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On the Surface Interactions of Proteins with Lignin

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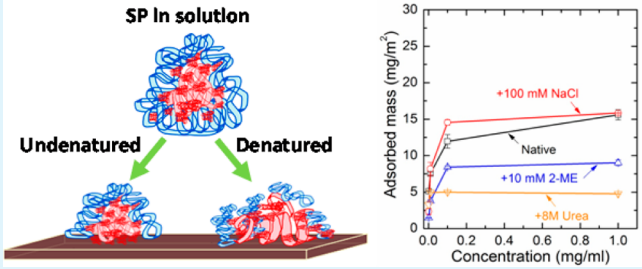
Supporting Information

ABSTRACT: Lignins are used often in formulations involving proteins but little is known about the surface interactions between these important biomacromolecules. In this work, we investigate the interactions at the solid−liquid interface of lignin with the two main proteins in soy, glycinin (11S) and β-conglycinin (7S). The extent of adsorption of 11S and 7S onto lignin films and the degree of hydration of the interfacial layers is quantified via Quartz crystal microgravimetry (QCM) and surface plasmon resonance (SPR). Solution ionic strength and protein denaturation (2-mercaptoethanol and urea) critically affect the adsorption process as protein molecules undergo conformational changes and their hydrophobic or hydrophilic amino acid residues interact with the surrounding medium. In general, the adsorption of the undenatured proteins onto lignin is more extensive compared to that of the denatured biomolecules and a large amount of water is coupled to the adsorbed molecules. The reduction in water contact angle after protein adsorption (by ∼40° and 35° for undenatured 11S and 7S, respectively) is explained by strong nonspecific interactions between soy proteins and lignin.



KEYWORDS: *adsorption, lignin, proteins, soy glycinin, beta-conglycinin, quartz crystal microbalance*

INTRODUCTION

Industrial interest in environmentally friendly materials has driven research in a variety of products from natural resources. Soybean proteins are among the most investigated natural compounds for nonfood applications including wood adhesives, films for food packaging, composites, biobased plastics, and paper coatings.[1] In addition to lipoxygenases, the main components in soybeans comprise the so-called storage proteins, a mixture of two macromolecules (glycinin and β-conglycinin) held together by disulfide linkages. Soy glycinin (11S) has a molecular weight of ≈320−350 kDa and is composed of acidic and basic subunits (denoted A1, A2, A3 and B1, B2, B3). β-conglycinin (7S) has a molecular weight of ∼180 kDa and contains three subunits (α, α′, β).[2,3] The acidic subunits in glycinin, and the α′s (α, α′) subunits in β-conglycinin, have hydrophilic character whereas the basic and β subunits of glycinin and β-conglycinin, respectively, are hydrophobic. These polypeptides chains arrange themselves forming soy proteins's quaternary structure (Figure 1). Different procedures for fractionation and purification of glycinin and β-conglycinin have been reported in the literature.[4,5] Most of the nonfood applications of soybean proteins take advantage of their thermoplastic behavior.[1,6] However, despite their industrial relevance, the nature of the adsorption and interfacial interactions need to be elucidated in order to benefit from soy protein and lignin unique functionalities in composite materials. Recently soy and other proteins were used as a platform for surface modification and to generate functional coatings via polymer grafting.[7,8] Thermal denaturation improved the adsorption of 11S and 7S onto hydrophobic surfaces, reducing the contact angle and allowing macromolecular coupling.[8]

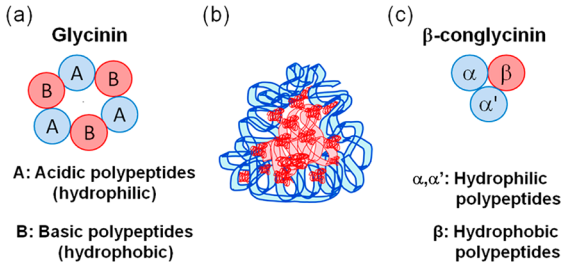


Figure 1. Schematic representation of the structure of (a) soybean glycinin 11S and (c) β-conglycinin 7S (see refs 25,26 for related information). (b) A schematic illustration of the distribution of hydrophobic residues in soybean glycinin and β-conglycinin is shown in (b) (see ref 24).

