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Surface Functional Properties of Native, Acid-Treated, and Reduced Soy Glycinin. 1. Foaming Properties

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Foaming properties of native and chemically modified glycinin were evaluated. Effects of ionic strength and glycinin composition and concentration on foam formation and stabilization were studied. Glycinin was modified by means of combined treatments: cold or hot acidic treatments, with or without later disulfide bridges reduction. Modified proteins obtained from glycinin present different degrees of dissociation, deamidation, and as consequence, varied surface hydrophobicity and molecular size. Parameters of forming and stabilizing of foam were correlated with both deamidation and dissociation degrees of modified and native glycinin samples. A positive relationship was observed between surface behavior and foaming properties of different protein species. Results show that dissociation, deamidation, and reduction have produced structural changes on glycinin (increased surface hydrophobicity, increased net charge, decreased molecular size) which enhance the adsorption and anchorage of proteins at the air−water interface and, consequently, improve the foam forming and stabilizing capacities.

Keywords: *Glycinin; 11S globulin; soy protein; surface properties; foaming properties; chemical modification*

INTRODUCTION

Glycinin (protein 11S-type), one of the major globulins of soybean seeds, is characterized by an hexameric structure $(AB)_6$ with a molecular mass around 360 kDa. Its quaternary structure is stabilized by electrostatic and hydrophobic interactions and by disulfide bridges between A and B polypeptides, constitutive of the subunits (Peng et al., 1984).

The foaming properties of glycinin have been extensively studied (Kinsella, 1979) and were shown to be limited by its close packed globular conformation (Kim, 1985). In a previous work (Wagner and Guéguen, 1995), we have observed that native glycinin—which keeps its hexameric structure in standard phosphate buffer (ionic strength $\mu = 0.5$, pH 7.6) (Thanh and Shibasaki, 1976)—has difficulties in adsorbing at the air−water interface, owing to its low surface hydrophobicity, low molecular flexibility, and high molecular size. Surface behavior and functionality of 11S-type proteins could be enhanced by appropriate and controlled modification of its structure by reduction, succinylation, or acetylation (Kim and Kinsella, 1987a,b; Guéguen et al., 1990; Schwenke et al., 1993; Krause et al., 1996). Wagner and Guéguen (1995) have shown the effect of dissociation, deamidation, and reduction on structure and surface behavior of glycinin. In this regard it was found that native glycinin is able to dissociate as a consequence of the low ionic strength and acidic medium. The dissociated

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glycinin exhibited a better surface behavior (higher surface pressure), which improved even more when adding an increase of surface charge (by deamidation in hot acid medium) and/or reduction of disulfide bridges between A and B polypeptides by thermal treatment in the presence of a reducing agent. The protein species obtained by these chemical modifications had good adsorption kinetics in the interface, regardless of the ionic strength of the medium. It was then expected that these characteristics would influence surface properties, such as foam and emulsion forming and stabilizing capacities.

In the present work, the objectives were to study the influence of structural and surface behavior changes of glycinin (from progressive dissociation−deamidation and reducing treatments) on foaming properties.

MATERIALS AND METHODS

Chemical reagents of analytical grade were obtained from Merck, Pharmacia, and Sigma Chemical Co. Seeds of soybean varieties "Kador B10" and "Alaric", obtained from the Station de Génétique et D'amélioration des Plantes (INRA, Montpellier), were employed. The seeds were dehulled, ground, and defatted by Soxhlet extraction with hexane, which was then removed at room temperature.

All the solutions were prepared with Millipore water, and to them were added 1 mM benzamidine as protease inhibitor and 0.02% sodium azide as antimicrobial agent, even to those used for dialysis.

Preparation of Glycinin. Crude glycinin was prepared from defatted soy flour by the procedure of Thanh and Shibasaki (1976) adapted by Kim and Kinsella (1987a), with slight modifications (Wagner and Guéguen, 1995). The purity of the crude glycinins was higher than 90% (determined by SDS−PAGE and affinity chromatography in ConA-Sepharose 4B). The nomenclature for the crude glycinin samples thus obtained from seeds of soybean varieties Kador B10 and Alaric is CGI and CGII, respectively.

Purification of Glycinin. The purification of glycinin was carried out from crude glycinin (CGI) by affinity chromatography with ConA-Sepharose 4B (to remove β-conglycinin) followed by gel filtration on Sephacryl 300 (to remove the glycinin aggregates and other minor proteins). The CGI solution (20 mg/mL in 0.035 M phosphate, 0.4 M NaCl buffer pH 7.6, ionic strength $\mu = 0.5$—namely standard phosphate buffer by Thanh and Shibasaki (1976) was added to the ConA-Sepharose 4B column and eluted with standard phosphate buffer. The nonadsorbed fraction (containing glycinin) was then chromatographed on the Sephacryl column using $(NH_4)HCO_3$ 1% solution, pH 8, as eluent. The principal fraction (corresponding to larger peak) was collected, freezed with N_2 liquid, and then lyophilized. The nomenclature of the purified glycinin sample thus obtained was PGI. The purity of the PGI sample was higher than 99% (determined by SDS−PAGE).

