin and β -conglycinin as a function of pH is shown in Figure 1.1. Electrostatic interactions affect functional properties like solubility, emulsifying and foaming of soy proteins.

Van der Waals forces are short range forces and the strength is inversely proportional to approximately the sixth power of the distance between induced dipoles in adjacent atoms. These interactions are related to the polarizability of the atoms involved. Van der Waals forces are short-range forces and provide weak electrostatic attractions. Although transient and weak, van der waals attractions have important effect on protein structure because of their absolute number.

Hydrophobic interactions are the major driving force for protein folding. Polypeptides have significant amount of amino acids with nonpolar side chains which interact thermodynamically unfavorably with water. Hydrophobic interactions occur between the nonpolar segments of polypeptides by minimizing lost energy caused by the contact between nonpolar groups and water, which disrupts lattices of water molecules. Thus, proteins tend to fold in a way that nonpolar groups closely associate and form a hydrophobic core and polar groups are exposed on the surface. However, not all hydrophobic amino acid are in the interior of proteins. Hydrophobic side chains found at the protein surface are usually involved in extensive hydrophobic interaction. Hydrophobic interactions are the predominant forces stabilizing protein structure (Ryan 1977).

The structure of water determines the magnitude of hydrophobic interactions, and the inherent properties of water are responsible for the hydrophobic effect (Kauzmann 1959). Hydrophobic interaction can be changed by agents and conditions that alter water structure. Salts have variable effects on stabilizing or destabilizing hydrophobic interaction. Hydrophobic interactions affect functional properties such as solubility, gelation, coagulation, and micelle formation of proteins.

Disulfide bonds are covalent bonds between the sulfhydryls groups of two cysteine residues. Intramolecular disulfide bonds are important in stabilizing the tertiary structure and quaternary structure of proteins and imparting molecular rigidity. Proteins containing disulfide bonds tend to be more heat stable and show higher denaturation enthalphies than protein without disulfide bonds (Kinsella et al. 1985).

Chemical Modification of Soy Protein

Protein modification usually refers to intentional alternation of protein structure by physical or chemical agents to improve properties (Park 1997). Chemical modification of protein

can be achieved in two ways: changing the structure of amino acids and alternating the environment in which the protein is placed.

Protein modification may involve alterations in structure and conformation of primary, secondary, tertiary, and quaternary structures. It includes disruption and reformation of protein structure stabilizing forces such as hydrogen bonds, hydrophobic interaction, electrostatic force, and disulfide bonds, using chemical treatment.

A number of functional groups on the amino acid side chains of protein are available for chemical reaction. The susceptibility of amino acid side chains is determined by their chemical reactivity and accessibility of the chemical reagent. Carboxyl, amino, disulfide, imidazole, indole, phenolic, sulfhydryl groups can be modified by chemical agents. The effects of acetylation, succinylation, and alkylation on functional properties of soy proteins have been studied (Franzen and Kinsella 1976; Geoghegan et al.1981; Kim and Kinsella 1986a).

Effect of Salt on Soy Proteins

Salt affects protein stability in two different ways. Ions interact with proteins via nonspecific electrostatic interactions at low salt concentration. The electrostatic neutralization of protein surface charges usually stabilizes protein structure. At higher concentration, the ion specific effects of salts affect the protein structure stability. Salt, such as Na_2SO_4 , enhances protein stability, but NaSCN weakens the protein stability (Damodaran 1996). The Hofmeister series described the relative effectiveness of various ions in altering protein conformations (von Hippel, P. H. and Schleich 1969).

Shen (1981) investigated the effect of various neutral salts on solubility of soy proteins. The solubility of soy proteins decreased for all salts up to 0.2 M and increased at higher salt concentration. The electrostatic shielding effect of salts decreases the electrostatic repulsion and enhances the hydrophobic interaction. When ionic strength increased to above 0.2 M, the solubility is increased by anions such as I^- , NO_3^- , and Br^- , indicating that the hydrophobic interactions are suppressed by these salts. But the structure stabilizing anion SO_4^{2-} decreased the solubility of soy proteins, indicating its positive effect on hydrophobic interactions. Soy protein viscosity, a manifestation of the attractive forces between the molecules in the liquid, is found to be affected by salts. The addition of NaCl and Na_2SO_4 to soy protein solution decreased the apparent viscosity (Petruccioli and Anon 1995). The counterions could neutralize surface charges on protein and subsequently decrease hydration of protein, leading to decrease in viscosity.

Differential scanning calorimetry studies proved that NaCl increased the thermal stability of soy proteins (Koshiyama 1972), suggesting that the forces responsible for the quaternary structure of glycinin and β -conglycinin are stabilized by NaCl.

Effect of Reducing Agent on Soy Proteins

There are two disulfide bonds per mole of β -conglycinin and these bonds are buried in the hydrophobic region of the molecule (Koshiyama 1972). The glycinin has 18-20 disulfide bonds of both inter and intramolecular bonding that contribute to the compact structure (Kella et al. 1986). Two thirds of the disulfide bonds in glycinin are contributed by the acidic polypeptides and the rest by basic polypeptides (Iyengar and Ravestien 1981).

Thiols and sulfites have been used extensively to cleave disulfide bonds in protein. The reducing agent 2-mecaptoethanol could depolymerize disulfide-linked polymers in soy proteins formed by glycinin and β -conglycinin (Briggs and Wolf 1957; Kelley and Pressey 1966). Nash and Wolf (1967) found that the solubility of soy proteins was increased by 2-mecaptoethanol, with significant increases in the glycinin and β -conglycinin peaks in the ultracentrifugal study. Abtachi and Aminlari (1997) investigated the effect of sodium sulfite, sodium bisulfite, and cysteine on soybean milk base. Sodium bisulfite, sodium sulfite, and cysteine increase the solubility of soy proteins in decreasing order and the increase in solubility was dependent on the reagent concentration. These effects are believed to involve reduction of disulfide bonds and decrease in the polymerization of proteins. All three reducing agents led to increase in the intensity of both β -conglycinin and glycinin in sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis.

Reducing agents break the intra and intermolecular disulfide bonds in glycinin and release acidic and basic polypeptides (Kim and Kinsella 1986b; Wolf 1993). Reduction by dithiothreitol improved glycinin solubility, but high dithiothreitol concentration (10 mM) resulted in loss in solubility (Kim and Kinsella 1986b). Cleavage of disulfide bonds in glycinin by 2-mecaptoethanol in the presence of GuHCl improves its trypsin digestibility. Kella et al. (1986) investigated the effect of reducing disulfide bonds of glycinin by oxidative sulfitolysis using sodium sulfite. The cleavage of protein changed the surface properties of glycinin, acidic, and basic peptides. The cleaved basic peptides tended to associate due to the exposed hydrophobic residue on the protein surface (Kella et al. 1986).

Soy Protein as Wood Adhesive

In wood adhesive applications, the desired functionalities include adhesion strength, water resistance, water solubility, and viscosity. For adhesive application, the main disadvantages of soy proteins are low adhesive strength and poor water resistance (Kumar 2002). The adhesive strength of protein adhesive depends on its ability to disperse in water and on interactions of nonpolar and polar groups of the protein with the substrate. The main reason for low adhesive strength of unmodified soy flour is that the majority of polar and nonpolar groups are unavailable due to internal bonds resulting from van der Waals forces, hydrogen bonds and hydrophobic interaction (Kumar et al. 2004). Denaturing and disulfide bond cleavage, which could unfold the protein and increase the interaction with wood, are expected to enhance the adhesive performance of soy proteins.

Various chemical modifications have been studied in an attempt to improve the adhesive performance of soy proteins. Alkaline treatment helps in unfolding the protein and exposing functional groups for interaction. Hettiarachchy et al. (1995) observed that alkaline treatment of soy protein isolates at pH 10 and 50°C enhanced adhesive strength and water resistance. Urea modified soy proteins showed better adhesive performance than alkaline modified soy proteins (Sun and Bian 1999). The urea and guanidine hydrochloride significantly increased the adhesive performance (Huang and Sun 2000a). Huang and Sun (2000b) studied the adhesion properties of soy proteins modified by sodium dodecyl sulfate (SDS) and sodium dodecylbenzene sulfonate (SDBS). Soy proteins modified at moderate concentration (0.5 and 1%) SDS and SDBS had better adhesive strength and water resistance than unmodified soy proteins. In all cases, protein unfolding induced increase in exposure of more functional groups including hydrophobic and polar groups, the main reason for enhancement of adhesive performance. The increase surface hydrophobicity was believed to be responsible for the higher water resistance. Soy proteins have also been modified with sodium sulfite. Viscosity and adhesive strength decreased as concentration of sodium sulfite increased. At a concentration of 0.1 M, sodium sulfite had not significant adverse effect on adhesive properties and reduced the viscosity (Kalapathy et al. 1996).

Enzyme hydrolysis is a more efficient, highly specific and high yield way to modify soy proteins. Soy protein hydrolyzed with trypsin exhibited much higher adhesive strength than unmodified soy protein (Kalapathy et al. 1996). Modification with urease was reported to

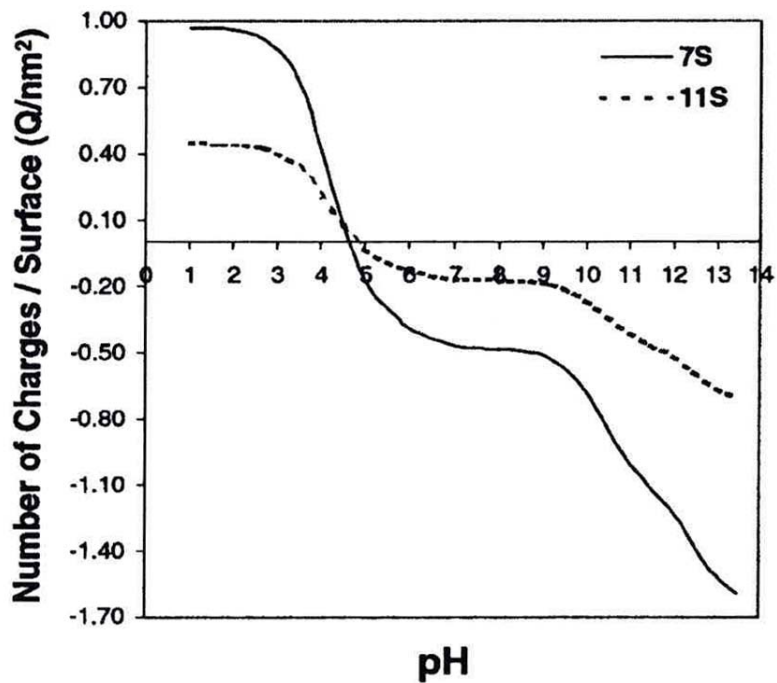
improve adhesive strength of soy protein. Extensive hydrolysis by chymotrypsin resulted in decrease in viscosity and loss of adhesion (Kumar et al 2004).

