Adsorption of Glycinin and β-Conglycinin on Silica and Cellulose: Surface Interactions as a Function of Denaturation, pH, and Electrolytes

Carlos Salas,† Orlando J. Rojas,*†‡ Lucian A. Lucia,† Martin A. Hubbe,† and Jan Genzer§

†Department of Forest Biomaterials, North Carolina State University, Raleigh, North Carolina 27695-8005, United States
‡Faculty of Chemistry and Materials Sciences, Department of Forest Products Technology, Aalto University, P.O. Box 16300, FI-00076, Aalto, Finland
§Department of Chemical and Bimolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, United States

ABSTRACT: Soybean proteins have found uses in different nonfood applications due to their interesting properties. We report on the kinetics and extent of adsorption on silica and cellulose surfaces of glycinin and β-conglycinin, the main proteins present in soy. Quartz crystal microgravimetry (QCM) experiments indicate that soy protein adsorption is strongly affected by changes in the physicochemical environment. The affinity of glycinin and the mass adsorbed on silica and cellulose increases (by ca. 13 and 89%, respectively) with solution ionic strength (as it increases from 0 to 100 mM NaCl) due to screening of electrostatic interactions. In contrast, β-conglycinin adsorbs on the same substrates to a lower extent and the addition of electrolyte reduces adsorption (by 25 and 57%, respectively). The addition of 10 mM 2-mercaptoethanol, a denaturing agent, reduces the adsorption of both proteins with a significant effect for glycinin. This observation is explained by the cleavage of disulfide bonds which allows unfolding of the molecules and promotes dissociation into subunits that favors more compact adsorbed layer structures. In addition, adsorption of glycinin onto cellulose decreases with lowering the pH from neutral to pH 3 due to dissociation of the macromolecules, resulting in flatter adsorbed layers. The respective adsorption isotherms fit a Langmuir model and QCM shifts in energy dissipation and frequency reveal multiple-step kinetic processes indicitive of changes in adlayer structure.

INTRODUCTION

Soy proteins comprise a large fraction of the raw bean weight (>50%). They include macromolecules with no biological activity, the so-called storage proteins as well as those with biological activity, that is, lipoygenates, a trypsin inhibitor. Storage proteins are composed of globulins, which account for 65–80% of the seed protein and precipitate at pH 4.5–4.8. They have been classified according to the sedimentation constant as 7S and 11S. The 7S fraction (conglycinin) is composed of three subunits, namely, α (57–76 kDa), α′ (57–83 kDa), and β (42–53 kDa) with each accounting roughly for 40, 30, and 30 wt % of the total, respectively. The 11S fraction (glycinin) has a molecular weight of 320–350 kDa and its quaternary structure is formed by 12 subunits forming a dimer. Three of the dimeric subunits are acidic (denoted as A1, A2, and A3) with reported molecular weights of 34.8–40 kDa. The three remaining dimeric subunits are basic (B1, B2, and B3) with molecular weights ranging from 19.6 to 20 kDa. The amount of half-cystine on glycinin molecule (48 mol/mol of protein) is larger than that of 7S globulins (of only 4 mol/mol protein). The isoelectric points of soy globulin 7S and 11S are 4.96 and 4.64, respectively. The 11S protein forms reversible association polymers at pH 7.5 when the ionic strength decreases from 0.5 to 0.1 M NaCl.

Extensive research has been carried out on the fractionation, purification, and characterization of soybean storage proteins, even at the pilot-plant scales. The use of soy proteins is closely related to their solubility, hydration properties, gelation, and interfacial activity, which, in turn, are governed by the structure and charge balance of the macromolecule. The effect of pH, ionic strength, and chemical modification on the functional properties of proteins has been studied extensively, especially in food applications. Likewise, nonfood uses require a detailed knowledge of the physicochemical and thermal properties of soy proteins. The manufacture of plastics, adhesives, binders in paper, paint coatings, and composites have been documented along with the molecular functionalities required. Soy protein products available commercially...
include flours, concentrates, and isolates with protein contents of \( \approx 56, 65, \) and 90\% respectively. Due to their high protein content and thermal behavior, soy protein isolates have been used to manufacture adhesives and films.\(^{21}\)