Preparation of Modified Glycinins. Acid dissociation and deamidation and reducing treatment of crude glycinin were carried out as described previously by Wagner and Guéguen (1995). CGII sample (10 mg/mL) was treated in 0.1 M HCl without heating (4 °C; 9 h: treatment 1) and with heating (70 °C; 1.5, 3, 4.5, 6, and 9 h: treatment 2). The resulting solutions were neutralized with 1 M NaOH, dialyzed against water and freeze-dried. The deamidation degree of resulting samples was 0, 1.7, 5.9, 10.6, 16.1, and 25.0, respectively. The acid-treated samples were reduced (treatment 3) as follows: the acid-treated samples were solubilized at 0.25−0.5% in 0.03 M Tris-HCl buffer, pH 8, with 0.2% $Na_2S_2O_5$ as reducer agent of disulfide bonds. The protein solutions were then heated at 100 °C for 5 min. After treatment, solutions were cooled in ice water and centrifuged at 10000*g* for 15 min. The supernatants were dialyzed against water and then freeze-dried. The degree of deamidation of the resulting reduced samples was the same as those in nonreduced ones.

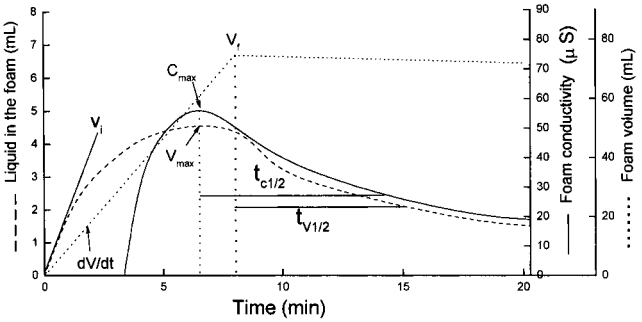

Figure 1. Experimental determination of foam formation and stability by measures of liquid volume in the foam (- - -), foam conductivity (—), and foam volume (···). Liquid volume in the foam was calculated from measures of liquid conductivity. Assays were performed with crude glycinin I at 1 mg/mL standard phosphate buffer ($\mu = 0.5$, pH 7.6). Parameters were v_i, dV_f/dt, C_{max}, V_{max}, V_f, $t_{c1/2}$, and $t_{v1/2}$ as described in Materials and Methods.

Degree of Deamidation. The degree of deamidation was determined by measurement of the ammonia released due to a moderated protein hydrolysis (Matsudomi et al., 1985).

Gel Filtration. Exclusion−diffusion superose 12 HR column connected to the LKB HPLC equipment was used. The procedure was as described by Wagner and Guéguen (1995). Proteins were detected at the column outlet at 280 nm. The relationship between the area of each peak and the total area determined the percentage of protein species.

Ultracentrifugation. The ultracentrifugation was performed in a 5−20% sucrose density gradient established in standard phosphate buffer, using a Beckman XL-70 type ultracentrifuge equipped with a swing-out rotor (Type SW 40 TI), at 30 000 rpm for 16 h at 20 °C. The procedure for determining the sedimentation coefficients of the samples was as described by Wagner and Guéguen (1995).

Adsorption Rate at Air−Water Interfaces. The surface tension γ (mN/m) was measured at 20 °C by the Wilhelmy Plate technique using a PROLABO tensiometer according to the procedure described by Wagner and Guéguen (1995). The γ−time curves were recorded. The solubilization and measurement buffer was 0.1 M sodium phosphate, pH 8, and the protein concentration was 0.02 mg/mL. The surface pressure ($\pi_5 = \gamma_0 - \gamma_5$) after 5 min of adsorption was kept as a measure of the adsorption rate during the first step of adsorption, according to the procedure of Kim (1985).

Compression Isotherms (π vs A). An IBM-AT computer-controlled Langmuir trough with a Wilhelmy balance was used to record π−A isotherms. The surface area could vary from 720 to 88 cm^2 with a constant-perimeter barrier made of PTFE tape. The monolayer was prepared by spreading the protein solution on the water surface using the Trutnit method. The initial superficial concentration was between 2.1 and 70.0 mg/m^2. After a 1 h equilibration period, the film was compressed at a speed of 10 mm/min.

Foaming Properties. Foaming properties were evaluated by conductimetry using the method and apparatus developed by Loisel et al. (1993). Foam was formed by air sparging into the protein solution in a column having a fritted glass disk (G4 type) at the bottom. Air was sparged at a flow rate of 10 mL/min through 13 mL of 0.3−2 mg/mL of native and modified glycinin in low and high ionic strength buffer until a fixed volume of foam was reached (75 mL). Figure 1 shows an example of experimental measures on foam formation and stability during 20 min. The level of the solution as a function of the time was measured by conductimetry with a pair of electrodes located at the basis of the column. The maximum volume of liquid incorporated into the foam (V_{max}, mL) and the rate of liquid incorporation to the foam (v_i, mL/min) were determined. The foam conductivity (in microsiemens) as a function of the time was measured with a second pair of electrodes at the middle of the column. The maximum conductivity of foam (C_{max}) was measured. The rate of liquid

drainage was determined as both the time for draining half the liquid incorporated into the foam at the end of the bubbling period ($t_{v,1/2}$, in minutes) and the time for diminishing half the foam conductivity at the end of bubbling ($t_{c,1/2}$, in minutes). Foam stability was calculated as the specific rate constant of liquid drainage, $K = 1/(V_{max}t_{1/2})$ (mL^{-1} min^{-1}) (Elizalde et al., 1991). The volume of foam was directly recorded by a uniline camera and that of gas by a flow meter. Foaming capacity was measured as $FC = dV_f/dG$, where V_f and G are the volumes of foam and sparged gas, respectively. Determinations were performed, at least, in duplicate.