Cross-linking of protein involves joining two molecular components by a covalent bond achieved through a cross-linking agent. Rogers et al. (2004) used 1,3-dichloro-2-propanol to crosslink soy protein and got higher shear strength. Epoxies are active crosslinking agents for alkaline soy glues and improve the adhesive strength and durability (Lambuth 1965). Glutaraldehyde at 20 μ M significantly improved water resistance of soy protein 115% more than unmodified soy proteins (Wang et al. 2007).

Objectives

The goal of this research was to study the effect of sodium bisulfite on the physicochemical, morphological, and adhesion properties of major soy protein components: glycinin and β -conglycinin. This research also studied the relationship between structures and properties of sodium bisulfite modified soy proteins, as well as the effect of concentration of sodium bisulfite on adhesion performance of soy proteins.

Figure 1.1 Surface charge density of β -conglycin (7S) and glycinin (11S)^a.



^aAdapted from Yuan, 2002

Table 1.1 Amount and composition of ultracentrifuge fractions of water-extractable soybean proteins ^a.

Fraction	Percentage (%)	Components	Molecular Weight (Da)
2S	22	Trypsin inhibitors	8000-21,500
		Cytochrome c	12,000
7S	37	Hemagglutinins	110,000
		Lipoxygenase	102,000
		β -Amylase	61,700
		7S Globulin	180,000-210,000
11S	31	11S Globulin	350,000
15S	11	---	600,000

^a Adapted from Wolf, 1970.

Table 1.2 Amino acid composition of glycinin and β -conglycinin^a.

Amino acid	Glycinin	β -conglycinin
Asx	11.8	14.1
Thr	4.2	2.8
Ser	6.6	6.8
Glx	18.8	20.5
Pro	6.3	4.3
Gly	7.8	2.8
Ala	6.7	3.7
Val	5.6	5.1
Met	1.0	0.2
Ile	4.6	6.4
Leu	7.2	10.2
Tyr	2.5	3.6
Phe	3.9	7.4
His	1.8	1.7
Lys	4.1	7.0
Arg	5.9	8.8
Cys	1.1	0.3
Trp	0.75	0.3

^a Adapted from Nielsen, 1985. Expressed as percentage

Table 1.3 Forces involved in protein interactions^a.

	Energy (kJ/mol)	Groups involved	Disrupting agent
Hydrogen bond	8-40	Amide, NH-OC, Hydroxyl, phenol, OH-OC	Urea, guanidine hydrochloride detergents, heat
Electrostatic force	42-84	COO ⁻ , NH ₃ ⁺ , etc.	Salt, acid or alkaline pH
Van der Waals	1-9	Permanent induced and instantaneous dipoles	
Hydrophobic interaction	4-12	Amino acid with aliphatic or aromatic side chain	Detergents, Organic solvents
Disulfide bond	330-380	Sulphyhydryl in cysteine	Reducing agents

^aAdapted from Park, 1997.

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CHAPTER 2 - EFFECT OF SODIUM BISULFITE ON PROPERTIES OF SOY GLYCININ

Abstract

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 sodium bisulfite concentration increased. Reduction of disulfide bonds did not decrease thermal stability of glycinin. Instead, 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 make glycinin form chain-like aggregates. Adhesive strength and water resistance of glycinin dropped significantly at lower levels of sodium bisulfite and then increased as amount of sodium bisulfite increased up to 24 g/L. Adhesive performance decreased again with further addition of sodium bisulfite.

Introduction

Synthetic petrochemical polymers have been used extensively as adhesives in packaging, construction, and furniture industries. However, concerns about environmental pollution created interest in and a need for bio-based alternatives (Huang and Sun 2000a). Soy protein, commonly used as a functional or nutritional component in food products, has potential for use in bio-based 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 (Hettiarachchy et al. 1995; Huang and Sun 2000a; Huang and Sun 2000b; Kumar et al.; Wang et al. 2006)

Soy proteins are classified by sedimentation coefficient into 2S, 7S, 11S, and 15S fractions. Glycinin (11S) and conglycinin (7S) are the two major components (Peng et al. 1984). Glycinin is an oligomer with a molecular weight 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 (Kella et al. 1986). 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 (Kella et al. 1986). Cleavage of disulfide bonds increases glycinin's surface hydrophobicity, viscosity, and susceptibility to tryptic hydrolysis (Kella et al. 1986). Kim et al. (1987) found significant improvement in surface-active properties of reduced glycinin. Completely cleaved glycinin has higher solubility in the neutral-to-alkaline pH range than native glycinin (Kella et al. 1986).

Soy protein modified with sodium bisulfite behaves like latex adhesives and has adhesive strength comparable to formaldehyde-based adhesives (Sun et al. 2006). Previous studies revealed that glycinin is a key ingredient for adhesion strength, especially water resistance. Soy protein-based adhesives with high glycinin content have shown higher adhesion strength and water resistance than those with low glycinin content (Mo et al. 2006). Because glycinin's structure and properties change in the presence of reducing agents, it might have unique characteristics that contribute to the adhesion. The objective of this study was to investigate the effect of different levels of sodium bisulfite on solubility, surface hydrophobicity, thermal properties, and adhesive properties of soy glycinin.

Materials and Methods

Materials

Defatted soy flour obtained from Cargill (Cedar Rapids, IA) was used for 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 50×127×4.8 mm (width×length×thickness) were provided by Veneer One (Oceanside, NY).

Isolation of Glycinin

Glycinin was separated from soy flour using the method described by Thanh and Shibasaki (Thanh and Shibasaki 1976). Flour was dissolved in a 30 mM Tris buffer at pH 8.0 containing 10 mM 2-mercaptoethanol. The slurry was centrifuged at 10,000×g for 20 min at 4 °C

to remove fiber. The supernatant was adjusted to pH = 6.4 and then centrifuged again at 10,000×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 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

NaHSO₃ Treatment

Glycinin was dispersed in deionized water at 6.25% solid content. The solution was adjusted to pH = 9.5 with 1N NaOH and stirred at room temperature for 1 h. NaHSO₃ was added to the dispersion at 0, 48, 96, 192, 384, and 576 mg/gram of glycinin, equivalent to 0, 3, 6, 12, 24, and 36 g/L (gram NaHSO₃ per liter of solution). The resulting solution was maintained at pH 9.5 by adding 1N 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 (1970). 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 NaHSO₃, SDS-PAGE was performed in the absence of 2-mercaptoethonal for NaHSO₃-modified glycinin samples. Native glycinin was used as a control. To estimate purity of the glycinin, 2-mercaptoethonal 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 absorbance of the supernatant of the centrifuged glycinin sample solutions with various pH values at 280 nm, as described by German et al. (1982). 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 10,000×g for 20 min. Absorbance of the supernatant was measured at 280 nm by a spectrophotometer (Biomate 3, Thermo Electron Corporation, Madison, WI). All measurements were done in duplicate, and means were reported.

Differential Scanning Calorimetry

Thermal properties of modified glycinin samples were assessed with a differential scanning calorimeter (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 °C 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

Surface hydrophobicity of modified glycinin samples was evaluated by measuring SDS-binding capacity (Hettiarachchy et al. 1995; Kato et al. 1984). The modified glycinin samples were diluted to 0.1% with deionized water. Then, 0.07 mM SDS was added to the protein dispersion, and the solution was stirred for 30 min. The SDS-protein solution was dialyzed against a 0.02 M bicarbonate buffer (pH 9.5) at 4 °C for 48 h. One millimeter of dialyzed glycinin solution was mixed with 10 mL chloroform. The mixture was blended with 2.5 mL of a 0.0024% methylene blue solution and centrifuged at 800×g for 10 min. Absorbance of the SDS-methylene blue mixture in the lower layer was measured at 655 nm. SDS-binding capacity was calculated from a standard calibration curve, which was obtained using the above described method with defined amounts of SDS, and represented as micrograms of SDS bound to 1 mg of protein as a measure of protein hydrophobicity.

Morphology Properties

A Philips CM 100 (FEI Company, 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 0.01M 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). 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 (Electro-Tech System, Inc., Glenside, PA) for at least 7 days at 23 °C and 50% relative humidity. The 350- μ L glycinin solution was applied with a brush to a marked area of 127 \times 20 mm (length \times 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 5 pieces with dimensions of 80 \times 20 mm (glued area of 20 \times 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 (2002). Crosshead speed was 1.6 mm/min, and stress at maximum load was recorded as shear strength. Reported results are the average of five replicates.

Water Resistance

Water resistance was measured according to ASTM Standard Methods D1183-96 (2002) and D1151-00 (2002). Wood specimens were soaked in tap water at 23 °C for 48 h and tested for wet strength immediately after soaking. Soaked strength was assessed after soaked specimens were dried and conditioned at 23 °C and 50% humidity for another 7 days. Shear strength was tested as described previously.

Results and Discussion

SDS-PAGE Analysis

Native glycinin gave main bands corresponding to acidic and basic subunits on SDS-PAGE (Figure 2.1, lane A). Glycinin with reducing agent NaHSO₃ 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 A₅B₃ complex, which is the disulfide bond-linked part of glycinin subunit G₄ (A₅A₄B₃) (Staswick et al. 1993). Another band that occurred at 32 KDa might be due to the non-covalently bonded acidic polypeptides (A₄) in the G₄ glycinin subunit. Several small bands that appeared around 100 KDa could be due to formation of disulfide bond-linked polymers caused by freeze drying or thiol-disulfide exchange during modification (Wolf 1993). Intensity of the bands at 38 KDa and 23 KDa increased as NaHSO₃ increased. Those two bands, corresponding to acidic and basic polypeptides, resulted from reduction of the disulfide bond-linked AB complex (Figure 2.1, lane C-G). Some small bands around 100 KDa (Figure 2.1, lane B) faded with addition of NaHSO₃ (Figure 2.1, lane C-G). These results indicate that intermolecular disulfide bonds that link acidic and basic polypeptides of glycinin subunits were cleaved by NaHSO₃. Although degree of cleavage increased as NaHSO₃ concentration increased, complete reduction of disulfide bonds was not achieved. Even at the highest NaHSO₃ concentration (36 g/L), a considerable amount of AB complexes were still present.