Paper coatings and sizing are among the largest industrial applications of soy proteins.\(^{20}\) In a recent study, 20 proteins (including some soy-derived) and 7 polypeptides were evaluated as additives to increase the wet strength of paper.\(^{22}\) It was concluded that, of all the proteins and polypeptides studied, those with a high content of five key amino acids (i.e., serine, threonine, arginine, lysine, and histidine), produced the largest increase of paper peel strength. Interestingly, the latter three represent common basic amino acid residues (having more than one active amine group), whereas the first two are polar, but possess a pendant hydroxyl group. Both the amino and hydroxyl groups may provide active bonding sites within the amino acid backbone that contribute to increase in peeling strength. In addition, the use of soy proteins as alternative paper strength additives was reported recently.\(^{23}\) The breaking strength. In addition, the use of soy proteins as alternative paper strength additives was reported recently.\(^{23}\) The breaking

- fundamental nature of protein
- paper strength additives was reported recently.\(^{23}\) The breaking

\[ \text{application of these proteins in cellulose modification and improved fiber performance.} \]

### MATERIALS AND METHODS

Defatted 7B soy flour was obtained as a gift from ADM (Decatur, IL). All chemicals employed were of analytical grade and were used without further purification. Sodium hydroxide solid (certified ACS), 35\% hydrochloric acid solution, sodium phosphate monobasic anhydrous, sodium phosphate dibasic anhydrous, sodium chloride, tris base (molecular biology grade), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were acquired from Fisher Scientific (Somerville, NJ). Mercaptoethanol (2-mercapto-ethanol) was purchased from Sigma Aldrich (Milwaukee, WI).

**Soybean Globulins: Fractionation, Purification, and Characterization.** Proteins were fractionated from defatted soy flour, according to the method of Nagano et al.\(^{29}\) Briefly, a desired amount of defatted soy flour was dispersed in water (15-fold volume excess), adjusted to pH 7.5 with 2 N NaOH, and stirred until a well-homogenized dispersion was obtained. The slurry was then filtered through a 180-mesh screen and the filtrate was centrifuged (9000 \( \times \) g) for 30 min (Beckman centrifuge, model J21C). After centrifugation, the precipitate was discarded. Sodium bisulfite was added to the supernatant up to the concentration of 0.98 g/L, the pH was adjusted to 6.4 and the suspension was left at rest overnight at 4 °C on a cold bath. The suspension was centrifuged (6500 \( \times \) g) for 20 min at 4 °C; the precipitate constituted the 11S fraction (giginn). Sodium chloride was added up to 0.25 M to the supernatant and the pH adjusted to 4.8 using 2 N HCl. The suspension was stirred for 1 h and centrifuged (9000 \( \times \) g) for 30 min at 4 °C. The precipitate was an intermediate mixture of giginn (11S) and \( \beta \)-conglycinin (7S) fraction. The supernatant was diluted with water (2-fold excess), the pH was adjusted to 5 and the solution was centrifuged (9000 \( \times \) g) for 30 min at 4 °C. The precipitate (the \( \beta \)-conglycinin (7S) fraction) was collected and the supernatant was discarded. Each precipitated fraction was dissolved carefully in water and pH adjusted to 7.5 by addition of 2 N NaOH. The protein content in the samples was determined by the combustion method using a Perkin-Elmer 2400 CHN Elemental Analyzer (Norwalk, CT). Conversion factors of 5.71 for giginn (11S), 6.37 for \( \beta \)-conglycinin (7S), and 6.08\(^{22,33}\) for the intermediate mixture and the flour were used as multipliers of the nitrogen content to quantify the protein concentration (wt\%).\(^{11}\)