RESULTS AND DISCUSSION

Foaming Properties of Native Glycinin. The experimental setup allows the foam forming and destabilizing process to be recorded in different ways: (a) liquid conductivity, (b) foam conductivity, and (c) foam volume (Figure 1). With methods a and b, we can obtain information about foam density (V_{max} and C_{max}, respectively), stability against liquid drainage ($t_{v1/2}$ and $t_{c1/2}$, respectively), and corresponding drainage constants (K_v and K_c). In all samples studied, these parameter pairs showed a linear trend with acceptable correlation coefficients (V_{max} vs C_{max}: $r^2 = 0.97$; $t_{v1/2}$ vs $t_{c1/2}$: $r^2 = 0.94$; K_v vs K_c: $r^2 = 0.997$). These results indicate that the information about foam stability provided by alternative a or b is basically the same. The advantage of alternative a is that it provides, in addition, the initial rate of liquid incorporation into the foam (v_i). Therefore we choose the determination of liquid volume into the foam rather than foam conductivity. Concerning point c (foam volume), it allows the determination of the foaming capacity and foam collapse once the final foam volume is reached ($V_f = 75$ mL, end of bubbling).

Influence of Native Glycinin Composition. In a first stage of the present work, we studied the effect of purity degree of native glycinin on its capacity to form and stabilize foams. Three samples were used: crude glycinin I (CGI), crude glycinin II (CGII), and purified glycinin I (PGI). The HPLC and ultracentrifugation analysis in standard phosphate buffer showed that native glycinins are composed basically of three molecular species: 15S (aggregates of MM > 1400 kDa), 11S (hexameric form, MW = 360−380), and 4S (monomeric form, MM = 60 kDa) (Wagner and Guéguen, 1995). Differences in the percentages of 15S, 11S, and 4S formed in each sample were observed: 24.9, 61.6, and 13.4% for CGI; 21.9, 71.5, and 6.5% for CGII; 23.4, 76.6, and 0% for PGI, respectively.

The study of the foaming property of the three glycinin samples is shown in Figure 2. It is observed that CGI exhibits the highest v_i, V_{max}, and $t_{1/2}$ values and the lowest value of K. Greater v_i means stronger tensioactive effect, as described later in this work. Greater V_{max} values means that more hydrated and, therefore, dense foam is formed. The longer the $t_{1/2}$ is, the greater the foam stability against drainage will be. K is a parameter which includes V_{max} and $t_{1/2}$ in such a way that its decrease reflects hydrated, dense, and more stable foam. Thus, the CGI sample resulted as the better foaming agent. This behavior of CGI could result from the slight dissociated state of its hexameric form 11S, revealed by the presence of monomeric form 4S in a proportion of 13%.

In contrast, CGII and PGI samples showed a deficient foaming behavior, which were comparable both in formation capacity and stability. This indicates that, although CGII is, comparatively, slightly more dissociated than PGI (6.5% of 4S), the dissociation is not enough to show appreciable differences in foaming property. Thus on the basis of these results, all following tests, including the preparation of modified glycinin, were conducted on CGII.

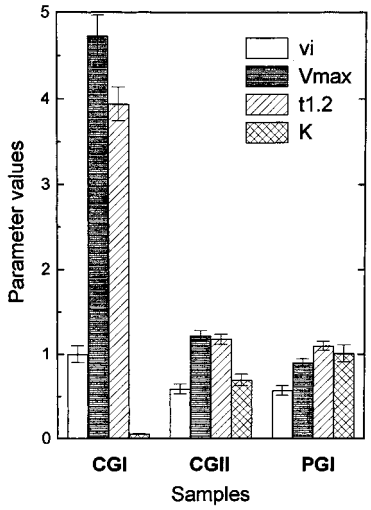

Figure 2. Foaming properties of crude glycinin I (CGI), crude glycinin II (CGII), and purified glycinin I (PGI). Assays were performed with sample solutions at 1 mg/mL standard phosphate buffer ($\mu = 0.5$, pH 7.6). Parameters were v_i (in mL/min), V_{max} (in mL), $t_{1/2}$ (in min), and K (in 1/mL.min) as described in Materials and Methods.

Influence of Ionic Strength and Protein Concentration. It had been observed that the ionic strength of the medium, by inducing association−dissociation of the glycinin, influences the adsorption kinetics in the air−water interface (Wagner and Guéguen, 1995). It is expected that the same effect will occur both during native glycinin solubilization and during the foam forming process.