Effect of NaHSO₃ Concentration on Surface Hydrophobicity

Surface hydrophobicity of glycinin was evaluated using SDS-binding capacity, which is proportional to the protein's surface hydrophobicity (Kato et al. 1984). Glycinin treated with 3 g/L NaHSO₃ did not show an obvious increase in surface hydrophobicity. A sharp upsurge in hydrophobicity occurred when NaHSO₃ was beyond 3 g/L, but hydrophobicity slowed at 12 g/L NaHSO₃ and increased slightly after that (Figure 2.2).

Cleavage of disulfide bonds induced by NaHSO₃ 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 et al. (1995) reported soy protein had higher surface hydrophobicity in alkali media (pH 8-10) than in a neutral environment, implying that hydrophobic groups might be exposed by alkali treatment. 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 NaHSO₃ (3 g/L) probably was not enough to bring a considerable quantity of hydrophobic groups to the surface, as level of NaHSO₃ increased, more disulfide bonds were broken and more hydrophobic groups were brought out to the protein surface, increasing surface hydrophobicity. As NaHSO₃ continued to increase, surface hydrophobicity was limited by inadequate disulfide-bond breakage, as shown by SDS-PAGE results.

Effect of NaHSO₃ Concentration on Solubility

The U-shaped solubility-pH distribution was observed for all glycinin modified with various levels of NaHSO₃ (Figure 2.3). Glycinin solubility was not significantly affected by NaHSO₃, but minimum solubility gradually shifted to lower pH values as NaHSO₃ increased. For example, unmodified glycinin had an insoluble region between pH 4.5-6.0 with a minimum solubility at around pH 5.6, but minimum solubility of glycinin treated at 3 g/L NaHSO₃ moved to around pH 5.0-5.5. At 36 g/L NaHSO₃, the pH range for minimum solubility was pH 4.7-5.5. Also, solubility in the alkaline region (pH > 6.5) decreased as NaHSO₃ increased.

A protein usually reveals minimum solubility at its isoelectric point. At the isoelectric point, electrostatic repulsions between protein molecules reach a minimum because of the zero net charge on the surface of protein molecules. During disulfide-bond cleavage by NaHSO₃, some sulfhydryls resulting from deoxidization are blocked as a sulfonate group (RS-SO₃⁻) (Savolainen 2004). Extra negative charges induced by NaHSO₃ increased the surface charge of glycinin and shifted the minimum solubility range to a lower pH. Protein solubility depends not only on electrostatic forces but also on hydrophobic interactions. The SDS-binding capacity results indicated that surface hydrophobicity increased as NaHSO₃ concentration increased.

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 (Kella et al. 1986). Reinforced hydrophobic interactions offset electrostatic repulsions, resulting in lower solubility for modified glycinin in the alkaline pH range.

Morphology of NaHSO₃ Modified Glycinin

Native glycinin existed mainly in the form of globular aggregates of various sizes, some of which formed into irregular shaped clumps (Figure 2.4A). In glycinin modified by 3 g/L NaHSO₃, the number of small globular aggregates increased, and clumps became larger than those in unmodified glycinin (Figure 2.4B). Increasing the concentration of NaHSO₃ to 6 g/L caused significant changes in appearance of glycinin molecules. Globular aggregates were broken into smaller clusters, which built chain-like structures (Figure 2.4C). At 36 g/L NaHSO₃, the number of short chains and rods increased as dimensions of large, chain-like aggregates decreased (Figure 2.4D). Formation of disulfide bonds through thiol-disulfide interchange has been shown to be involved in cross-linking of protein molecules to form aggregates (Catsimpoolas and Meyer 1970). However, NaHSO₃ is not only a reducing agent but also a sulfhydryl-blocking reagent, which hinders thiol-disulfide exchange during protein interaction. As shown in the non-reducing SDS-PAGE, bands (around 100 kDa) that might be caused by disulfide cross-linking were small and vague. Intermolecular disulfide exchange played a limited role in glycinin aggregation after NaHSO₃ modification.

In addition to disulfide cross-linking, hydrophobic interactions and electrostatic forces are crucial variables for protein cluster formation (Sun et al. 2008). SDS, which can bind to exposed hydrophobic groups on the protein surface, is capable of eliminating hydrophobic interactions. After adding 0.1% SDS, glycinin modified by 6 g/L NaHSO₃ no longer had chain-like structures but appeared similar to native glycinin (Figure 2.4E). This suggested that hydrophobic interactions between nonpolar groups of proteins were the main driving force for formation of chain-like structured aggregates in the 6 g/L NaHSO₃-modified glycinin. After being dialyzed against 0.01M bicarbonate buffer, the large, chain-like structure in glycinin modified by 6 g/L NaHSO₃ fragmented into relatively small chains and clumps (Figure 2.4F). Although hydrophobic interaction facilitates protein aggregation, electrostatic repulsion between protein molecules inhibits aggregate growth. As NaHSO₃ concentration increased, greater ionic strength was introduced to glycinin solutions. Because ionic strength could shield electrostatic charges and then moderate the range of electrostatic interactions, the increase in ionic strength favors protein aggregation (Horne 1998). After dialysis, ionic strength of modified glycinin decreased. As a result, electrostatic repulsions were fortified and caused disintegration of the large, chain-like aggregates into smaller aggregates in 6 g/L NaHSO₃-modified glycinin.

Therefore, the balance between hydrophobic interaction and electrostatic force was the major factor determining shape and size of glycinin aggregates. When the balance was reached, glycinin molecules turned into large, chain-like aggregates, which also were observed by Sun et al. in soy protein (Sun et al. 2008). As in glycinin modified by 3 g/L NaHSO₃, hydrophobic interaction was relatively weak. Although 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 NaHSO₃, surface negative charges induced by blocking sulfhydryls as sulfonate groups (RS-SO₃⁻) could increase greatly due to high NaHSO₃ concentration, and the large increase in electrostatic repulsion could break the balance between hydrophobic and electrostatic interaction, resulting in smaller chain-like aggregates.

Effect of NaHSO₃ Concentration on Thermal Properties

Denaturation temperature (T_d) and enthalpy (ΔH) of glycinin were affected significantly by NaHSO₃ (Table 2.1). Unmodified glycinin showed two peaks during heat denaturation (Figure 2.5); 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 (Eldridge and Wolf 1967). The so-called $\overline{7S}$ component exhibited an endothermic peak at 69.8 °C at low ionic strength ($\mu = 0.01$) and pH 7.6 (Utsumi et al. 1987). Thus, the additional peak at 70.0 °C was due to the occurrence of the $\overline{7S}$ component. As NaHSO₃ concentration increased from 3 to 36 g/L, the peak corresponding to $\overline{7S}$ disappeared, and the 11S peak gradually shifted to a higher temperature.

The increase in thermal stability of 11S protein treated with a reducing agent (i.e., sodium sulfite) also was observed by Petrucci and Anon (Petrucci and Anon 1995). Disulfide bonds in native 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 NaHSO₃ 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 (Relkin 1994). In addition, ΔH of glycinin increased at NaHSO₃ concentrations up to 24 g/L. The ΔH of glycinin modified by 36 g/L NaHSO₃ dropped almost 2 J/g compared with that modified by 24

g/L NaHSO₃ (Table 2.1); this might have been caused by a less ordered structure that could not be stabilized by the effect of the salt.

Effect of NaHSO₃ on Adhesive Shear Strength

Glycinin without NaHSO₃ modification showed greater adhesive strength than modified glycinin (Table 2.2). Adhesive strength decreased extensively with addition of 3 g/L NaHSO₃. However, strength began to recover at 6 g/L NaHSO₃ to 5.62 MPa, which is not significantly different from the strength of unmodified glycinin. Adhesive strength leveled off at NaHSO₃ 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, wet strength and soaked strength of glycinin dropped dramatically at 3 g/L NaHSO₃ (Table 2.2). Although wood specimens glued with glycinin modified with 3 g/L NaHSO₃ did not delaminate during soaking, wet strength was too low to be accurately determined and reported. Wet and soaked strength were regained as NaHSO₃ increased to 12 g/L and then decreased slightly at a NaHSO₃ concentration of 24 g/L. Further addition of NaHSO₃ to a concentration of 36 g/L resulted in considerable decreases in water resistance.

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 (Mo et al. 2004). As previously discussed, part of the R-SH groups was converted to RS-SO₃⁻, resulting in extra negative charges in the glycinin due to NaHSO₃. Presence of RS-SO₃⁻ can decrease the effective interfacial area and enhance electrostatic repulsion between protein and wood, subsequently decreasing adhesive strength (Kalapathy et al. 1996). In addition, the higher salt concentration can weaken the interaction between polar groups of the protein and polar groups of the wood (Kalapathy et al. 1996). Conversely, significant increases in surface hydrophobicity after moderate NaHSO₃ 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 hydrophobicity of glycinin modified at 3 g/L NaHSO₃ is too small to offset the loss of interfacial area and the weakening of the protein-wood polar interaction. As NaHSO₃ concentration increased to more than 3 g/L,

escalation of hydrophobicity overwhelmed the negative effect of NaHSO₃ and increased adhesive strength and water resistance.

Glycinin modified at 36 g/L NaHSO₃ lost a substantial amount of strength after soaking and exhibited depressed water resistance (Table 2.2). During water soaking, water molecules penetrate the glue area and weaken the interaction between protein and wood (Mo et al. 2006). Although hydrophobic interactions in glycinin were induced and strengthened by NaHSO₃ 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 NaHSO₃ showed average dry strength but inferior water resistance.

Conclusion

Modification of glycinic protein with NaHSO₃ increased surface hydrophobicity and introduced extra charges and ionic strength. Hydrophobic interaction, electrostatic force, and presence of salt ions affect protein's physico-chemical 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 NaHSO₃ could enhance hydrophobicity interaction by increasing surface hydrophobicity, the resulting excessive occurrence of salt is detrimental to adhesive strength, especially water resistance.

Figure 2.1 SDS-PAGE pattern of native glycinin in the presence of 2-mercaptoethanol: acidic subunits (AS), basic subunits (BS) (lane A). In the absence of 2-mercaptoethanol: unmodified glycinin (lane B); glycinin modified by 3 g/L NaHSO₃ (lane C); glycinin modified by 6 g/L SDS-PAGE pattern of native glycinin in the presence of 2-mercaptoethanol (lane D); glycinin modified by 12 g/L NaHSO₃ (lane E); glycinin modified by 24 g/L NaHSO₃ (lane F); glycinin modified by 36 g/L NaHSO₃ (lane G).