**SDS-PAGE**\(^{34}\) was run following the procedure reported by Sathe et al.\(^{35}\) with a slight modification. Briefly, 7 mg of sample was suspended in 2 mL of extraction buffer (50 mM Tris-HCl pH 8.5 containing 20 mM of 2-mercapto-ethanol) and gently stirred with magnetic stirrer for 30 min at room temperature. After extraction, the samples were centrifuged for 15 min at 9000 \( \times \) g. An equal volume of supernatant and sample buffer (50 mM Tris-HCl pH 6.8, 1% SDS, 0.01% bromophenol blue, 30% glycerol, 2 vol \% 2-mercapto-ethanol) was mixed and boiled during 10 min at 95 °C. All samples were cooled in an ice bath before pouring them into the SDS-PAGE wells. An SE 250 mini-vertical SDS-PAGE chromatography unit (Amersham Biosciences, San Francisco, CA) was employed at constant voltage (125 V), and precast polyacrylamide 4–20% gradient gels were employed (Pierce Biotechnology, Rockford, IL). A molecular weight marker kit (wide range 6.5–205 kDa) from Sigma-Aldrich was used. Running buffer was tris-HEPES-SDS buffer (100 mM Tris base, 100 mM HEPES, 0.1% SDS, pH 8), as suggested in the instructions of the manufacturer. Sample load was 15 \( \mu \)L (\( \approx 22 \mu \)g) per well.

Solubility measurements at a pH ranging from 3 to 10 without adjustment of ionic strength were carried out according to procedures described elsewhere.\(^{11,36}\) The pH was adjusted by using dilute aqueous solutions (1 wt \%) of either NaOH or HCl. Protein dispersions were prepared and stirred using magnetic stirrers for 1 h; following this, the suspensions were centrifuged at 9000 \( \times \) g for 30 min. Protein content in the supernatant was determined by the modified Lowry procedure (Modifed Lowry Protein Assay Kit, Pierce Biotechnology, Rockford, IL)\(^{37}\) and reported as percent of dissolved protein, that is, protein in the supernatant/total protein in the initial solution.

---

**Biomacromolecules**

388 dx.doi.org/10.1021/bm20141531 Biomacromolecules 2012, 13, 387–396
Circular Dichroism. Circular dichroism was carried out in a Jasco J 500 spectropolarimeter (Jasco Inc. Easton, MD). The instrument was warmed for at least 40 min before use. A cylindrical quartz cell of 1 cm path length was used. Scan parameters used in all experiments included 20 nm/min speed, bandwidth of 1 nm, and a sensitivity of 50 mdeg, with a step resolution of 1 nm. At least three accumulations (scans) were measured per sample.

Adsorption by QCM. We used a quartz crystal microbalance QCM-D E4 (Q-Sense, Gothenburg, Sweden) in the batch operation mode. In QCM, the mass increment due to adsorption on the surface of a sensor is proportional to the shift of resonance frequency ($\Delta f$) given by the Sauerbrey equation:\textsuperscript{38}

$$\Delta m = - \frac{C \Delta f}{n}$$  \hspace{1cm} (1)

where $\Delta m$ refers to the change of adsorbed mass associated with adsorption or desorption, and $n$ is the overtone number (1, 3, 5, 7, etc.). The third overtone ($n = 3$) was used in interpreting the QCM data in this study. For this particular setup, $C$ is a constant equal to 17.7 ng/cm$^2$e$^{-1}$ at $f = 5$ MHz. Equation 1 can be applied to calculate sensor mass changes under the following assumptions: (1) the adsorbed amount is smaller than the mass of the crystal, (2) the species are adsorbed rigidly, and (3) the adsorbed mass is distributed uniformly over the crystal surface. However, it has been pointed out that the frequency shift depends not only on the changes in mass adsorbed, but also on the viscosity and density of the medium.\textsuperscript{39}

The energy dissipation $D$ is related to the frequency $\Delta f$ and the decay time $\tau$ as follows:

$$D = \frac{1}{\pi\Delta f\tau}$$  \hspace{1cm} (2)

where $\tau$ values are obtained by periodically disconnecting the oscillating crystals from the main circuit via a computer-controlled relay.

Adsorption was studied on silica and gold sensors covered with an ultrathin film of cellulose. Experiments were carried out under different conditions, using protein solution concentrations of 1, 10, 100, and 1000 $\mu$g/mL in phosphate buffer at pH 7. Stock solution for dilutions was prepared in similar fashion as in the solubility experiments; all samples were prepared fresh from refrigerated stock solutions that were no more than one week old. A 1 wt % protein dispersion was mixed in the buffer at the desired pH, stirred for 1 h, and centrifuged (9000 g) for 30 min at 4 °C. Protein content in the supernatant was determined as described earlier. Phosphate buffer at pH 7 (100 mM NaCl) was used for experiments when needed. Finally, a denaturant agent 2-mercapto-ethanol (referred thereafter as 2-ME) at a concentration of 10 mM was used in some of the experiments.