The effect of the ionic strength in CGII solubilization (sodium phosphate solutions with $\mu = 0.006-0.28$, pH 7.6) on the foaming parameters performed at constant ionic strength ($\mu = 0.5$ by addition of solid NaCl) was studied (Figure 3a,b). It is observed that the lower the μ value is in the solubilization of CGII, the greater the values of v_i, V_{max}, and $t_{1/2}$ and the lower the value of K will be. These results may indicate that native glycinin suffers dissociation during its solubilization, which is induced by the low μ of the medium, as previously reported (Wagner and Guéguen, 1995). This has an impact during the foaming, since the glycinin would present a better behavior as surface agent.

To study the μ effect on the foaming process itself, the CGII was solubilized in 0.02 M phosphate, pH 7.6, solution, and variable amounts of solid NaCl were added to the resulting solution a few seconds prior to foam formation. It could be seen that the gradual increase in ionic strength favors liquid incorporation during the foam forming process (greater v_i and V_{max}) (Figure 4a). The stability against drainage also increases with the ionic strength (greater $t_{1/2}$, lower K), though it remains constant from 0.25 M on, indicating that neutralization of electrostatic forces plays an important role in the integrity of the foam (Figure 4b). Previous works have shown that at 0.2 M NaCl there is an increase of surface hydrophobicity (Wagner and Añón, 1990) which enhances hydrophobic interactions (Damodaran and Kinsella, 1982). These interactions would be required for stabilizing the foam by increasing the rigidity of protein

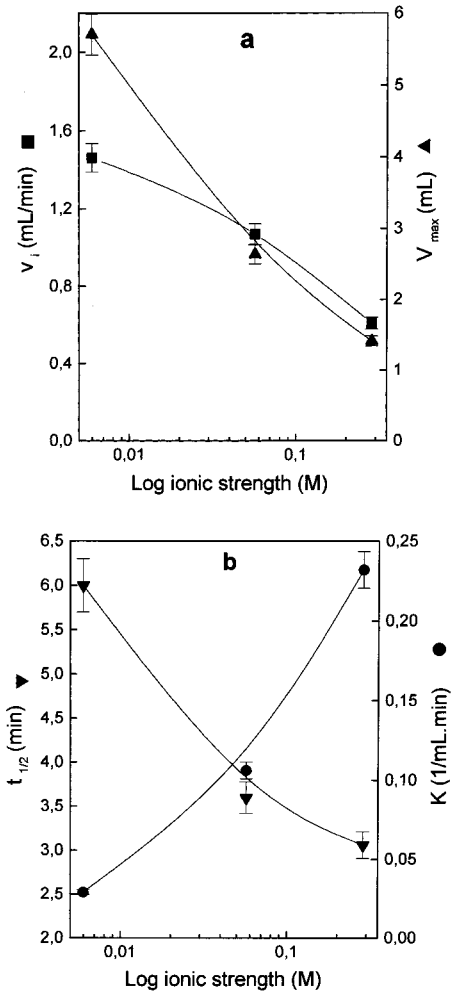

Figure 3. Influence of the ionic strength of the sample solubilization process on the foam forming (a) and stabilizing (b) parameters. Foam assays were performed at constant ionic strength (0.5, by addition of solid NaCl) using crude glycinin II solution (1 mg/mL sodium phosphate, pH 7.6) prepared at different ionic strengths. Parameters were v_i (■), V_{max} (▲), $t_{1/2}$ (▼), and K (●) as described in Materials and Methods.

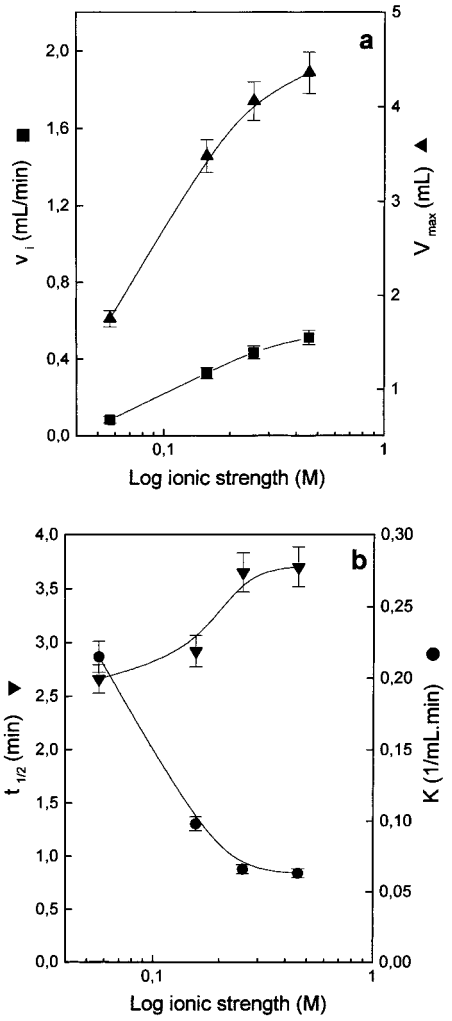

Figure 4. Influence of the ionic strength of the foaming process itself on the foam forming (a) and stabilizing (b) parameters. Foam assays were performed at different ionic strengths using crude glycinin II solution (1 mg/mL of 0.02 M sodium phosphate, pH 7.6) with variable amounts of solid NaCl added. Parameters were v_i (■), V_{max} (▲), $t_{1/2}$ (▼), and K (●) as described in Materials and Methods.

film. Yu and Damodaran (1991) found, for soy protein isolate, a sharp increase in foam stability (predominantly due to a decrease in the gravitational drainage rate) up 0.1 M NaCl. Therefore, it can be concluded that low ionic strength can slightly improve foam formation by partial glycinin dissociation but that high ionic strengths are required for dense and stable foams.