From the colloidal chemistry point of view, soy proteins can be used as wet[9] or dry[10] additives. The nature of the interactions of proteins 11S and 7S with cellulosic surfaces

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has been recently reported under conditions relevant to papermaking.[11,12] The results indicated different behavior for each of the proteins; in the native state a higher adsorption onto silica compared to cellulose was observed. Adsorption was reduced when the proteins were partially denatured by a reducing agent (2-mercaptoethanol). The adsorbed protein molecules underwent conformational changes upon exposure to the surrounding medium of either hydrophobic or hydrophilic amino acid residues. The adsorption of 11S increased with the ionic strength, whereas the opposite was determined for 7S;[11] these observations were taken as an indication of the importance of their structural differences. Of potential industrial interest is the use of soybean proteins to improve bonding in recycled paper furnishes or low grade pulps that contain lignin. For example, the performance of commercial soy flour as dry strength additive for recycled pulp was studied at different conditions of pH and ionic strength and a synergistic effect of soy flour mixed with cationic starch was observed.[12] Lignin model films from different sources have been prepared to study adsorption of polyelectrolytes[13−15] and related interactions.[16] Soy proteins are naturally amphiphilic because they possess both hydrophilic and hydrophobic domains that from a physicochemical perspective have the potential to interact strongly with lignin. Kawamoto et al.[17] studied the adsorption of bovine serum albumin (BSA) onto different lignins, including thio-lignin, lignin from steam explosion of a type of Japanese white birch (*Betula platyphylla*), acetic acid lignin, and milled wood lignin. Although no correlation was found between the amount of protein adsorbed and the molecular weight or number of phenolic hydroxyl groups, all lignins studied adsorbed BSA to a relatively large extent. The interactions of proteins with lignin are of fundamental importance.

Yang et al.[18] studied the adsorption of BSA onto cellulose- and lignin-containing surfaces (corn stover) to improve enzymatic hydrolysis. Their results show that BSA adsorbs more extensively on lignin-containing substrates compared to Avicel cellulose. A reduction in the nonspecific binding of cellulases and β-glucosidases by adsorbing BSA on the lignocellulose substrates was observed, which facilitated higher glucose yields. In addition, BSA has been claimed to be a "lignin blocker" that can facilitate the enzymatic hydrolysis process.[19] Other efforts to elucidate the role of lignin in enzymatic hydrolysis in bicomponent (cellulose-lignin) systems have been reported by us,[20] and indicate the occurrence of irreversible bonding between the enzymes and lignin. Lignin−protein interactions are also relevant in wood adhesion where the promotion of molecular contacts and the surface energy of the component play dominant roles.[21] Understanding the nature of lignin−protein interactions is critical in explaining the mechanism of adsorption/wetting in wood adhesives, solid dispersions, etc. Some of the unanswered questions include: What are the forces involved in the adsorption process of proteins to lignin? Can the interactions be modulated to improve/reduce adsorption?

In this work, we report on the adsorption behavior and interactions of soy proteins 11S and 7S with lignin as well as a hydrophobic surface (1-dodecanethiol self-assembled monolayers on gold) used as reference by the application of quartz crystal microbalance and complementary techniques, such as surface plasmon resonance and water contact angle measurements.

MATERIALS AND METHODS

7B defatted soy flour was provided as a gift from Archer Daniel Midland (ADM, Decatur, IL) and fractionated into glycinin (11S) and β-conglycinin (7S) (Figure 1) by following a procedure reported previously (see the Supporting Information, Figure SI-1, for nitrogen and protein contents).[11] Sodium phosphate monobasic anhydrous, sodium phosphate dibasic anhydrous, and urea (certified ACS) were purchased from Fisher Scientific (Somerville, NJ). 1-Dodecanethiol, ethanol (200 proof anhydrous), 2-mercaptoethanol, and 1,4-dioxane were purchased from Sigma Aldrich (St. Louis, MO). Reference organosolv lignin was supplied by Lignol and reported to contain low amounts of sulfur (~0.05%), sodium (17 ppm), potassium (44 ppm), and calcium (163 ppm).[22,23] We note that relative to technical kraft lignins, organosolv lignins are more hydrophobic.[22] All reagents were used as received. Milli-Q water with a resistivity of >18 MΩ cm was used in all experiments.

Lignin Films. Gold-coated AT-cut quartz crystals were used as resonators under thickness shear mode of vibration. They were cleaned by immersion in piranha solution (70% sulfuric acid, 30% hydrogen peroxide) for 5 min, rinsed thoroughly with Milli-Q water and exposed to nitrogen gas to dry. Before spin coating, the dry sensors were cleaned further using ultraviolet (UV)-ozone treatment for ten minutes. Lignin films were prepared following the procedure of Tammelin et al.[15] with a slight modification, namely, polystyrene was first spin-coated on gold as a supporting substrate for lignin deposition. Detailed preparation of the films can be found as Supporting Information.

Reference Self-Assembled Monolayers of Dodecanethiol. UV-ozone cleaned gold QCM resonators were immersed (for 18 h) in 1×10^{-3} M 1-dodecanethiol solution using ethanol as a solvent. A fully covering, self-assembled monolayer (SAM) of the alkylthiol was formed (ellipsometric layer thickness of ~3 nm) on the gold. This substrate was used as a hydrophobic reference surface.