**RESULTS AND DISCUSSION**

Purification of soy globulins yielded fractions of glycinin or 11S (92%), and $\beta$-conglycinin or 7S (99.7%), and an intermediate mixture (87%) with a protein content higher than that of the protein flour (51%). SDS-PAGE results (not shown) under denaturant conditions contained the typical bands of the 7S and 11S subunits of soy proteins. Very light bands of each protein were observed onto the other one presumably due to slight cross-contamination. The solubility of the proteins at 25 °C in different pH buffers is shown in Figure 1 as % of nitrogen soluble at different buffer pH.

The data shown in Figure 1 indicate that soy globulins are quite soluble at pH values below 4 or above 6, that is, sufficiently far away from the isoelectric point (pI) of these proteins (of 4.64 for glycinin and 4.9 $\beta$-conglycinin).\textsuperscript{30} It has been shown that protein adsorption onto solid surfaces is the highest at their isoelectric point (pI) because the adsorbing species exhibit the lowest repulsion in solution.\textsuperscript{30,42} Concurrently, bulk solubility at pI is very low due to increased coagulation among neighboring proteins. Therefore, to apply well-solubilized proteins and also to take advantage of their net negative charge, solutions at pH 7 were used in most of the adsorption experiments, as will be discussed in the next section, which focuses on glycinin and $\beta$-conglycinin.

**Dynamics of Protein Adsorption.** The dynamics of soy protein 11S adsorption on silica is illustrated by the isotherms in Figure 2 for different protein concentrations. The downward shift in resonant frequency associated with adsorption increases proportionally with increasing protein concentration due to the higher mass adsorbed, as predicted by eq 1 (vide supra). Rinsing buffer was allowed to flow through the cell after minute 83 and until $\approx$145 min. Irreversible adsorption was observed in all cases as indicated by the fact that the frequency after rinsing did not return to the respective baseline. A faster adsorption process is observed at the highest protein concentration (Figure 2). The kinetics data were fitted to a Langmuir model\textsuperscript{32} considering molecular diffusion from the bulk solution to the interface, collision, and uniform adsorption of the molecules onto the surface and desorption, which was assumed to be
insignificant during the time frame of the experiment, as judged by the unchanged frequency signal observed after rinsing. The values of the apparent binding and Langmuir constants for adsorption were determined and are reported in the Supporting Information.

Interpretation of the results in the case of the cellulosic substrate is complex due to its softer nature and its possible coupling with the solvent (changes in swelling, density, etc.). Therefore, only the results for the silica surface are reported as follow. The Langmuir constants $K_{eq} (=k_d/k_a$, where $k_d$ and $k_a$ are the desorption and adsorption constants, respectively) were determined from best fits to the Langmuir model. The values without adjustment of ionic strength were of 0.0023 and 0.0011 mg/mL for glycinin and $\beta$-conglycinin, respectively. At 100 mM NaCl, $K_{eq}$ is increased for glycinin (to 0.0094 mg/mL) and is reduced for $\beta$-conglycinin (3.7 × 10^{-4} mg/mL). Overall, the fitting parameters indicate an increase in the apparent binding constant as the concentration of protein increases (see Table 1 and Supporting Information).

**Adsorption Isotherms and Effect of Ionic Strength.**

The areal (Sauerbrey) mass of glycinin was calculated and the effect of solution ionic strength on adsorption onto silica and cellulosic surfaces is discussed here in light of the isotherms shown in Figure 3.

As shown in Figure 3a, the adsorption of soy glycinin (11S) on both silica and cellulose surfaces increases with increasing ionic strength. The effect of ionic strength observed in our work is more dramatic for cellulose. It has been shown that glycinin (11S) undergoes association–dissociation in solution, depending on the presence of electrolytes. At low ionic strength (<0.1 M NaCl and pH 7.6), glycinin (11S) dissociates into a

---

**Table 1. Binding Constant $k_{obs}$ (in min^{-1}) for Soy Globulins Adsorbed onto Silica Surfaces from Aqueous Solutions of Different Ionic Strengths**