An important point is that dissociation, particularly for native glycinin (which is the reference structure), would be minimized during solubilization and foaming. For this reason, the condition selected for such stages in the comparative tests of native and modified glycinin was the standard phosphate buffer, which has an ionic strength of 0.5.

To observe the influence of protein concentration on the foaming phenomenon, we prepared solutions of CGII (0.3, 0.5, 1, and 2 mg/mL) in standard phosphate buffer. At 0.3 mg/mL, the foam did not reach the 75 mL expected (it only reached 38 mL), giving a low foaming capacity (FC $\approx$ 0.4). For concentrations higher than 0.5 mg/mL, the foam reached the 75 mL expected, with a FC $\approx$ 0.8, which reflects acceptable air retention. For concentrations of 0.5, 1, and 2 mg/mL, the v_i values were 0.78, 1.00, and 2.34, while K values were 0.135, 0.0536, and 0.033, respectively. The results confirmed the previously reported effect of protein concentration on the foaming process (Yu and Damodaran, 1991; Halling, 1981).

Foaming Properties of Modified Glycinin. *Effect of Chemical Treatment.* The foaming properties of modified glycinin samples were determined at 0.5 mg/mL (minimal concentration of CGII sample in which the fixed volume of foam was reached). We analyzed only the parameters v_i and K that give information specifically related to the foam forming and stabilization processes, respectively. The soluble protein resulting from three modifications were employed (for details, see Materials and Methods): treatments of the CGII in cold acidic medium (treatment 1: dissociation and unfolding), treatments of the CGII in hot acidic medium (treatment 2: modifications as in 1 plus deamidation and limited hydrolysis), and reducing thermal treatment applied to the resultant species of 1 and 2 (treatment 3: modification as in 1 or 2 plus cleavage of disulfide bonds).

Values of v_i and K of native glycinin CGII and of the modified glycinin by means of treatments 1 and 2 for different deamidation degrees) were compared (Figure 5a). Just by the effect of the cold acid medium treatment

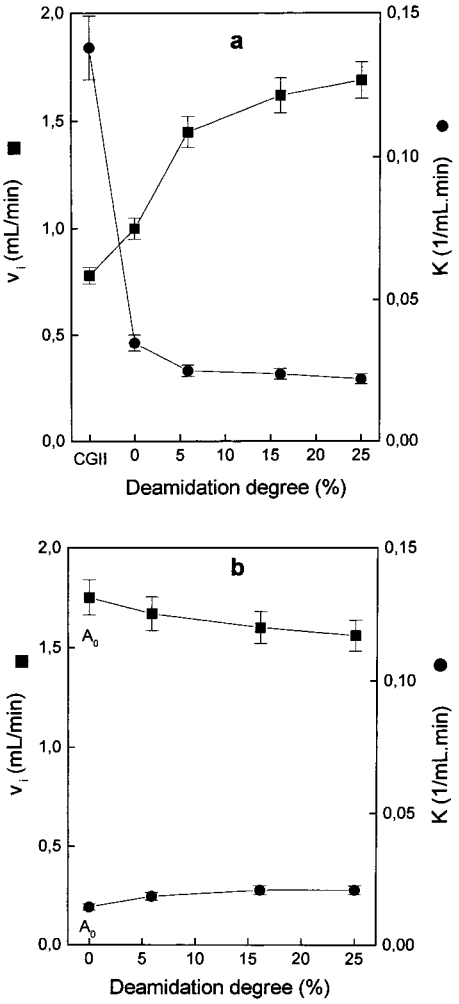

Figure 5. Foaming parameters (v_i and K) of native crude glycinin II (CGII), and modified glycinin samples (soluble proteins resulting from treatment of crude glycinin II in cold acidic medium (deamidation degree, D = 0%) or hot acidic medium (D = 6−25%) without (a) and with (b) reducing treatment). Assays were performed with sample solutions at 0.5 mg/mL standard phosphate buffer (μ = 0.5, pH 7.6). Parameters were v_i (■) and K (●) as described in Materials and Methods.