AFM Imaging. AFM imaging was performed in tapping mode in air using a NanoScope III D3000 multimode scanning probe microscope from Digital Instruments Inc. (Santa Barbara, CA). Lignin films were scanned by using a NanoScope III D3000 multimode scanning probe microscope (Digital Instruments, Santa Barbara, CA). Scan sizes of 5×5 and 1×1 μm^2 were employed. At least three different areas on each sample were measured; no image processing except flattening was performed.

Quartz Crystal Microgravimetry. QCM-D E4 (Q-Sense, Gothenburg, Sweden) was operated in batch mode. QCM principles and operation have been described in detail elsewhere.[27−29] The mass adsorbed onto the QCM sensor is related to the shift of resonance frequency (Δf) according to the Sauerbrey equation (eq 1)[30]

$$\Delta m = -C\Delta f/n \quad (1)$$

where Δm is the change of mass (adsorption or desorption), Δf is the change in frequency, n is the overtone number, C is a constant, 17.7 ng cm^2 s^{-1} at f = 5 MHz. The energy dissipation or factor D accounts for the changes in viscoelastic properties of the mass adsorbed on the crystal and also for variations in the density and viscosity of the solution; simply stated D is the ratio of energy dissipated to the energy stored by the systems at the interface on the quartz crystal as given by eq 2:[29]

$$D = \frac{E_{\text{dissipated}}}{2\pi E_{\text{stored}}} \quad (2)$$

Typically, protein adlayers display a moderate dissipation; therefore, in this work, we used the Johannsmann[31] approach to calculate the mass adsorbed onto the surface (see the Supporting Information for details).

Adsorption experiments were carried out with lignin substrates as well as 1-dodecanethiol reference SAM. The substrates were exposed to protein solutions of different concentrations (1, 10, 100, and 1000 μg/mL) that were freshly prepared in 10 mM phosphate buffer at pH 7.0. Prior to any measurement, the sensors were allowed to equilibrate in water for about 2 h in order to register base signals for Δf and ΔD,

zeroed and allowed to run for 10 min before protein solution injection. Some measurements were conducted in buffer solution with added 10 mM 2-mercaptoethanol or 8 M urea; in these cases and prior to protein injection, the baseline was zeroed and signal stabilization allowed in the respective buffer solution (in the presence of the added components).

Surface Plasmon Resonance. Protein adsorption was also investigated by surface plasmon resonance (SPR) (SPR Navi 200, Oy BioNavis Ltd., Tampere, Finland). The sensitivity of the technique to detect small changes in refractive index ($\sim 1 \times 10^{-6}$) within time resolution of the order of milliseconds[32] have proved useful in biosensing applications. The excitation of the surface plasmons occurs at a given angle, the surface plasmon resonance (SPR) angle. In this condition, a sharp dip in the reflected light intensity occurs. Even small changes in the refractive index at the metal-surrounding medium interface are resolved by SPR. The response of the sensor to adsorbed mass (SPR signal) is expressed in resonance units or angle shift ($\Delta\theta$).[32] In this work, the thickness of the adsorbed protein layer was determined using eq 3

$$d = \frac{l_d}{2} \frac{\Delta\theta}{m(\eta_a - \eta_o)} \quad (3)$$

where d is the thickness of adsorbed layer, l_d is a characteristic evanescent electromagnetic field decay (usually estimated to be around 0.37 times the wavelength of the incident light, 240 nm),[32] m is a sensitivity factor of the sensor (109.95°/RIU, RIU: refractive index units) obtained by calculating the slope of a $\Delta\theta$ calibration curve for a series of glycerin aqueous solutions of different concentrations and known refractive indices.[33] η_o is the refractive index of the bulk solution (buffer, 1.334)[34] and η_a is the refractive index of the adsorbed species (proteins), which was assumed to be 1.57.[3] After the calculation of the thickness, the surface excess concentration was computed using eq 4.

$$\Gamma = \rho d \quad (4)$$

where ρ is the bulk density of the soy protein (1370 kg/m^3), determined from specific volume data (0.73 mL/g).[2]

The adsorbed mass was determined by SPR under the same set of conditions (concentration, temperature of 25 °C, pH, rinsing protocol, etc.) used in QCM experiments. In contrast to QCM, coupled water does not affect the SPR signal.[35] Thus the contribution of water coupled to the adsorbed layer can be calculated from the corresponding masses according to eq 5.

$$\%\text{coupled water} = 100 \frac{(\text{mass}_{QCM} - \text{mass}_{SPR})}{\text{mass}_{QCM}} \quad (5)$$

Water Contact Angle. The water contact angle of the surfaces was determined by using a dynamic contact angle (DCA) Phoenix 300 system (Seoul, South Korea). The images of the sessile drop were analyzed by using Image J software (National Institutes of Health, Bethesda, MD). In the case of the QCM sensors the initial advancing water contact angle was measured before and after protein adsorption.