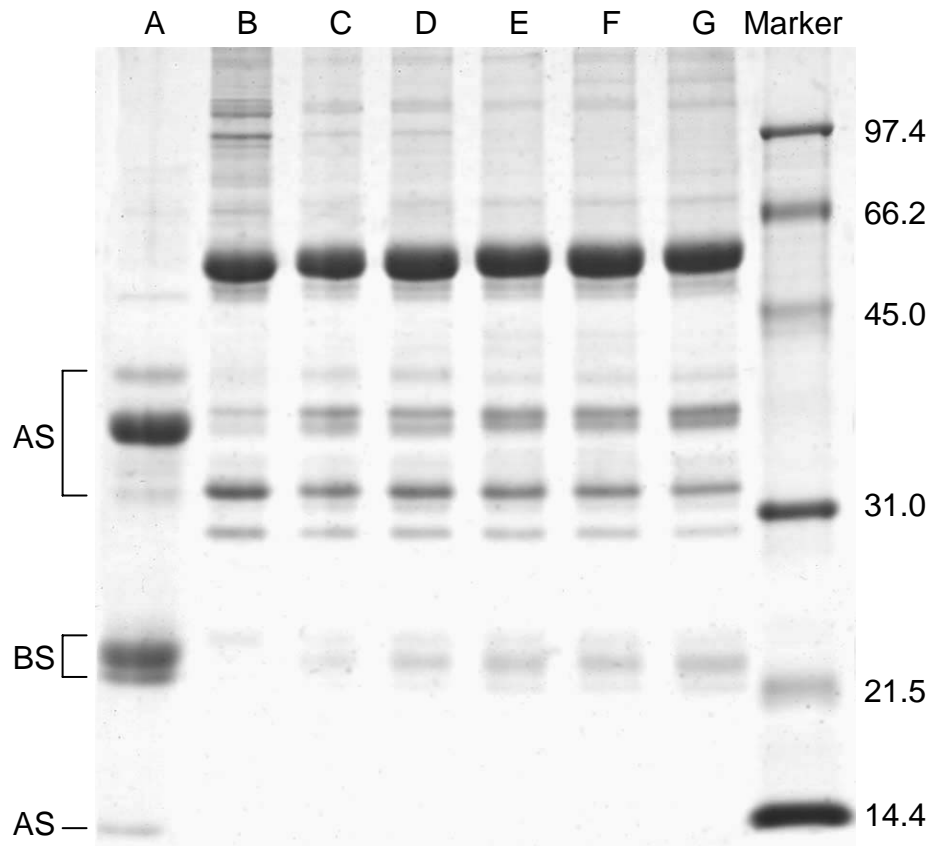


Figure 2.2 Effect of NaHSO₃ concentration on SDS-binding capacity of glycinin.

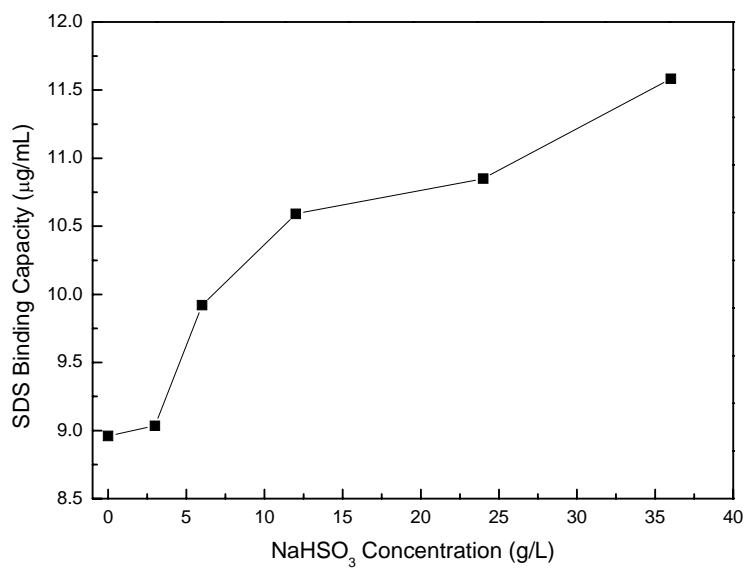


Figure 2.3 pH solubility profile of glycinin, glycinin modified with 3 g/L NaHSO₃, and glycinin modified with 36 g/L NaHSO₃.

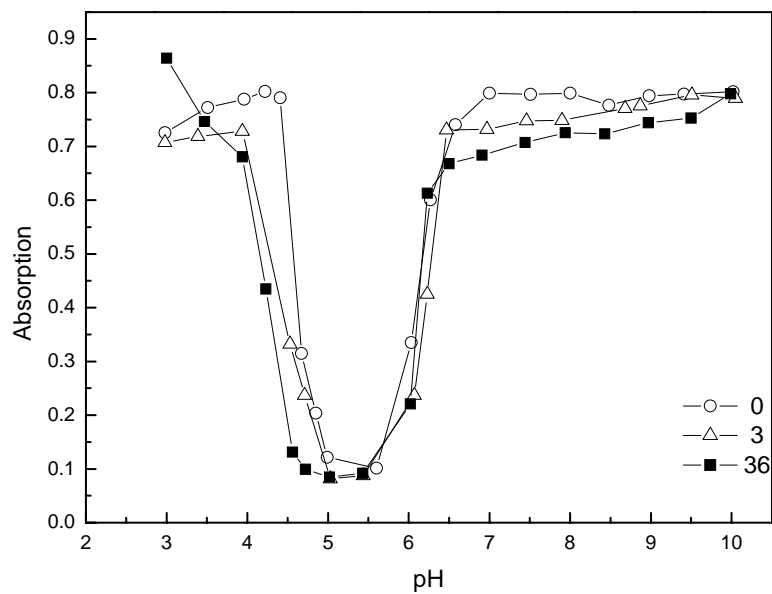
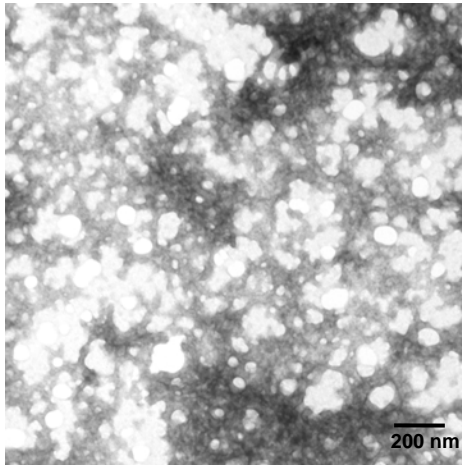
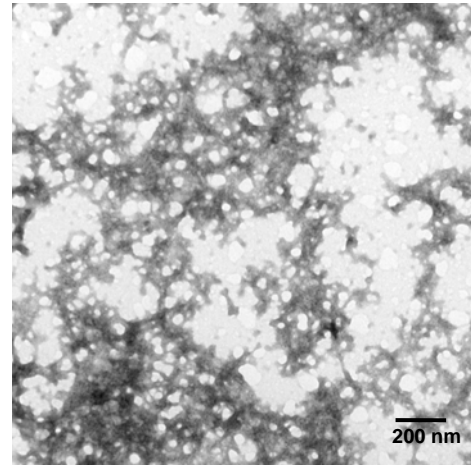


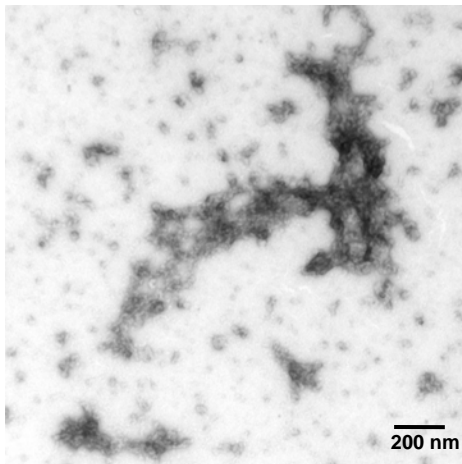
Figure 2.4 TEM images of soy glycinin: unmodified glycinin (A); glycinin modified by 3 g/L NaHSO₃ (B); glycinin modified by 6 g/L NaHSO₃ (C); glycinin modified by 36 g/L NaHSO₃ (D); glycinin modified by 6 g/L NaHSO₃ dialyzed against 0.01M bicarbonate buffer (E); glycinin modified by 6 g/L NaHSO₃ treated with 0.1% SDS (F).



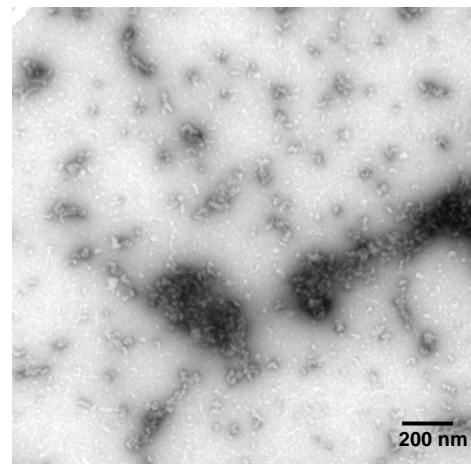
A



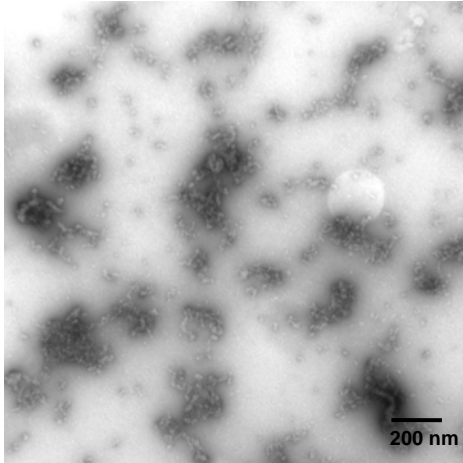
B



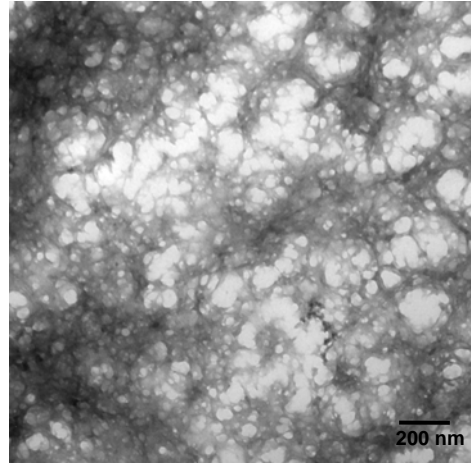
C



D



E



F

Figure 2.5 DSC thermogram of glycinin modified with NaHSO₃ concentrations of 0, 3, 6, 12, 24, and 36 g/L.