applied to CGII (deamidation degree D = 0%), a pronounced increase of foam stability is observed (K decreases by 75% of the value of native glycinin). Foam formation also improves with this treatment, though in a much lesser extent (v_i increased by 22% with respect to native glycinin value). The latter property only begins to show a significant and progressive increase with deamidation, as it is in the case of glycinins treated in hot acid medium. In this regard, the foam stability also improves with deamidation; for instance, at 5.9% deamidation, the K value decreases by 30% (with respect to D = 0) and then remains practically constant for higher deamidation degrees. Previous works have reported that the foam forming and stability of soy protein isolates could be increased with mild acid treatment (Matsudomi et al., 1985; N Guyen Thi Quynh et al., 1992). Our results coincide with those of Matsudomi et al. (1985) and show that a deamidation degree of 6−16% is enough to improve the foaming properties considerably. These results could be partially attributed to the higher surface hydrophobicity (H_0) exposed by modified glycinins as result of dissociation and deamidation processes (Wagner and Guéguen, 1995). Previous reports have emphasized the contribution of protein hydrophobicity to its surface functionality (Kato and Nakai, 1980; Nakai et al., 1986). Native glycinin has a H_0 value of 1.53, while that for glycinin dissociated by cold acid medium is twice higher (H_0 = 3.13). On the other hand, glycinin treated by hot acid medium exhibits greater H_0 values of 3.7, 4.28, 4.35, 4.02, and 3.52, which correspond to deamidation degrees of 1.7, 5.9, 10.6, 16.1, and 25.0%, respectively, showing an extensive unfolding of subunit AB polypeptides, as was previously reported by us (Wagner and Gueguen, 1995). In addition, the exposure of B polypeptides (very hydrophobic and placed in the interior of the glycinin molecule), caused by the hydrolysis of A polypeptides, could also explain the improvement of foaming properties of deamidated glycinin with the increase in surface hydrophobicity.

Figure 5b shows that the soluble samples obtained by the reducing treatment (treatment 3) are better foaming agents than the corresponding nonreduced samples. At difference of these samples, v_i values tend to decrease with increasing deamidation degree, while the value of K increased in contrary. Thus, the sample with best foaming properties is the soluble protein resulting from the reducer treatment of the glycinin treated in cold acid medium. This sample was named A_0 by Wagner and Guéguen (1995) because it consists mainly of A polypeptide with %D = 0. In contrast, the resulting samples of treatment 3 on deamidated glycinins—which are formed by the deamidated and partially hydrolyzed A polypeptide plus variable amounts of deamidated B polypeptide—have lower forming and stabilizing capacities when the degree of deamidation increases. Wagner and Guéguen (1995) reported that A_0 sample has a H_0 value of 2.46 and all other reduced deamidated glycinins have H_0 values ranging from 1.41 to 2.16. These samples exhibit good foaming properties even when their surface hydrophobicity is lower than the nonreduced glycinins, which have H_0 values ranging from 3.13 to 4.35. These results suggest that, in addition to surface hydrophobicity, there are another structural properties that enhance the surface behavior of reduced samples. It might be due to the presence of free B polypeptides, which remained soluble as a result of its deamidation. Moreover, according to Kim and Kinsella (1987a) molecular flexibility of glycinin is enhanced by disruption of AB disulfide bridges. Therefore, the improved foaming properties of all reduced glycinin samples could be attributed to the higher molecular flexibility of constituent proteins.

Relationship between Foaming Properties and Surface Behavior. By tensiometric measurements (surface tension vs time), the surface pressure values at 5 min (π_5) were obtained as a measure of the adsorption rate of protein in the interface (Kim, 1985). Figure 6 shows the relationship between v_i and π_5 values for reduced and nonreduced modified glycinin samples. For nonreduced samples, it is clearly observed that the increase of π_5 as a consequence of the deamidation effect causes, in turn, an increase of v_i. Due to their partial unfolding, caused by heating and increased charge, deamidated proteins anchored in the interfacial layer more easily. For reduced samples, this correlation was least evident because they all present elevated π_5 and v_i values, as a consequence of their lower molecular size and higher molecular flexibility (Wagner and Guéguen, 1995; Kim and Kinsella, 1987a). Thus, the values of v_i are related

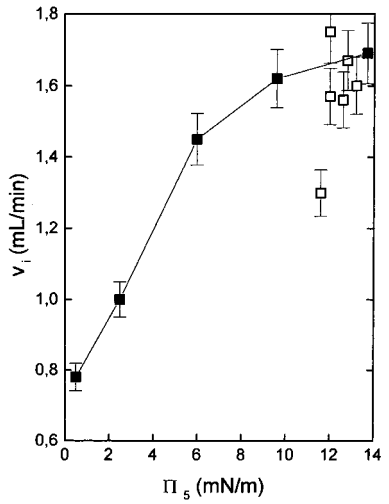

Figure 6. Relationship of v_i (rate of liquid incorporation to the foam) with π_5 (surface pressure after 5 min of adsorption). Samples were native and modified glycinin without (■) and with (□) reducing treatment. (See Materials and Methods for details.)

to kinetics of adsorption and protein tensioactivity, with the latter being increased by higher deamidation degrees.