RESULTS AND DISCUSSION

The noncontact mode AFM images of the polystyrene support before and after deposition of lignin can be observed in the Supporting Information (Figure SI-2). Both films were smooth with root-mean square surface roughness of 0.17 nm for polystyrene and 0.47 nm for lignin. The initial advancing water contact angle on each film was ~90 and ~65° for polystyrene and lignin, respectively.

Glycinin Adsorption onto Lignin Films. The organosolv lignin used is insoluble in water and more hydrophobic (water contact angle of ~65°) than Kraft lignins.[22] Hydrophobic interactions between the protein and the lignin substrate are anticipated. A QCM shift of frequency after native protein adsorption on lignin can be observed in Figure 2. The kinetics

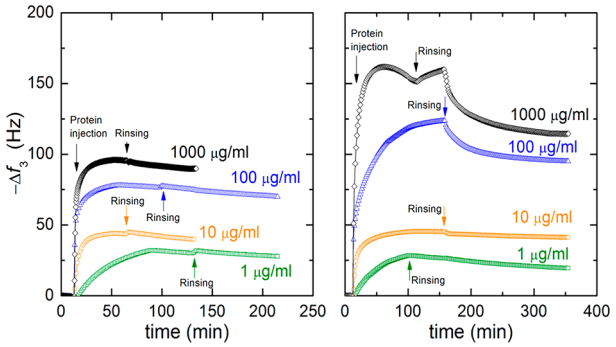

Figure 2. Shift in QCM frequency upon adsorption of 11S (left) and 7S (right) from aqueous solutions on lignin.

of the adsorption/desorption process can be determined from the initial slope of the curves. For both proteins, the adsorption is slower at low concentration (1−10 μg/mL) compared to that at higher concentrations (100 and 1000 μg/mL). The fact that frequency signals do not return to the baseline after rinsing suggests that the adsorption is irreversible. The larger shift in QCM reveals that 7S adsorbs more extensively than 11S.

Equilibrium adsorption isotherms for both soy glycinin (11S) and β-conglycinin (7S) indicate a significant adsorption in their native condition, as can be observed in Figure 3. The curves

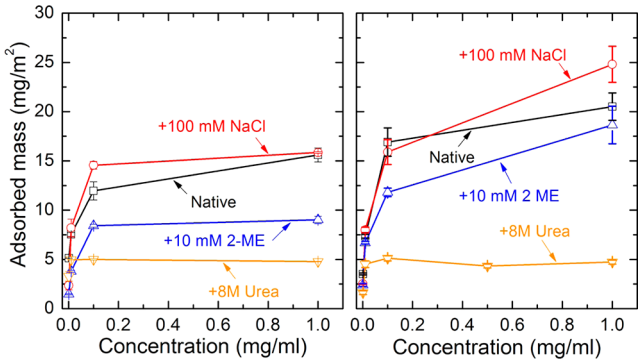

Figure 3. Adsorption isotherms of soybean proteins 11S (left) and 7 S (right) onto lignin films as determined by QCM-D. The error bars indicate the standard deviation from three repetitions. In some cases, the standard deviation is smaller than the size of the respective symbol.

indicate that for both 11S and 7S the increase in ionic strength has a negligible effect on the extent of adsorption; however, treatment with reducing 2-ME or denaturant 8 M urea reduces the adsorbed amounts for both proteins. More specifically, the addition of 10 mM 2-mercaptoethanol has a more negative effect on the adsorption of 11S compared to 7S, which is expected because 11S molecules contain more disulfide linkages.[36]

Protein adsorption on solid surfaces is driven by different types of nonspecific interactions such as electrostatics, hydrogen bonding, and hydrophobic forces. Electrostatic interactions between lignin and the proteins are probably less relevant since ionic strength does not appear to affect adsorption. There are still, however, protein intramolecular interactions that are affected by the ionic strength. It is expected that nonionic interactions play a prominent role. Nonionic interactions have been suggested as possible cause for the high adsorption of poly(acrylic acid) onto Kraft lignin films as studied by QCM; high dissipation values were observed, indicating a weakly

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attached layer of polymer.[14] Unlike kraft lignin, organosolv lignin contains less carboxylic groups and a large amount of phenolic hydroxyl groups;[37] therefore, it is also likely that the charged amino acids on the protein engage in weak electrostatic interactions and also hydrogen bonding with the scarcely available charged groups on the surface. An attempt has been made in the literature to correlate the adsorbed amount of bovine serum albumin (BSA) with the molecular weight of lignin and the number of phenolic hydroxy groups; however, besides the high adsorption observed for BSA onto lignin-containing substrates, no correlation was found.[17]