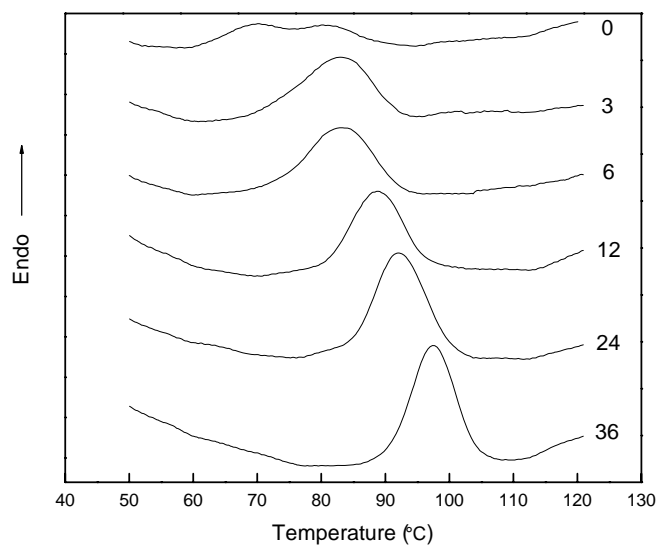


Table 2.1 Denaturation temperature (T_d) and enthalpy of denaturation (ΔH) of glycinin modified with various NaHSO_3 Concentrations^a

NaHSO_3 (g/L)	T_d ($^{\circ}\text{C}$)	ΔH (J/g)
0	70.0/81.6 ^g	7.8 ^f
3	82.6 ^f	14.5 ^e
6	83.4 ^e	14.7 ^e
12	88.8 ^d	15.5 ^d
24	92.2 ^c	18.3 ^b
36	97.3 ^b	16.4 ^c

^a ANOVA and LSD tests were performed using SAS. Means with the same letters in the same row are not significantly different at $P = .05$.

Table 2.2 Effect of NaHSO₃ concentration on adhesive strength of glycinin^a

Shear strength (MPa)	NaHSO ₃ concentration (g/L)					
	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 = .05.

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CHAPTER 3 - SODIUM BISULFITE-INDUCED CHANGES IN PHYSICOCHEMICAL, SURFACE AND ADHESIVE PROPERTIES OF SOY B-CONGLYCININ

ABSTRACT

The effect of sodium bisulfite on electrophoresis profile, turbidity, thermal, surface and adhesive properties of soy β -conglycinin protein was studied. Sodium bisulfite dissociated high-molecular-weight aggregates in the protein, and the aggregate percentage decreased with increase in sodium bisulfite concentration. The denaturation temperature of sodium bisulfite treated β -conglycinin increased as sodium bisulfite increased. However, at high sodium bisulfite concentration (i.e. 36 g/L), the denaturation enthalpy decreased significantly. A sudden drop in turbidity at pH 4.8 was also observed at the same salt level. The contact angle of β -conglycinin reached its minimum at 6 g/L sodium bisulfite on cherry wood and 24 g/L on glass. Water resistance of β -conglycinin was improved but not significantly by 6 g/L sodium bisulfite at pH 9.5. An obvious increase in adhesive strength of the protein occurred at 3 g/L and 6 g/L sodium bisulfite at pH 4.8. High sodium bisulfite concentration at 36 g/L reduced the adhesive performance of β -conglycinin sharply.

Introduction

Soybean proteins constitute about 40% of the seed, and 90% of the proteins are extractable with water or salt solutions (Yamauchi et al. 1991). Soy protein has been extensively used in a wide range of foods as functional or nutritional components. Being first used as adhesives in the early 1920s, soybean proteins have shown potential as alternatives to formaldehyde based resins for wood products (Sun 2005).

As the dominant storage protein, globulins account for about 50-90% of soybean seed proteins. The major globulins in soybean are β -conglycinin (7S) and glycinin (11S) (Utsumi et al. 1997). β -conglycinin is a trimer with a molecular weight of 150-200 kDa, composed of three subunits: α , α' , and β . The α and α' subunits are composed of the core regions and the extension regions, while the β subunit only has the core region (Maruyama et al. 1998). β -conglycinin is a

glycoprotein. The α and α' subunits have two carbohydrate moieties and the β subunit has one (Thanh and Shibasaki 1977). The subunits of β -conglycinin are not covalently linked but are held together primarily by hydrophobic forces (Kinsella et al. 1985). β -conglycinin is composed of relatively high amounts of arginine and amide-containing amino acids, but is lack of tryptophan and sulfur-containing amino acids (Nielsen 1974). β -conglycinin trimers exhibit association-dissociation phenomena at different pH and ionic strength (Koshiyama 1968). β -conglycinin has a trimeric structure (7S) at pH 7.0 and ionic strength $\mu > 0.5$ or as pH < 4.8 . When ionic strength $\mu < 0.2$, it exists in hexamer form (10S) in the pH range 4.8–11.0 (Lampart-Szczapa 2001). The association-dissociation behavior affects the structure of β -conglycinin, resulting in changes in chemical and functional properties.

Soy protein modified with sodium bisulfite behaves like latex adhesives and has adhesive strength comparable to formaldehyde-based adhesives (Sun et al. 2006). β -conglycinin and glycinin are the major components of the adhesive system. Previous study on the effect of sodium bisulfite on glycinin showed that the adhesive performance of glycinin did not improved by sodium bisulfite modification. Although only two disulfide bonds were found in one mole of β -conglycinin and these bonds seemed to be buried in the hydrophobic region of the molecule (Koshiyama 1968), it is possible that β -conglycinin has a unique contribution to adhesion performance. This present study is to investigate the effect of sodium bisulfite on the electrophoresis profile, thermal properties, turbidity, contact angle and adhesive properties of soy β -conglycinin.

Materials and Methods

Materials

Defatted soy flour obtained from Cargill (Cedar Rapids, IA) was used for isolation of soy β -conglycinin. 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 50×127×4.8 mm (width×length×thickness) were provided by Veneer One (Oceanside, NY).

Isolation of β -conglycinin

Crude β -conglycinin was separated from soy flour using the method described by Thanh and Shibasaki (Thanh and Shibasaki 1976b). The ammonium sulfate fractionation method was used for the purification of β -conglycinin as described by Iwabuchi and Yamauchi (1987). Three grams of crude β -conglycinin was dissolved in 100 ml phosphate buffer (32.5 mM K_2HPO_4 , 2.6 mM KH_2PO_4 , 0.4 M NaCl, 10 mM mercaptoethanol, and 1mM EDTA). Ammonium sulfate was added to the slurry to 75% saturation. Supernatant was obtained after the slurry was centrifuged. Additional ammonium sulfate was added to the supernatant to 90% saturation. The precipitate was collected as β -conglycinin after centrifugation. The purified β -conglycinin was dialyzed against deionized water for 3 days and lyophilized. The β -conglycinin had about 91% purity, as evaluated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

NaHSO₃ Treatment

The β -conglycinin was dispersed in deionized water at 5% solid content. The solution was adjusted to 9.5 with 1N NaOH and stirred at room temperature for 1 h. NaHSO₃ was added to the dispersion at 0, 60, 120, 240, 480, and 920 mg per gram of β -conglycinin, equivalent to 0, 3, 6, 12, 24, and 36 g/L (gram NaHSO₃ per liter of solution). The pH of the resulting solution was maintained at pH 9.5 by adding 1N 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 as described by Laemmli (1970). Protein samples were mixed with a sample buffer containing 2% SDS, 25% glycerol, and 0.01% bromphenol blue. To prevent disulfide bond breakage not induced by NaHSO₃, SDS-PAGE was performed in the absence of 2-mercaptoethanol for protein samples. To estimate the purity of the β -conglycinin, 2-mercaptoethanol was added to the sample buffer to perform the reducing SDS-PAGE. The gel was stained in 0.25% Coomassie brilliant blue R-250 and destained with a solution containing 10% acetic acid and 40% methoanol. Molecular weight standards were run with the samples. Densitometry was obtained by analyzing the gel image using the Kodak 1D Image Analysis software, version 4.6 (Kodak, Rochester, NY).

Differential Scanning Calorimetry

Thermal properties of β -conglycinin samples were studied using a differential scanning calorimeter (DSC) (DSC7, Perkin-Elmer, Norwalk, CT) calibrated with indium and zinc. Protein solutions (about 50 μ L) were hermetically sealed in the large-volume stainless steel pan. All samples were held at 20°C for 1 min and then scanned from 20°C to 150°C at a heating rate of 10°C/min. The peak temperatures and denaturation enthalpies were calculated from thermograms. Duplicates were made for each sample and the average values were reported.

UV Spectroscopy

β -conglycinin samples were diluted to 1% with deionized water for analysis. UV spectroscopic investigations were carried out with a Shimadzu UV-1650PC spectrometer (Shimadzu Scientific Instruments, Columbia, MD). The UV spectra of β -conglycinin samples were taken at a scan rate of 1 nm/s with a slit width of 2 nm. The secondary-derivative spectra were calculated using a band width ($\Delta\lambda$) of 2 nm and smoothed over 0.9 nm.

Turbidity

The turbidity of β -conglycinin samples was determined as the absorbance at 600 nm using the method described by Thanh and Shibasaki (Thanh and Shibasaki 1976b). Modified β -conglycinin samples were diluted to 0.1% with deionized water and maintained at pH 9.5. Then the diluted protein samples were adjusted to pH 4.8 with 0.1N HCl. All protein samples at pH 9.5 and 4.8 were stirred for 1h before testing by a spectrometer (UV-1650PC, Shimadzu Scientific Instruments, Columbia, MD). All measurements were done in duplicate and the average was reported.

Contact Angle Measurement

The contact angle was measured with an optical contact angle meter (CAM100, KSV Instruments, Helsinki, Finland). A droplet of β -conglycinin solution (2 μ L) was dropped on the surface of the substrate. Two substrates were used in this test: cherry wood and glass (Plain microscope slides, Fisher Scientific, Fair Lawn, NJ). For cherry wood, contact angles were measured every 3 sec from 0 to 147 sec. For glass, contact angles were measured every sec for 0 to 49sec. The average values were used for analysis. Five replicates were made for each sample.