A comparison between compression isotherms of native and some modified glycinins was also carried out to obtain additional information on surface behavior. Surface pressure vs surface area isotherms (π vs A) were performed under two different conditions. Assay a was performed with initial surface pressure around zero, employing an initial superficial concentration of 2.1 mg/m^2 for all samples (Figure 7a). Assay b was performed with initial surface pressure superior at zero, employing different superficial concentrations for each sample (in the range 4.2−69.4 mg/m^2) to reach the collapse pressure (Figure 7b). As generally observed with other proteins, a decrease in surface area is accompanied by an increase in surface pressure (Subirade et al., 1992). These authors showed that the behavior of protein films at the air−water interface is very sensitive to the state of the protein. As Figure 7a shows, acid dissociation−denaturation has a considerable effect on the spreading of the glycinin, more when a certain deamidation degree is reached by hot acid treatment. The A_0 reduced sample presents as well a higher surface behavior than that of native glycinin but it does not reach those of deamidated samples. At the same surface pressure, the superficial area is higher for modified samples than those for native glycinin. In the same figure, it could be observed that an initial superficial concentration of 12.6 mg/m^2 of native glycinin (6 × 2.1 mg/m^2) is needed to obtain an isotherm comparable with that of acid-treated glycinin. It indicates that the dissociated and unfolded AB subunit spread over the interface whereas the globular hexameric $(AB)_6$ form remained 6 times more compact.

Results from assays b show that compression isotherms with comparable collapse pressure values can be obtained if a different initial surface concentration for native and modified glycinins was used. For native glycinin, a concentration 5−6 times higher than that for acid-treated glycinin (with or without reduction) was needed; for deamidated glycinin (with 5.9% deamidation degree), it was sufficient for one-third of them. These results confirm that dissociated, deamidated, and/or reduced glycinins have higher surface behavior and

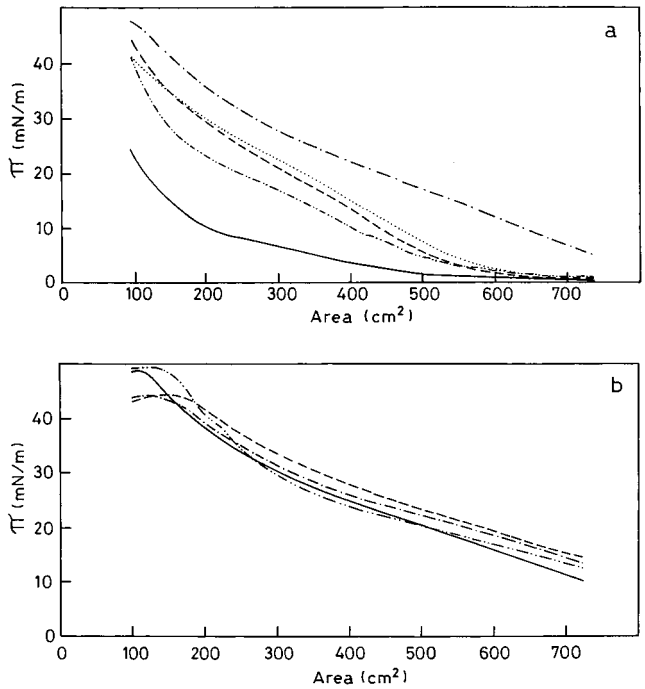

Figure 7. Surface pressure−area isotherm for native (CGII) and modified glycinin. Samples were native glycinin CGII (—), glycinin treated by cold acidic medium (^0D = 0%, ---), glycinin treated by hot acid medium for 3 h (^0D = 6%, -•-), and glycinin treated by cold acid medium plus reducing treatment (A_0 sample, ^0D = 0%, -••-). All assays were performed with protein solutions prepared with standard phosphate buffer (0.035 M potassium phosphate, 0.4 M NaCl buffer, pH 7.6, ionic strength μ = 0.5). Assays a: All samples had an initial superficial protein concentration of 2.1 mg/m^2, and an additional assay with CGII at 12.5 mg/m^2 (•••). Assays b: Different initial superficial concentrations for native and modified glycinins were used to arrive at compression isotherms with comparable collapse pressure values. The initial superficial concentrations were 69.4, 12.5, 4.2, and 13.9 mg/m^2 for CGII (—), glycinin treated by cold acidic medium (^0D = 0%, ---), glycinin treated by hot acid medium for 3 h (^0D = 6%, -•-), and glycinin treated by cold acid medium plus reducing treatment (A_0 sample, ^0D = 0%, -••-), respectively. ^0D = deamidation degree.

foaming properties than those of native glycinin, due to their increased interfacial spreading.

Relationship between Foaming Properties and Molecular Size. The different foaming properties exhibited for native and modified samples could also be analyzed in relation to the effect of molecular size modification as result of treatments. The molecular size of the protein species present in samples of native and modified glycinin was obtained by molecular exclusion chromatography (HPLC). Glycinin, in native state—hexameric form $(AB)_6$—has a molecular mass (MM) of 360 kDa, approximately, and an AB subunit of 60 kDa (Wagner and Guéguen, 1995; Peng et al. 1984). Therefore, as an indicative parameter for the protein dissociation degree, we used the percentage of molecular species with MM above 20 000 and below 100 000 (noted as MM20−100). Figure 8a,b shows the relationship between foaming parameters and MM20−100 percentage values of samples. All nonreduced samples had a content of MW < 20 species not higher than 20%, even at deamidation degrees of 25%. The CGII sample presents MM20−100 of 6.5%, indicating a slight dissociated state. This parameter increases with the dissociating acid medium action and with the deamidation degree. The increase in MM20−100 brings about an increase in stability (lower K values for MM20−100 values higher than 22%)