<table>
<thead>
<tr>
<th>Protein</th>
<th>0 mM NaCl</th>
<th>100 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{obs}$ min^{-1}</td>
<td>$R^2$</td>
</tr>
<tr>
<td>glycinin (11S)</td>
<td>0.001 0.166 0.987</td>
<td>0.163 0.987</td>
</tr>
<tr>
<td></td>
<td>0.01 0.217 0.993</td>
<td>0.273 0.993</td>
</tr>
<tr>
<td></td>
<td>0.1 0.449 0.967</td>
<td>0.6046 0.967</td>
</tr>
<tr>
<td></td>
<td>1 0.653 0.879</td>
<td>0.7216 0.879</td>
</tr>
</tbody>
</table>

*See Supporting Information for details.*
form with same sedimentation constant of β-conglycinin or 7S, the so-called 7S. At higher ionic strengths (>0.5 M NaCl, pH 7.6), dissociation is reduced, which follows from the general understanding of moderated aggregation effects. For instance, at 0.5 M NaCl and pH 7.6 the protein exists in the 11S dimeric form. At pH 7, the macromolecule is negatively charged because its isoelectric pH is 4.64. This also leads to electrostatic repulsion between negatively charged groups in both the proteins and the surface, favoring a more extended conformation of the adsorbed proteins. At higher ionic strength, the electrostatic charges between the negatively charged groups in the protein molecule and the surface are screened, which, in turn, results in higher adsorbed amounts of protein.

The different behaviors of the acidic and basic subunits of glycinin influence its functional properties. The effect of pH and ionic strength on the solubility and molecular structure of soy glycinin has been reported.19 By using fluorescein isothiocyanate (FITC) labeling, Lakemond et al showed that the “relative exposure” of acidic and basic polypeptides in glycinin is different depending on the physicochemical environment (pH, ionic strength), that is, the acidic or basic polypeptides buried inside the molecule adapt to a more favorable environment (the “relative exposure” was defined as the ratio between the corrected fluorescence integrated signal for the polypeptide of interest and the corrected integrated absorbance signal at 280 nm).18,19 It was shown that for acidic polypeptides the “relative exposure” was favored at low and high ionic strengths (0.03 and 0.5 M NaCl, respectively), with a greater exposure at low ionic strengths. In contrast, exposure of the basic polypeptides was reduced relative to the acidic ones and increased with increasing ionic strength. Similar effects explain the finding that glycinin adsorption on cellulose was higher at high ionic strength. At low ionic strength and pH 7.0, the glycinin molecule contains a large number of negatively charged polypeptides, which experience higher repulsion with the negatively charged cellulose and silica resulting in low adsorption. Increasing the ionic strength to 100 mM NaCl effectively screened the electrostatic interactions on the protein and the substrate, thus, leading to increased adsorption. Figure 4 shows a schematic illustration of the effect of the physicochemical environment on glycinin association and dissociation.

Similar trends were found for adsorption of β-conglycinin onto silica and ultrathin films of cellulose, as shown in Figure 3b. However, the adsorption of β-conglycinin was lower than that observed for glycinin (cf. Figure 3), with the opposite effect when salt was present, that is, for glycinin the adsorption increased in the presence of 0.1 M NaCl but the reverse occurred in the case of β-conglycinin. The lower adsorption observed can be explained by the presence of less disulfide linkages in the β-conglycinin molecule; it is possible that the disulfide linkages promote association of glycinin on the surface which can lead to multilayer adsorption, as has been reported in the case of proteins containing cysteine residues.29 Studies of β-lactoglobulin, which contains five cysteine residues (four forming disulfide linkages and one free),46 indicate that the free cysteine residue favors formation of disulfide linkages because it can engage readily in an S−S exchange reaction, which favors adsorption on solids.29 For the 7S protein, this association is restrained and perhaps the adsorption as a monolayer is favored. Besides the fact that β-conglycinin molecule contains less disulfide linkages, it also contains ≈5 mol % of carbohydrates (i.e., ≈3.9 mol % mannose and ≈1.25 mol % n-acetylgalcosamine)16 as well as a high level of phytate (≈1.41 mol %). The presence of the carbohydrates on the β-conglycinin could probably account for an increase in steric hindrance between protein molecules both in solution and at the negatively charged solid surface. In addition, it is important to consider the difference in the isoelectric point of the subunits that compose each protein.