Morphology Properties

β -conglycinin samples were diluted to 1% with deionized water for imaging. Diluted 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). A Philips CM 100 (FEI Company, Hillsboro, OR) transmission electron microscope (TEM) was used to investigate the microstructure of β -conglycinin samples. Morphology of β -conglycinin was observed with operation conditions at an accelerating voltage of 100 kV.

Wood Specimen Preparation

Cherry wood veneers were preconditioned in a chamber (Electro-Tech Systems, Inc., Glenside, PA) for 7 days at 23 °C and 50% relative humidity. Besides protein solution at pH 9.5, β -conglycinin samples at pH 4.8 were also made by adding 1N HCl. A volume of 350 μ L β -conglycinin solution was brushed onto a marked area of 127 \times 20 mm (length \times 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. The glued wood assemblies were cooled, conditioned at 23 °C and 50% relative humidity for 3 days, and cut into 5 pieces with dimensions of 80 \times 20 mm (glued area of 20 \times 20 mm). The cut wood specimens were conditioned for another 4 days before measurement.

Shear Strength

Wood specimens were tested using an Instron Tester (Model 4465, Canton, MA) according to ASTM Standard Method D2339-98 (2002). Crosshead speed was 1.6 mm/min, and stress at maximum load was recorded as shear strength. The average of five replicates was reported.

Water Resistance

Water resistance was measured following ASTM Standard Methods D1183-96 (2002) and D1151-00 (2002). Wood specimens were soaked in tap water at 23 °C for 48 h and tested immediately after soaking for wet strength. Soaked strength was assessed after soaked specimens were dried and conditioned at 23 °C and 50% humidity for another 7 days. Shear strength was tested as described previously.

Results and Discussion

SDS-PAGE Analysis

In reducing SDS-PAGE, β -conglycinin gave three major bands representing α (~82 KDa), α' (~77 KDa) and β (~47 KDa) subunits, respectively (Figure 3.1, lane A). In non-reducing SDS-PAGE, a new high-molecular-weight band appeared in all unmodified β -conglycinin (Figure 3.1, lane B) and NaHSO₃-modified β -conglycinin samples (Figure 3.1, lane C-G), which was also observed by Tsukata et al. (2006). The intensity of the new band decreased gradually with the increase in NaHSO₃ concentration (Figure 3.1, lane C-G). This indicated that the high-molecular-weight aggregation could be disulfide bond-linked polymers. In β -conglycinin, only the α and α' subunits have a little cysteine (Thanh and Shibasaki 1976a), so it is possible that these two subunits took part in the formation of the aggregation as suggested by Petrucci and Anon (1995).

However, the main SDS-PAGE patterns of unmodified and modified β -conglycinin were similar in the range of NaHSO₃ from 0 to 36 g/L (Figure 3.1, Lane B–G). Thus, NaHSO₃ had no effect on cleavage of the protein. Two moles of disulfide bond are present in a mole of β -conglycinin, and these bonds are buried in the hydrophobic region (Koshiyama 1968; Thanh and Shibasaki 1976a). It is very difficult for the reducing agent NaHSO₃ to reach the buried disulfide bonds. This result is in agreement with work on the effect of a reducing agent like 2-mercaptoethanol on β -conglycinin (Clara et al. 2007; Fukushima 1968; Koshiyama 1968). No significant effects of 2-mercaptoethanol were observed on ultracentrifugation, optical rotatory dispersion, and fluorescence emission of β -conglycinin.

Effect of NaHSO₃ Concentration on Thermal Properties

The denaturation temperature (T_d) and enthalpy (ΔH) of denaturation of β -conglycinin treated with different NaHSO₃ concentration are shown in Table 3.1. β -conglycinin with 0 g/L NaHSO₃ had a ΔH of 4.1, which is only about 57% of the reported value 7.25 (Mo et al. 2004). Because ΔH is the quantity of the heat energy required to denature the protein, higher ΔH indicates a more stable and ordered structure. The decrease in ΔH suggests that the β -conglycinin was partially denatured at pH 9.5 during stirring for 3 h even without NaHSO₃ treatment. Alkali could break hydrogen bonds of protein molecules and extensively unfold the protein, leading to exposure of buried hydrophobic residues and sulfhydryl groups (Mo et al. 2004).

The NaHSO₃-treated β-conglycinin had significantly higher T_d and ΔH than non-treated. The T_d of β-conglycinin increased as NaHSO₃ concentration increased and reached its maximum value at 36 g/L. The increase in thermal stability of β-conglycinin could be due to neutralization of negative charges on the protein surface, thus reducing electrostatic repulsion and stabilizing the protein (Relkin 1994).

β-conglycinin achieved 1.5-fold increase in ΔH after 3g/L NaHSO₃ modification. The ΔH kept increasing up to 24 g/L NaHSO₃, and dropped significantly at 36 g/L NaHSO₃ (Table 3.1). The increase in ΔH after addition of NaHSO₃ has also been observed in β-conglycinin by Deak and others, although they did not observe difference in treatments with different concentration (Deak et al. 2006). NaHSO₃ treatment reinforced the forces (e.g. hydrogen bonding and hydrophobic force) that stabilize the β-conglycinin conformation, so the destabilized β-conglycinin in alkaline condition gained stability after adding NaHSO₃ (L'Hocine et al. 2007). In the pH region 4.8-11, β-conglycinin (7S) dissociates to 5.6S and 2S at high ionic strength (Ibuchi and Imahori 1978). The decrease in ΔH at 36 g/L NaHSO₃ might be the result of the dissociation of the protein.

UV Spectroscopy

The secondary-derivative spectra of unmodified β-conglycinin showed peaks and troughs between 200-300 nm which are characteristic of the aromatic amino acids: phenylalanine, tyrosine, and tryptophan. Because absorption of tyrosine and tryptophan are overlapped especially in the spectra region (> 280 nm), the pure tyrosine negative peak at around 278 nm, corresponding to the positive peak at 278 nm on absorption spectroscopy, is used to monitor changes in tyrosine microenvironment (Figure 3.2). Continuous small red shifts were observed with increase in NaHSO₃ concentration to 24 g/L, and a high level (i.e. 36 g/L) of NaHSO₃ induced a small blue shift (Figure 3.3). The peaks of aromatic amino acids are known to shift to longer wavelengths by 2-5 nm as the hydrophobicity of the environment increases (Breydo et al. 1997). The very limited change (< 1 nm) in tyrosine peak indicates minor alterations in the microenvironments of tyrosine residues. Deshpande and Damodaran (1990) have reported little influence of salt on the degree of tyrosyl exposure in β-conglycinin. Therefore, the effect of NaHSO₃ on the structure and conformation of β-conglycinin is insignificant.

Effect of NaHSO₃ Concentration on Turbidity

Figure 3.4 shows the turbidity of the β -conglycinin at pH 9.5 and pH 4.8 as a function of NaHSO₃ concentration. At pH = 9.5, the turbidity of β -conglycinin was independent of NaHSO₃ concentration in the range from 0 g/L to 36 g/L (Figure 3.4). β -conglycinin has negative surface charge at pH 9.5, the addition of salt should suppress the protein electrostatic interaction and promote aggregation. However, as shown in SDS-PAGE analysis, NaHSO₃ dissociated intermolecular disulfide bonds-linked polymers, and potentially lowered the turbidity. The reduction effect of NaHSO₃ counteracted the electrostatic shielding effect of NaHSO₃ as a salt. Moreover, the relatively high surface charge density of β -conglycinin determines that a rather high amount of salt is needed to neutralize the electrostatic repulsion (Yuan 2002). It is possible that the highest NaHSO₃ concentration of 36 g/L was not high enough to increase turbidity significantly by shielding the surface charges.

At pH 4.8, β -conglycinin had much higher turbidity than at pH 9.5, which is due to favorable protein-protein association when pH approaching its pI point (Figure 3.4). The turbidity increased slowly in small extent with increasing NaHSO₃ concentration up to 12 g/L, almost leveled off at 24g/L, and then decreased significantly from 1.065 to 0.689 as NaHSO₃ concentration reached 36 g/L. As discussed in the thermal properties section, the ordered β -conglycinin structure was partially disrupted in the alkaline condition. The conformation change will cause changes in surface charge of β -conglycinin, and the pI might shift from 4.8. Accordingly, the turbidity of β -conglycinin increased slightly due to electrostatic shielding upon adding NaHSO₃ at pH 4.8. When NaHSO₃ concentration exceeds the level that makes protein possessing net zero charge, the salt-in effect of salt progressively dissociates the aggregates and increases the solubility (Kinsella et al. 1985).

Effect of NaHSO₃ Concentration on Contact Angle

Figure 3.3 shows the contact angle of β -conglycinin on cherry wood (Figure 3.5a) and glass surfaces (Figure 3.5b) as a function of NaHSO₃ concentration. Contact angle is a parameter indicating affinity of a liquid for a solid. Wettability on the substrate surface, which can be reflected by contact angle, is a precondition of better adherence (Zhang and Hua 2007). On wood surface, 3 g/L NaHSO₃ did not decrease the contact angle significantly. The contact angle of β -conglycinin reached its minimum at 6 g/L NaHSO₃, and then progressively increased with

increasing NaHSO₃ concentration. The TEM images of β-conglycinin demonstrated the difference in morphology of unmodified and modified β-conglycinin (Figure 3.6). In the absence of NaHSO₃, β-conglycinin was mainly composed of big aggregates of various sizes (Figure 3.5a). With 6 g/L NaHSO₃, almost all the aggregates fragmented to uniform sized granules dispersed in water (Figure 3.5b). The fragmentation of aggregates increased the surface area of β-conglycinin protein polymer, resulting in an increase in the effective contact area between the protein molecules and wood surface. In addition, viscosity is a key factor influencing contact angle. As viscosity decreased, the contact angle decreased (Cheng and Sun 2006). Wagner et al. (1992) reported that Na₂SO₃ prevented water molecules from interacting with protein molecules through ion pair shielding, resulting in reduced water imbibing capacity of soy protein. The loss of the capacity of water retention led to decrease in hydrodynamic volume and consequently viscosity of soy protein. NaHSO₃, with similar chemical properties to Na₂SO₃, would be capable of reducing viscosity of β-conglycinin, subsequently lowering the surface tension of the protein solution and decreasing contact angle.