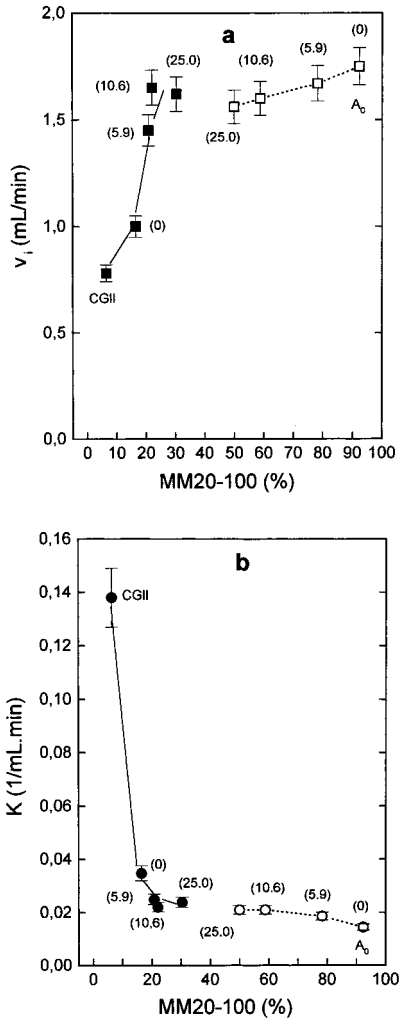

Figure 8. Relationship between foaming parameters of native and modified glycinins and content (%) of molecular species with molecular weights in the range 20 000−100 000 (MM20−100). Samples were native and modified glycinin without (■, ●) and with (□, ○) reducing treatment: (a) foam forming parameter v_i vs MM20−100; (b): foam stabilizing parameter K vs MM20−100. (See Materials and Methods for details.)

and of liquid-to-foam incorporation rate (higher v_i values at MM20−100 above 16%). Due to their lower molecular size, these proteins diffused more quickly to the interface. The lower molecular size would favor protein adsorption to the air−water interface; the partial unfolding would induce its subsequent anchorage and interaction to confer rigidity to the protein lamella (Graham and Phillips, 1979; Kim, 1985).

The reduced sample A_0 is mainly composed for A polypeptides with molecular mass around 40 kDa and in less quantity components of 80−100 kDa, resulting an MM20−100 = 92%. The other reduced samples, coming from deamidated glycinins (previously treated in hot acid medium), have decreasing MM20−100 values as the deamidation degree increases. For these samples, v_i is high (above 1.5) and almost independent from the MM20−100 values in the range 20−80% (Figure 8a). We will also see that for all samples (reduced or not), the higher v_i value (≥1.6) was reached at MM20−100 above ∼30%. On the other hand, the Figure 8b shows that the decrease of MM20−100 in reduced samples leads to a slight increasing of K values (lower foam stability). This result can be understood if we consider that the decrease of MM20−100 in reduced samples is caused largely by the appearance of species with MM < 20 000 (Wagner and Gueguen, 1995). These species, of very low molecular mass which come mainly from the hydrolysis of A polypeptides, possess tensioactive action since they are able to diffuse and be adsorbed in the air−water interface (high values of π_5 and v_i, as showed in Figure 6). However, they lack the capacity to interact and anchor in such interface to form a rigid protein lamella. Zhu and Damodaran (1994) have reported that the addition of protease-peptones causes a sharp decrease in foam stability of whey protein isolate. On the other hand, Halling (1981) has shown that the presence of small molecule surfactants (as low molecular weight peptides) could modify the surface rheology of the protein film. These agents will tend to penetrate or even displace the protein into the interfaces. This usually reduces the rigidity of the surface film.

Among all modified samples, that named A_0 was the sample with the best foaming properties. The A polypeptide, which makes up the bulk of it, possesses a low molecular weight and a high molecular flexibility that favors its surface adsorption and, besides, has the ability to associate at the interface (presumably by hydrophobic interactions), which would increase the lamella rigidity. This assumption is based on its compression isotherms and on previous results from gel filtration (Wagner and Gueguen, 1995) showing that, by the effect of the high ionic strength ($\mu = 0.5$), a fraction of the A polypeptide (MW about 40 kDa) is associated to components of higher apparent molecular weight (80−100 kDa).

With regard to the foaming capacity (FC), in the conditions under which the foaming tests were done, all samples gave similar FC values (about 0.8) and showed an absence of collapse in the formed foam during the time of assay.

In conclusion, this study shows that the molecular properties of glycinin, particularly surface hydrophobicity, size, net charge, and composition, are important in controlling the surface activity and foaming properties. The denaturation and dissociation, achieved through chemical modifications, improve the ability of the protein to act as surface active agent and consequently as foaming agent. By means of the combined effect of acid, thermal, and reduced treatments, it was possible to obtain from soy glycinin various surface-active proteins with good foam forming and stabilizing capacities.

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