In their native state, proteins assume a globular conformation in aqueous solution in order to minimize interactions of the hydrophobic amino acids. Once proteins adsorb some denaturation occurs at the solid–liquid interface and the macromolecules spread on the surface.[38,39] The lower protein adsorbed mass in the presence of denaturants suggests that the respective adlayer is less hydrated. The molecules expose their hydrophobic amino acids upon addition of either 8 M urea or the reducing agent 2-mercaptoethanol, the former having a more remarkable effect. Urea is a denaturant that breaks hydrogen bonding and disrupts the secondary structure of proteins, while at the concentration studied here, 2-mercaptoethanol only breaks disulfide bonds in the molecules, leading to unfolding. It has been suggested that depending on the physicochemical environment and under the effect of 2-mercaptoethanol as reducing agent, the hydrophobic amino-acids associate into coils, and the hydrophilic polypeptides can form rod-like clusters (see Figure 4).[40] These smaller

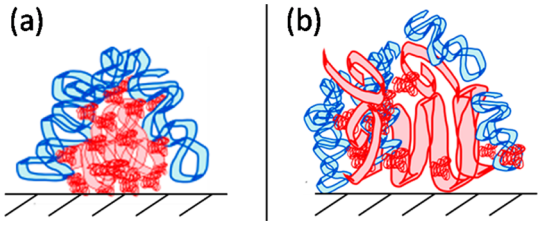

Figure 4. Schematic illustration of the possible conformation of hydrophobic (red) and hydrophilic (blue) polypeptides of soybean proteins upon adsorption from (a) native, undenatured form and (b) less ordered, denatured conditions (see ref 40 for additional information about soy protein molecular structure).

polypeptide chains behave differently from the globular-shaped native molecules. Figure 4 includes a schematic illustration of the possible conformation of the protein molecules upon adsorption.

Addition of urea can also produce a change in the properties of the liquid phase, which translates into an increased viscoelasticity of the adsorbed layers, as observed in Figure 5, where the change in dissipation (ΔD) is plotted against the shift of frequency (Δf). Compared to adsorption under the native or reduced (2-mercaptoethanol) condition, the presence of urea produces $\Delta D-\Delta f$ profiles with very steep slopes for both proteins.

Upon adsorption on relatively hydrophobic lignin films, the 7S protein exhibits higher dissipation values compared to 11S. This is in contrast to the behavior observed for adsorption onto hydrophilic, negatively charged surfaces (silica and cellulose).[11] This behavior is probably related to the structural differences of these two proteins and their response to changes in their physicochemical environment. In fact, the results suggest that

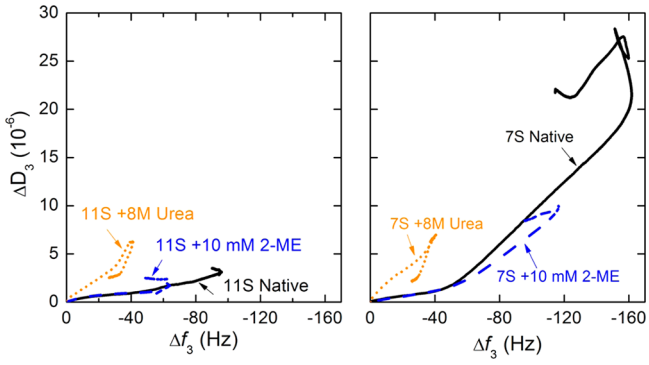

Figure 5. Dissipation frequency plots for adsorption of soybean proteins 11S (left) and 7S (right) onto lignin model films from 1 mg/mL aqueous solutions.

the adlayers of 7S protein adopt a more extended conformation, resulting in a better packing of the adsorbed molecules at the interface. In addition, it is expected that in the native state 7S has a higher hydration shell because of its high carbohydrate content (3.29 and 1.25 mol % mannose and N-acetylglucosamine, respectively),[36] which are measured in QCM as a high sensed mass. Table 1 summarizes the results

Table 1. Amount of Soybean Proteins Adsorbed onto Lignin Films under Different Aqueous Conditions[a]

	native	100 mM NaCl	2-ME	8 M urea
11S adsorbed mass, mg/m^2	15.6 ± 0.7	15.9 ± 0.07	9.0 ± 0.4	4.8 ± 0.02
7S adsorbed mass, mg/m^2	20.5 ± 1.7	24.8 ± 1.83	18.7 ± 1.9	4.7 ± 0.2

[a]The values are determined after adsorption from 1 mg/mL protein solution concentration.

for adsorption from 1 mg/mL solution concentration: it can be observed that for adsorption from solutions containing 8 M urea the amount adsorbed is practically the same for both proteins. If it is assumed that the molecules are dehydrated and adsorb side-on, the figures are close to a protein monolayer: 4.08 and 3.24 mg/m^2 for 11S and 7S, respectively.[11] For end-on adsorption, the values correspond to 7.05 (11S) and 7.08 mg/m^2 (7S).[11]

As observed in AFM images of adsorbed proteins (cf. Figure 6), aggregates are formed on the surface. Roughness values of 0.87 and 1.50 nm were determined for 11S and 7S, respectively, which imply that the 7S proteins form slightly larger aggregates. This observation agrees with the higher adsorbed mass registered for 7S compared to 11S.