However, the existence of salt could decrease the effective protein-wood interfacial area (Kalapathy et al. 1996). For β-conglycinin with high NaHSO₃ concentration (i.e. 36 g/L), a thin layer of salt sedimentation on the wood surface was observed after a drop of protein solution was absorbed by the wood. The presence of NaHSO₃ could prevent intimate contact between β-conglycinin and the wood surface, which would inhibit molecular attraction between them, and increase the contact angle extensively.

Glass surface is a high energy hydrophilic surface. Contact angle is greatly affected by the surface free energy of the solid substrate. A surface with higher surface energy has lower contact angle. Therefore, β-conglycinin solution has much lower contact angle on glass than on wood. Similar to a wood surface, the contact angle of β-conglycinin decreased with increase in NaHSO₃ concentration. But the lowest contact angle was obtained at 24 g/L NaHSO₃ (Figure 3.5b). Besides dispersing aggregates and reducing viscosity, NaHSO₃ could make the protein solution more polar, which favors the attraction between protein solution and the hydrophilic glass surface. On glass, the reinforced positive effect of NaHSO₃ dominates the contact process over the negative effect up to 24 g/L NaHSO₃. However, the barrier at high NaHSO₃ concentration (36 g/L) formed between protein and glass significantly increased the contact angle.

Effect of NaHSO₃ on Adhesive Shear Strength

The adhesive strength of β -conglycinin treated with different concentration of NaHSO₃ at pH 9.5 and 4.8 are shown in Table 3.2. At pH 9.5, NaHSO₃ had insignificant effects on dry strength in the range from 0 to 24 g/L. But a big drop was observed at 36 g/L NaHSO₃. Good wetting is synonymous with intimate molecular contact between adhesive and wood substrate, which must be compatible for good adhesion (Gollob and Wellons 1989). The relatively high contact angle of β -conglycinin with 36 g/L NaHSO₃ on the wood surface resulted in poor wetting of protein adhesive on wood. In addition, high NaHSO₃ concentration could increase the effective distance between active sites in protein molecules and those hydroxyl groups on the wood surface, and potentially reduce the interfacial interaction. These might account for the much lower dry strength of β -conglycinin at 36 g/L NaHSO₃, though it has a contact angle similar to protein with 24 g/L NaHSO₃.

The wet strength of β -conglycinin at pH 9.5 decreased dramatically as compared with dry strength (Table 3.2). During soaking, water could take away soluble components in the protein adhesive and salts, and compete with protein to form hydrogen bonds with wood, resulting in weakening of adhesive strength. β -conglycinin treated with 6 g/L NaHSO₃ showed increase in wet strength, but this increase is not significant. The small increase might be related to the better wettability (lower contact angle) at 6g/L NaHSO₃. When water dissolves the salts in the protein adhesive, cavities would be generated in the adhesive that penetrated in wood surface and between wood pieces. These cavities disrupted the continuous adhesive matrix which is detrimental to strength. The higher the NaHSO₃ concentration, the more cavities are generated during water soaking. This effect should be the main reason for weakening adhesive strength at NaHSO₃ concentration 12 g/L -36 g/L.

At pH 4.8, dry strength of β -conglycinin increased significantly with increase of NaHSO₃ concentration to 3 g/L, and gradually decreased from 12 g/L to 36 g/L NaHSO₃ (Table 3.2). As discussed in the turbidity study, protein interaction was strengthened in the presence of NaHSO₃, which is favorable for protein entanglement during curing and gives high strength (Mo et al. 2006). However, excessive increase in protein aggregation can hinder the penetration of protein into the pores on the wood surface, restrain the formation of mechanical interlocks, and then also weaken adhesion strength. At 36 g/L NaHSO₃, the dissociation of protein aggregates could result in too much penetration. Large portions of small molecules penetrated deeply into the wood

surface, and the relatively long distance between proteins restrains protein interaction (Cheng and Sun 2006). β -conglycinin with 0 g/L and 3 g/L NaHSO_3 had the best wet strength, while higher NaHSO_3 concentration induced decrease in wet strength. The similar phenomenon observed at pH 9.5, wet strength was extensively affected by the negative effect of NaHSO_3 as a salt (Table 3.2).

Conclusion

NaHSO_3 treatment affected β -conglycinin by two possible mechanisms. It acted as a reducing agent, dissociating disulfide bond-linked polymers in β -conglycinin. As a salt, it altered ionic strength surrounding the protein molecules in the solution environment and changed surface charges of the protein. NaHSO_3 improved the thermal stability of β -conglycinin, except for high NaHSO_3 concentration at 36 g/L. Turbidity of β -conglycinin was not significantly affected by NaHSO_3 at pH 9.5, but had a minimum value at 36 g/L NaHSO_3 at pH 4.8. Excessive salt prohibited intimate contact between the protein and substrate. Moderate NaHSO_3 concentration improved adhesive strength of β -conglycinin at pH 4.8 and water resistance at pH 9.5, but surplus NaHSO_3 was not favorable for adhesive performance.

Figure 3.1 SDS-PAGE pattern of native glycinin in the presence of 2-mercaptoethanol. In the absence of 2-mercaptoethanol: unmodified β -conglycinin (lane B); β -conglycinin modified by 3 g/L NaHSO₃ (lane C); β -conglycinin modified by 6 g/L NaHSO₃ (lane D); β -conglycinin modified by 12 g/L NaHSO₃ (lane E); β -conglycinin modified by 24 g/L NaHSO₃ (lane F); and β -conglycinin modified by 36 g/L NaHSO₃ (lane G).

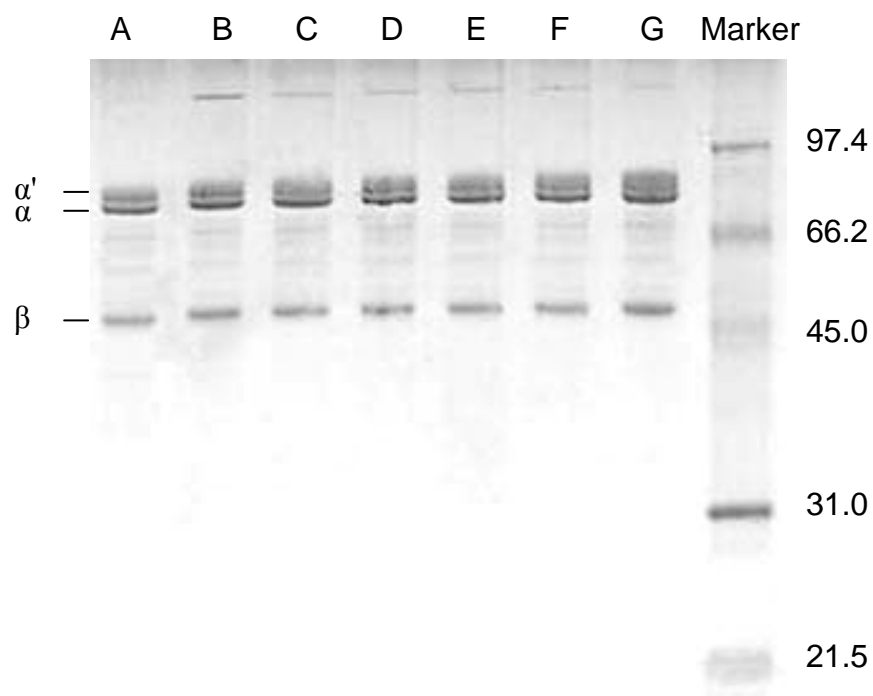


Figure 3.2 Absorption (dotted line) and second derivative (solid line) spectra of β -conglycinin

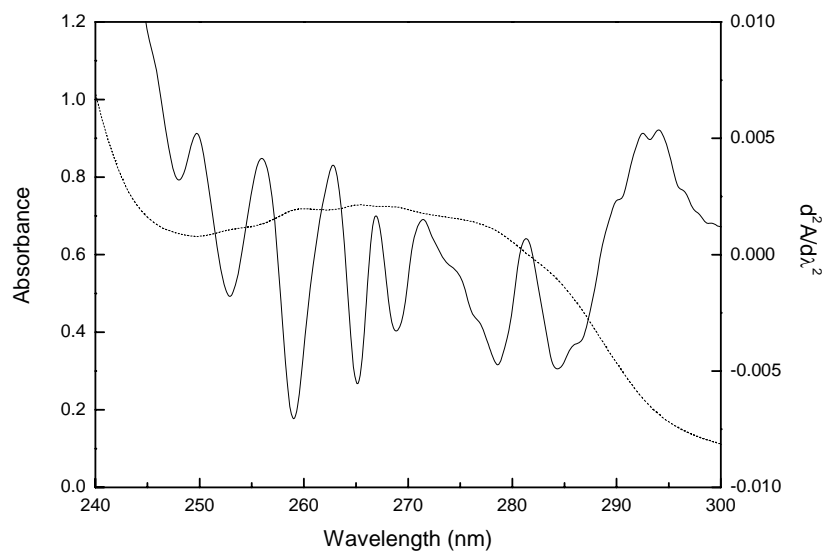


Figure 3.3 Effect of NaHSO₃ on tyrosine peak (~ 278 nm) of β-conglycinin.

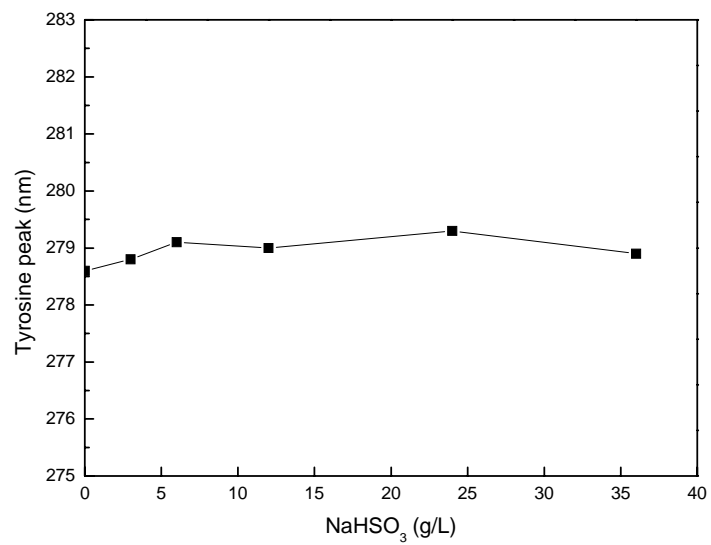


Figure 3.4 Turbidity of β -conglycinin modified with different levels of NaHSO_3 at pH 4.8 and pH 9.5.