For the glycinin molecule, the isoelectric point of its acidic subunits ranges from 4.75 to 5.40 and that of the basic subunits from 8 to 8.5. For β-conglycinin, the isoelectric point is ≈4.9 for the α subunit, ≈5.2 for the α’, and between 5.7 and 6.0 for the β subunit. It is clear that at pH 7, all the subunits of both proteins carry net negative charges; thus, if only electrostatic interactions are involved in the adsorption, the interaction with the surface should be favored at higher ionic strengths. However, this was not what was observed.

A comparison of adsorbed mass and the theoretical packing for glycinin and β-conglycinin is shown in Table 2. The respective theoretical packing was calculated assuming the respective molecular dimension and mass of 11 × 11 × 7.5 nm3 and 350 kDa (glycinin5) and 9.6 × 9.6 × 4.4 nm3 and 180 kDa

![Figure 4](image-url). Schematic representation of soy glycinin changes under different physicochemical environments, as discussed by Badley et al.5 and Martin et al.45 (native glycinin molecule illustration reprinted from ref 5, with permission from Elsevier).

| Table 2. Maximum Experimental Adsorbed Mass, \( \Gamma_{\text{max}} \), of Glycinin and β-Conglycinin at Various pH and Ionic Strength Conditions Studied* |
|------------------|-----------------|------------------|-----------------|
| \( \Gamma_{\text{max}} \) | Glycinin or 11S | β-Conglycinin or 7S |
|                  | (mg/m²)         | (mg/m²)          |
| \( \text{silica} \) | \( \text{cellulose} \) | \( \text{silica} \) | \( \text{cellulose} \) |
| 0 mM NaCl, pH 3  | 15.4            | 7.23             | 9.33            | 2.97            |
| 100 mM NaCl, pH 7 | 17.3            | 13.6             | 7.04            | 1.27            |
| 10 mM 2-ME, pH 7  | 11.6            | 1.19             | 6.80            | 0.65            |
| 0 mM NaCl, pH 3   | 4.3             | 6.2              |                 |                 |
| 100 mM NaCl, pH 3 | 6.2             | 6.2              |                 |                 |

*The theoretical values are provided in the last two rows for side-on \( \Gamma_{\text{side on}} \) and end-on \( \Gamma_{\text{end on}} \) protein adsorption.
Rechendorff et al. reported on the adsorption of fibrinogen limited on the rougher cellulose surface. Therefore, these macromolecules in response to the change in the ionic environment.

The effect of association–dissociation of proteins on their adsorption behavior has been reported. Contrary to glycinin, which dissociates at low ionic strength, β-conglycinin forms a dimer with a sedimentation constant of 9S at low ionic strength. This dimer has more available sites for interaction and packing when exposed to surfaces and, thus, adsorbs readily. The lower adsorption of β-conglycinin at 100 mM NaCl ionic strength indicates that protein–protein interactions are favored over protein–surface interactions.

Circular dichroism measurements were performed in order to evaluate if the secondary structure of both glycinin and β-conglycinin were affected by changes in solution ionic strength. Although we did not quantify the amounts of α-helix and β-sheet secondary structures in each spectrum, it is apparent from results in Figure 3c that the signal for β-conglycinin exhibits a more positive ellipticity at 222 nm for the ionic strength of 100 mM NaCl, while glycinin shows lower ellipticity values, as seen in Figure 3d. This suggests a change in the secondary structure of the protein, a loss in the α-helical conformation of the macromolecules in response to the change in the ionic environment.

At first, it is tempting to associate the reduced adsorption with surface roughness. The effect of surface roughness on adsorption of proteins has been studied. For instance, Rechendorff et al. reported on the adsorption of fibrinogen and bovine serum albumin (BSA) onto surfaces of tantalum films. For fibrinogen, the authors indicated an increase of the adsorbed amount of protein with increasing surface roughness. We measured the roughness of the silica and cellulose films by using atomic force microscopy and obtained values of $\approx 0.18$ and $\approx 1.96$ nm, respectively. In contrast with the observations of Rechendorff et al., adsorption was more limited on the rougher cellulose surface. Therefore, these results are likely to be more related to the complexity of the soy protein molecules and their orientation on the surface upon adsorption.