The extent of adsorption onto lignin films was determined by using SPR in order to decouple the effect of water bound to the protein molecules. As expected, the isotherms shown in Figure 7 display values of adsorbed mass that are lower than those obtained from QCM. The adsorption profiles follow same trends as those observed in QCM isotherms, with similar amounts of adsorbed mass onto lignin for 7S and 11S protein. The amount of water coupled to the adsorbed layer from the highest solution concentration of protein (1 mg/mL) was calculated from eq 5 (see Table 2).

Not only does 7S protein exhibits higher water coupling (hydration) compared to 11S but the amount of proteins adsorbed is very close to that calculated for a monolayer

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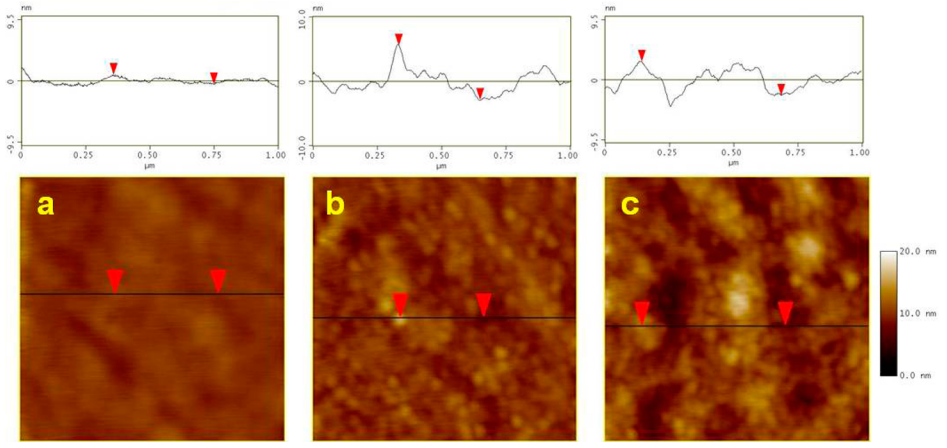

Figure 6. AFM images of lignin substrates of (a) bare lignin film (0.47 nm roughness) and after adsorption from 1 mg/mL solution of (b) 11S (0.87 nm roughness) or (c) 7S (1.50 nm roughness). Scan size is 1 × 1 μm^2.

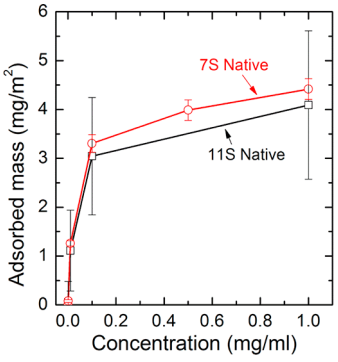

Figure 7. Adsorption isotherms for soybean proteins 11S and 7S onto lignin model films as determined from SPR results. The error bars indicate the standard deviation from three repetitions. In some cases, the standard deviation is smaller than the size of the respective symbol.

Table 2. Water Mass Coupled to Proteins Adsorbed on Lignin from 1 mg/mL Aqueous Solution Concentration

	adsorbed mass (mg/m²)			
	Γ_{QCM}	Γ_{SPR}	$\Gamma_{QCM}-\Gamma_{SPR}$ (mg/m²)	% coupled water
11S-native	15.6 ± 0.1	4.09 ± 1.5	11.5	74
7S-native	20.5 ± 1.4	4.41 ± 0.2	16.1	79

conformation, via side-on adsorption.[11] Apparently, the interactions of 7S globulin with lignin films are more favorable than those of 11S. This observation is in contrast to what was observed for cellulose substrates (see Table 3), where a larger adsorption was observed for 11S than that for 7S.[11] A study of BSA adsorption onto lignin-containing substrates (corn stover) and Avicel cellulose[18] reported a more extensive adsorption on lignin, in agreement with the current results obtained with soy proteins.

Table 3. Comparison of Soybean Protein Adsorption onto Lignin and Cellulose from 1 mg/mL Aqueous Solutions (values obtained from the isotherm plateau)

	lignin films Γ(mg/m²)		cellulose Γ(mg/m²)[11]	
	11S	7S	11S	7S
native	15.6 ± 0.70	20.5 ± 1.39	7.23 ± 3.13	2.98
100 mM NaCl	15.85 ± 0.071	24.8 ± 1.83	13.65 ± 2.71	1.27

Evidently the mechanism of adsorption is different for each protein studied. In the native state (i.e., low ionic strength) it is expected that 11S has more exposed acidic groups (negatively charged); in addition, 11S dissociates into a small biomolecule with the same sedimentation rate as the 7S molecule, so-called $\overline{7S}$.[3] In contrast, 7S proteins associates into larger structures with higher sedimentation rates, 9S.[36] Because in the present case it is expected that the lignin surface has a low density of charged groups, the interactions are likely driven by hydrophobic interactions or hydrogen bonding. It has been reported that 7S protein (β-conglycinin) possesses higher surface hydrophobicity than glycinin 11S, as determined from the slope of fluorescence measurements of protein solutions with fluorescent tag 1-anilino−8-naphthalene sulfonic acid.[4,41] This observation translates into better emulsification ability for the 7S compared to the 11S. These observations support the experimental results presented here.