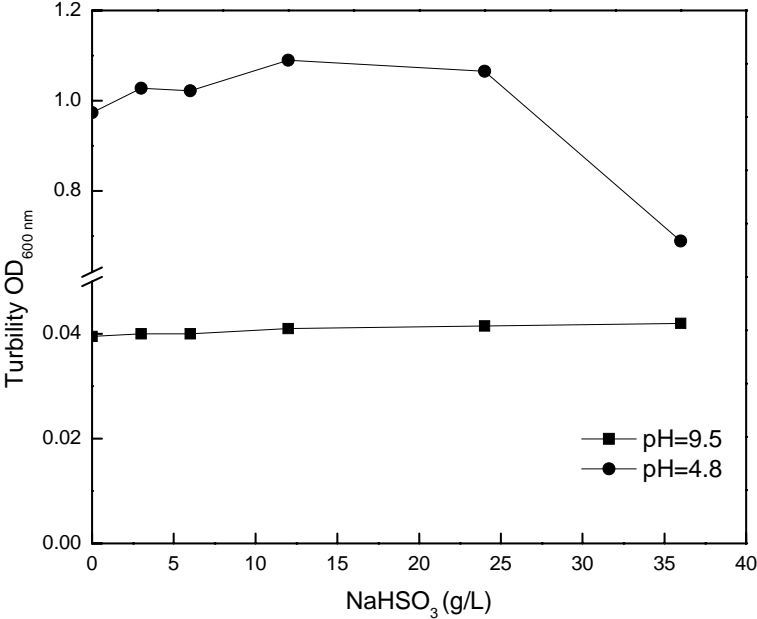


Figure 3.5 Contact angle β -conglycinin modified with different levels of NaHSO_3 at pH 9.5 on wood surface (a) and glass surface (b).

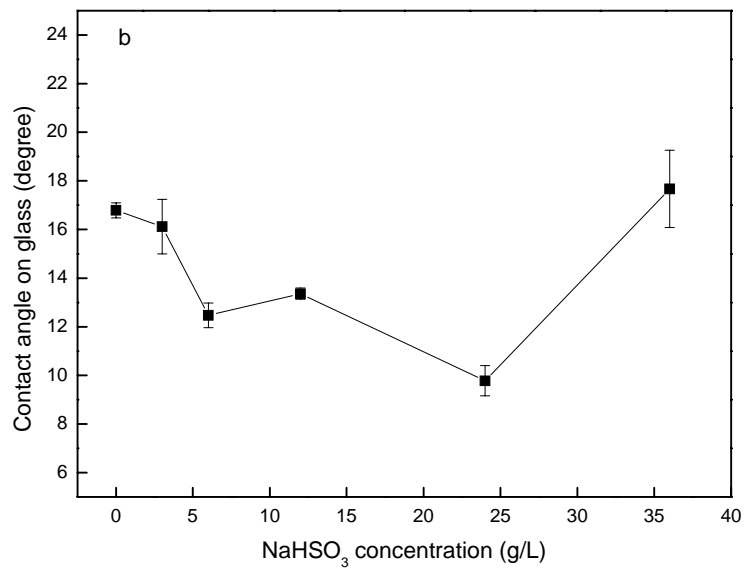
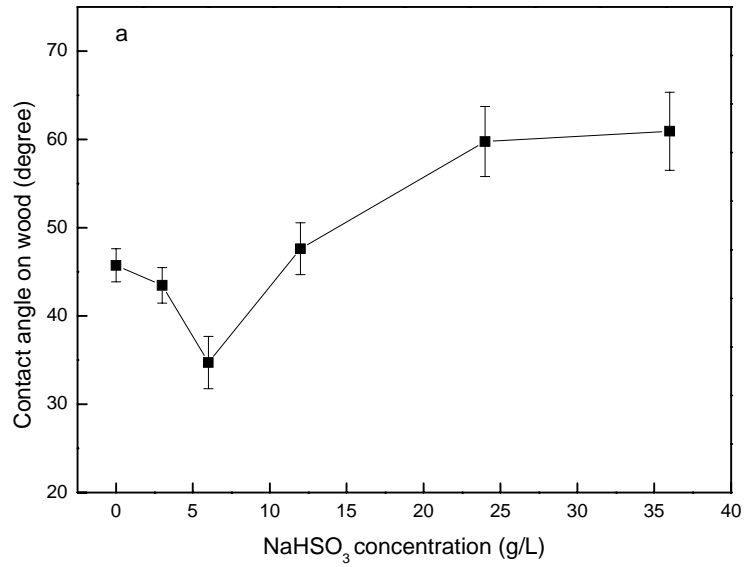
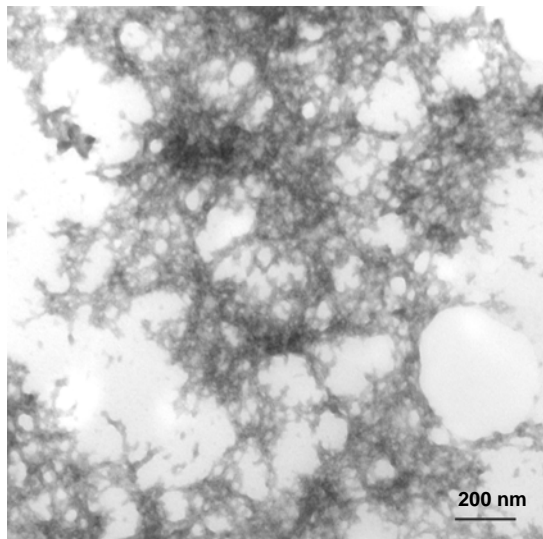


Figure 3.6 TEM images of soy β -conglycinin: unmodified β -conglycinin (a); β -conglycinin modified by 6 g/L NaHSO₃ (b).

a



b

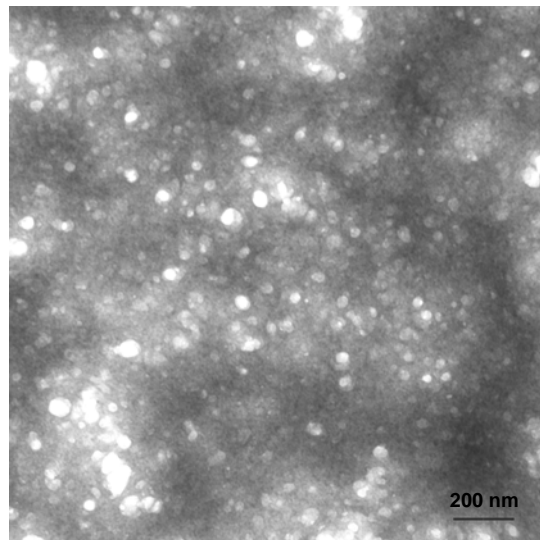


Table 3.1 Denaturation temperature (T_d) and enthalpy of denaturation (ΔH) of β -conglycinin modified with various NaHSO_3 concentrations ^a

NaHSO_3 (g/L)	T_d ($^{\circ}\text{C}$)	ΔH (J/g)
0	67.4 ^g	4.1 ^e
3	73.7 ^f	10.4 ^d
6	78.3 ^e	12.2 ^c
12	80.7 ^d	13.2 ^c
24	85.5 ^c	15.4 ^b
36	93.8 ^b	12.09 ^{cd}

^a ANOVA and LSD tests were performed using SAS. Means with the same letters in the same row are not significantly different at $P = .05$.

Table 3.2 Effect of NaHSO₃ concentration on adhesive strength of glycinin^a

Shear strength (MPa)	NaHSO ₃ concentration (g/L)					
	0	3	6	12	24	36
pH = 9.5						
Dry strength	5.24 ^b	5.12 ^b	4.79 ^b	5.38 ^b	5.28 ^b	2.84 ^c
Wet strength	1.46 ^b	1.63 ^b	1.97 ^b	0.63 ^c	0.65 ^c	0.60 ^c
pH = 4.8						
Dry strength	4.72 ^c	6.74 ^b	6.30 ^b	5.23 ^c	3.71 ^d	2.89 ^d
Wet strength	2.29 ^b	2.24 ^b	1.59 ^c	1.90 ^{bc}	0.49 ^d	0.12 ^d

^a ANOVA and LSD tests were performed using SAS. Means with the same letters in the same row are not significantly different at P = .05.

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CHAPTER 4 - SUMMARY AND FUTURE RESEARCH

Summary

Disulfide bonds in glycinin were cleaved by NaHSO_3 , which induced increase in surface hydrophobicity of glycinin protein. Moreover, NaHSO_3 modification introduced extra charges to the protein molecules and increased ionic strength in the environment. Hydrophobic interaction, electrostatic force, and the presence of salt ions affect protein's physico-chemical properties including water solubility, surface hydrophobicity, morphological properties, thermal stability, and adhesion performance. The balance between hydrophobic interaction and electrostatic force determined the aggregation of glycinin, which affects properties and characteristic of glycinin. Although modification with a high concentration of NaHSO_3 could enhance hydrophobic interaction by increasing surface hydrophobicity, the resulting excessive occurrence of salt is detrimental to adhesive strength, especially water resistance.

NaHSO_3 treatment affected β -conglycinin in two different mechanisms. It acted as a reducing agent dissociating disulfide bond-linked polymers in β -conglycinin. As a salt, it altered ionic strength surrounding the protein molecules in the solution environment and changed surface charges of the protein. NaHSO_3 improved the thermal stability of β -conglycinin via shielding of charges on protein surface. Excessive salt prohibited intimate contact between the protein and substrate. Moderate NaHSO_3 concentration improved adhesive strength of β -conglycinin at pH 4.8 and water resistance at pH 9.5, but surplus NaHSO_3 was not favorable for adhesive performance.

Future Works

Present study revealed the effect of NaHSO_3 on glycinin and β -conglycinin and their physicochemical, morphology, and adhesion properties. However, neither glycinin nor β -conglycinin has the special properties and superior adhesive strength as compared with the soy latex like adhesive, indicating interactions between these two soy protein components would be important in the soy protein adhesive system.

More work should be done on study the interaction between glycinin and β -conglycinin especially the interaction among their subunits with NaHSO_3 modification.

Exploring the preference association among subunits from glycinin and β -conglycinin and identify the nature of the association would be helpful in understanding the properties of soy latex adhesive.

The ratio of glycinin to β -conglycinin may also have significant influence on the interactions of these two soy protein components. It is necessary to study the effect of NaHSO_3 on the mixture of glycinin to β -conglycinin at different ratio in order to identify the contribution of two components to the properties of the mixture.