The effect of ionic strength seems to be more critical for protein adsorption on cellulose relative to silica. Another factor to consider in explaining the lower adsorption onto cellulosic films involves deswelling of the cellulose substrate at higher ionic strength, which does not occur in the case of silica substrates. It is also expected that the hydration of the glycosilated 7S molecule decreases with the increase in ionic strength due to the dehydration of the linked carbohydrates molecules; thus, the molecules adsorbed carry less bound water.

**Effect of pH and Denaturation on Soy Protein Adsorption.** The effect of pH is critical in the functional properties and applications of soy proteins. Therefore, the adsorption of glycinin on ultrathin cellulose films is discussed in terms of adsorption isotherms (cf. Figure 5). Adsorption of this type of globulins was found to be higher at pH 7 relative to pH 3 (Figure 5a). It has been reported that the effect of pH on glycinin folding is more pronounced than that of the ionic strength. The amount of adsorbed nonstructured protein increases when the pH decreases from 7.6 to 3. It was found that low pH promotes higher unfolding (denaturation) of the protein molecules; this effect increases with increasing ionic strength. Low pH and low ionic strength conditions were found to promote dissociation of glycinin into subunits; these subunits comprise both acidic and basic chains of polypeptides, which possess a lower molecular weight; it is likely that, compared to the globular molecule, the shorter chain polypeptides adsorb on the surface in a flatter conformation.

From the circular dichroism data for the 11S protein (cf. Figure 5b) a shift toward more positive values of ellipticity is observed at higher wavelengths (>220 nm) at pH 3, indicating a change in the secondary structure of the protein. For instance, the ellipticity at 222 nm (indicative of α-helical structures) is almost zero at pH 3 compared to pH 7, which may indicate protein denaturation.

Finally, while the protein molecules may carry a net positive charge at low pH (i.e., pH 3) the negative charge of cellulosic surface decreases (because of smaller number of available negative groups for adsorption) due to protonation of carboxyl groups.

Soy glycinin contains a high amount of disulfide groups that affect strongly its physicochemical properties. It has been noted...
that the addition of 10 mM of 2-mercapto-ethanol (2-ME) is sufficient to cleave disulfide linkages in the glycinin molecule and prevent aggregation in solution; concentrations of $\approx 0.2$ M and above are needed to break the molecules fully into subunits. In the present experiments, 10 mM of 2-ME was used to evaluate the effect of denaturation on adsorption of glycinin and $\beta$-conglycinin. The results revealed a decrease in the adsorbed protein amount in the presence of 2-ME, with a more remarkable effect for cellulosic substrates (see Figure 6a,b). This can be explained by unfolding of the glycinin molecule in solution. For example, glycinin was reported to dissociate into subunits due to reduction with dithiothreitol (DTT); 5 mM DTT produced an increase of protein surface hydrophobicity, and the tyrosine and tryptophan residues exposed to the aqueous, polar environment increased the surface activity of the molecule. This effect was further noted to increase glycinin foaming and exhibit emulsifying properties. It is expected that 2-ME produces an effect similar to that of DTT. As a consequence, the adsorption of glycinin on hydrophilic surfaces decreases when the protein is reduced with 2-ME (Figure 6a).

Circular dichroism measurements were carried out to determine if 2-ME induced a disruption in the secondary structure of the soy globulins. It can be observed from Figure 6c that 2-ME induced a shift in the ellipticity of glycinin toward more positive values, which is associated with a loss of secondary structure, whereas for $\beta$-conglycinin the change was less pronounced (Figure 6d). This result is related to the more prominent presence of disulfide linkages in glycinin compared to $\beta$-conglycinin.

**Conformation of Adsorbed Protein Layers.** Figure 7 includes QCM $\Delta D-\Delta f$ curves (dissipation vs frequency profiles) from adsorption experiments conducted on silica, at several concentrations of glycinin (Figure 7a,b) and $\beta$-conglycinin (Figure 7c,d) at different ionic strengths studied. Only one surface type (silica) and pH is presented here and discussed to simplify the discussion. The presence of salt is discussed with the data plotted in Figure 7b. In Figure 7a, the large dissipation values suggest a more extended conformation of the adsorbed glycinin, probably due to electrostatic repulsions between the molecules. The change in slope of the $\Delta D-\Delta f$ curves, more noticeable in the case of glycinin, is indicative of different adsorption kinetics. At lower concentrations of protein (1 and 10 $\mu$g/mL), two different slopes are observed (suggesting a two-step kinetics), whereas at higher concentrations (100 and 1000 $\mu$g/mL), a three-step process is followed. Similar behaviors have been reported for other types of proteins.