To further explore the mechanism and interactions involved, the adsorption of the soy proteins 11S and 7S onto a reference hydrophobic surface was evaluated, namely SAMs of alkanethiol (1-dodecanethiol) on gold (water contact angle of ∼90°). The SPR technique was used to monitor the adsorption; moreover, QCM experiments were performed at the highest concentration of protein to determine the effect of coupled water and to determine the viscoelasticity of adsorbed layers.

Adsorption isotherms for all conditions studied are shown in Figure 8. Interestingly, the results follow an opposite trend to that observed previously for the lignin surfaces; compared to the 7S protein, 11S protein adsorbs to a larger extent in the native state and also in the presence of 10 mM 2-mercaptoethanol. The different adsorption behavior of 11S and 7S in the native state onto SAM can be related to the flexibility and conformation of the protein molecules. It is possible that in the presence of the more hydrophobic surface the 11S protein rearranges to allow tighter packing at the interface. 11S could also engage in S−S linkages associations once adsorbed due to the presence of free sulphydryl groups. The lower slope of the ΔD vs Δf plots (cf. Figure 9) in the cases of undenatured and denatured (2-mercaptoethanol) samples suggest a flatter conformation of the adsorbed layers of 11S compared to 7S, which can be related to a higher spreading of the molecule on the surface.

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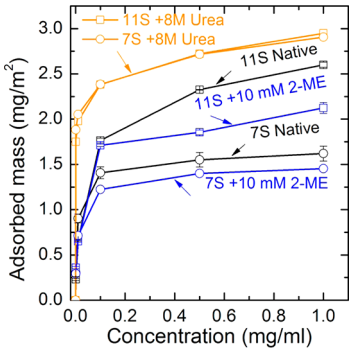

Figure 8. SPR adsorption isotherms of soybean proteins 11S and 7S onto SAM hydrophobic surfaces. The error bars indicate the standard deviation from three repetitions. In some cases, the standard deviation is smaller than the size of the respective symbol.

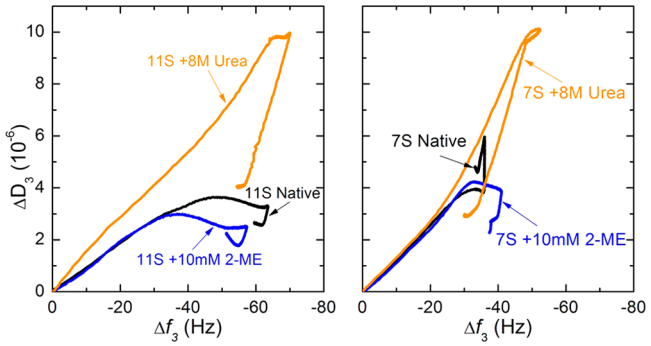

Figure 9. Changes in dissipation upon adsorption of soybean proteins 11S (left) and 7S (right) onto SAM hydrophobic surfaces from 1 mg/mL aqueous solutions.

For both proteins, the adsorption from 8 M urea solutions is higher than that in other conditions. This is in agreement with the fact that urea denatures the proteins and exposes their hydrophobic groups, which favors larger adsorption onto the hydrophobic surface. There is no possibility of hydrogen bonding with the SAM and these results suggests that the interactions with lignin involve not only hydrophobic interactions but hydrogen bonding and others. Since urea disrupts hydrogen bonding, this could explain why the adsorption is lower on a lignin surface when proteins adsorb from urea denatured conditions compared to the native state. A summary of results for adsorption on the hydrophobic SAM surface is presented in Table 4 together with the calculated coupled water. From Table 4 it is important to highlight two

Table 4. Mass of Water Coupled to Proteins Adsorbed onto Hydrophobic SAM from 1 mg/mL Solutions

	adsorbed mass (mg/m²)			
	Γ_{QCM}	Γ_{SPR}	$\Gamma_{QCM}-\Gamma_{SPR}$ (mg/m²)	% coupled water
11S-native	10.7 ± 0.32	2.6 ± 0.028	8.1	75.7
7S-native	5.95 ± 0.23	1.62 ± 0.08	4.33	72.8
11S +10 mM 2-ME	9.18 ± 0.22	2.13 ± 0.06	7.05	76.8
7S +10 mM 2-ME	6.78 ± 0.2	1.45 ± 0.01	5.33	78.6
11S +8 M urea	9.2 ± 0.14	2.95 ± 0.15	6.25	68
7S +8 M urea	5.4 ± 0.27	2.90 ± 0.04	2.49	46.3

things: (1) Compared to other conditions the percentage of coupled water is lower for adsorption from urea; this seems to favor a more extended viscoelastic layer of adsorbed protein on the surface as evidenced in the $\Delta D-\Delta f$ profiles in Figure 9, where the adsorption from urea exhibited the highest dissipation values and the steepest slope. (2) The high dissipation observed implies more extended protein adlayers, better packing at the interface and higher adsorption, probably in an end-on configuration. However, the adsorbed mass does not even reach the lowest expected value for a protein monolayer assuming side-on configuration (4.08 (11S) and 3.24 mg/m^2 (7S)).[11] It is worth mentioning that urea destroys the secondary structure of the protein, leading to a more random coiled molecule.[42−44] In fact, it has been reported[3,36] that concentrations of urea above 6 M can break the soybean protein molecules into subunits or small polypeptide chains which, depending on their nature (hydrophilic or hydrophobic), can adsorb assuming a loose conformation on the surface.

Change in Surface Wettability after Adsorption. Water contact angle (WCA) measurements were carried and reported as the difference between the initial WCA (bare surfaces) and the WCA after protein treatment. Results for both proteins in native condition after adsorption onto lignin are reported in Figure 10a. Compared with 7S, 11S produces the largest change

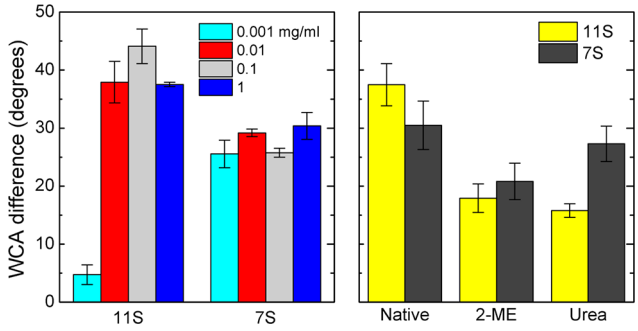

Figure 10. Water contact angle change upon adsorption of soybean proteins (11S and 7S) at different concentrations on (a) lignin model films and (b) 1-dodecanethiol self-assembled monolayers before and after denaturation (2-ME or urea). The error bars indicate the standard deviation from three repetitions.

in WCA after adsorption at the highest concentration studied. The change in contact angle upon adsorption onto dodecanethiol SAM is shown in Figure 10b. In the native state 11S displays the highest change in WCA; however, in the case of the more hydrophobic surface, dodecanethiol SAM, the highest change in WCA was observed after treatment by adsorption from solutions with 10 mM 2-mercaptoethanol or 8 M urea. The molecular differences between 11S and 7S can account for this result, for example, because 11S has a higher number of disulfide bonds. It is expected that the secondary structure is affected in the presence of 10 mM of 2-mercaptoethanol, as has been confirmed previously by circular dichroism.[11] The change in secondary structure might affect the conformation of protein molecules on the surface upon adsorption, this in turn enhances the interactions of hydrophobic amino acids with the substrate and facilitates the exposure of hydrophylic units away from the surface. Because 7S protein molecule contains carbohydrate moieties,[36] unfolding of the molecule via 2-mercaptoethanol or denatura-

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tion with urea, drives adsorption to minimize the interactions of the hydrophilic carbohydrate moieties with the hydrophobic surface. This can explain why under the given conditions the hydrophilicity is higher in the case of 7S treatment than for 11S.

Overall the results show favorable interactions of the soybean proteins with lignin and the ability of proteins to modify the surface energy of the substrates upon adsorption. Further exploration of these interactions is required to unveil novel uses that combine these biomacromolecules. The favorable interactions between lignin and the partially hydrophobic residues of proteins and the resultant exposure of hydrophilic groups of the protein in the presence of aqueous surrounding media suggests that it is possible to apply these proteins onto, for example, hydrophobic substrates (fibers, textiles, etc.) and obtain water-wettable surfaces.

CONCLUSIONS

Adsorption of soybean proteins glycinin (11S) and β-conglycinin (7S) onto lignin surfaces and hydrophobic self-assembled monolayers has been studied and compared. Our results reveal different adsorption behaviors for each protein, which highlights the complexity of protein adsorption onto solid surfaces. A higher adsorption onto lignin films is observed compared to cellulose. The changes in water contact angle (wettability) of the lignin surfaces upon soybean proteins adsorption imply that once in contact with lignin these proteins can spread on the surface and change its wettability; it is thus anticipated a better contact between wood surfaces and an improved adhesion in respective composites. Overall, favorable interactions with hydrophobic substrates were measured, which could be used as rationale for the application of soy proteins in surface modification.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This include soy fractions nitrogen and protein contents; a description of the preparation of lignin thin films; AFM images of polystyrene support and lignin films; as well as contact angles and details about the model used to calculate the adsorbed mass by using quartz crystal microbalance. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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