An increased ionic strength favors the exposure of basic polypeptides on the surface of the protein; the basic polypeptides are normally expected to be buried inside the glycinin molecule because they comprise more hydrophobic amino acid units. At high ionic strength, water becomes a poor
solvent for such hydrophobic polypeptides, which, in turn, favors hydrophobic interactions at the substrate surface causing higher adsorption than in the absence of salt. The results shown in Figure 7b suggest that the layers of protein adsorbed carry less bound water and are more rigidly adsorbed to the surface when the ionic strength is increased.

In the case of $\beta$-conglycinin (7S), the results shown in Figure 7c and d indicate a trend similar to those that were discussed for glycinin, with lower values of dissipation (a maximum value of $3.5 \times 10^{-6}$ was measured). Such low values of dissipation suggest a flat and rigid layer of protein adsorbed. Interestingly, all $\Delta D-\Delta f$ curves for $\beta$-conglycinin with or without the presence of NaCl have similar slope indicating that in terms of viscoelasticity the proteins adsorbed followed a similar process, regardless of the solution concentration. Similar results to those for the ionic strength were found when 10 mM of 2-ME was added: a low dissipation of the adsorbed layers was observed for all concentrations studied.

Figure 8 depicts a schematic representation of the possible surface arrangements of glycinin under the different conditions studied: a more extended layer of protein is expected at low ionic strength while a high ionic strength favors a compact adsorbed layer due to the screening of electrostatic interactions. Low pH and addition of 2-ME promotes adsorption as thin layers.

Overall, adsorption of soy proteins onto solid substrates like other type of proteins is a complex process that depends
strongly on the physicochemical environment present. Glycinin exhibits a high affinity for both silica and cellulose substrates, which makes it a biopolymer of interest in further studies, especially in relation to fiber surface modification, adhesion, and in developing functional properties in fibrous systems.

■ CONCLUSIONS
Soy globulins 1S and 7S were successfully fractionated and characterized, and their adsorption onto silica and cellulose surfaces was investigated. The kinetics of the process indicated a Langmuir-type adsorption with faster dynamics in solutions at high protein concentration. Glycinin adsorbed to a higher extent when the ionic strength was increased while the opposite was observed for β-conglycinin, which reveals the different responses of the macromolecules to the physicochemical environment. Changes in ionic strength and pH affected the association–dissociation of the proteins and, thus, produced distinctive changes in adsorption. Adsorption of glycinin was comparable to that of synthetic polyanamines. Higher adsorption was observed at high ionic strength and solution pH was found to be critical in the modulation of the process. Adsorption of both globulins onto silica and cellulose was reduced after denaturation in the presence of 10 mM of 2-mercaptoethanol; this effect was more pronounced in the case of adsorption on cellulosic substrates.

■ ASSOCIATED CONTENT

Supporting Information
The model used to describe the kinetics of glycinin and β-conglycinin adsorption onto silica and cellulose and the respective variables of apparent binding and Langmuir constants at various solution pH are available. This material is available free of charge via the Internet at http://pubs.acs.org.

■ ACKNOWLEDGMENTS
The authors are thankful for funding support provided by the United Soybean Board (USB) under project numbers 0426 and 0490. Drs. Robina Hogan and Connie Howe (Omni Tech International, Ltd.) and also Tom Theyson (TensTech, Inc.) and Russ Egbert (Archer Daniels Midland, ADM) are gratefully acknowledged for helpful discussions and for their support. Dr. Charles Hardin, NC State’s Biochemistry Department, is thanked for allowing access to circular dichroism instrumentation. Samples of soy flour from ADM were received as a gift. Pure fractions of 7S and 11S were provided by Dr. David Sessa (USDA’s Plant Polymer Research), which were used as reference in SDS-PAGE analyses.

■ REFERENCES