Structural Stability of Globulins

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Application of differential scanning calorimetry (DSC) and the susceptibility of amaranth globulins (A-G) to α-chymotrypsin gave a quantitative estimation of protein denaturation in solid state. To compare effects of size and crystal structure, A-G, quinoa globulins (Q-G), and bovine γ-globulins (γ-G) were examined at similar conditions for the extent of denaturation. A-G and Q-G showed similar data, but γ-G exhibited maximal conformational changes. The larger percentage of denaturation in globulins that is associated with enthalpy and the number of ruptured hydrogen bonds correspond to the smaller crystallinity determined by X-ray diffraction and disappearance of the α-helix in Fourier transform-infrared spectra.

Keywords: *Amaranth; quinoa; calorimetry; denaturation; spectroscopy*

INTRODUCTION

Amaranth and quinoa are dicotyledonous plants indigenous to the Andes region of South America, where they have been used as a staple food crop for hundreds of years by the native population. Nowadays, tons of amaranth are produced annually in Mexico for special food purposes, such as candies consumed in the central part of this country, and flakes and flours distributed by stores specialized in nutritional foods. This crop has a strong agronomic potential in view of the remarkable traits of the plant, such as its drought tolerance, pest resistance, and capability of growing in soils of poor quality, in addition to the outstanding nutritional properties of its seed proteins (Coulter, 1990; Gorinstein et al., 1991; Brinegar and Goundan, 1993; Paredes-López et al., 1994; Segura-Nieto et al., 1994). Amaranth and quinoa are important cheap sources of proteins of high nutritional quality. The nutritional quality of plant seed storage proteins such as amaranth, quinoa, and other globulins depends on the amount of essential amino acids and their digestibility (Konishi et al., 1985; Coulter and Lorenz, 1990; Gorinstein et al., 1991; Marcone and Yada, 1991; Brinegar and Goundan, 1993; Mansour et al., 1993; Paredes-López et al., 1994; Segura-Nieto et al., 1994). The use of these proteins is dictated by their functional properties, such as emulsification, solubility, and foaming abilities (Kinsella and Phillips, 1989). Amaranth globulins (A-G), as well as some other proteins, have the properties of heat-stable emulsifiers. Amaranth proteins may be an important alternative to those from soybean because the amaranth protein macromolecules have various nutritional and functional properties as good as, or better than those of soybean (Voutsina et al., 1983; Utsumi et al., 1984; Konishi and Yoshimoto, 1989; Wang and Damodaran, 1991; Marcone and Yada, 1992; Mansour et al., 1993). The conformational changes in proteins that are widely used in food systems and pharmaceuticals have been characterized by differential scanning calorimetry (DSC), circular dichroism (CD), and intrinsic fluorescence (IF; Arntfield and Murray, 1981; Arntfield et al., 1987; Ma and Harwalkar, 1988; Zemser et al., 1994; Gorinstein et al., 1995, 1996).

Our recent study (Gorinstein et al., 1996) focused on the denaturant-induced secondary and tertiary structural changes of A-G as followed by measurements of fluorescence intensity and emission wavelength plus CD. However, information on globulin denaturation in the dry state is limited.

This work reports a new application of DSC to determine the structural stability in dry-heated solids of A-G, quinoa globulin (Q-G), and bovine γ-globulins (γ-G). In addition to X-ray diffraction, Fourier transform-infrared spectroscopy (FT-IR), electrophoresis under nondenaturing conditions, and protease digestibility were applied to study the denaturation of globulins.

MATERIALS AND METHODS

Materials. Guanidine hydrochloride (GuHCl), urea (U), sodium dodecyl sulfate (SDS), α-chymotrypsin, 2-mercaptoethanol (2-ME), and γ-G were reagent grade chemicals from Sigma Chemical Co. Deionized water was used throughout.

Sample Preparation. Whole mature seeds of amaranth [*Amaranthus* (*A*). *caudatus* (*cau*) and *A. hypochondriacus* (*hyp*)] and quinoa (*Chenopodium quinoa*) were ground in a mill with a 60-mesh screen and defatted in a Soxhlet extractor with *n*-hexane for 10 h. The meal was stored at 4 °C after removal of *n*-hexane. Albumins (Alb) and globulins (G) were extracted with 0.5 M NaCl from the meal (1 g) with a solvent:sample ratio of 10:1(/v/w) at 4 °C and vigorously shaken for 1 h. The extracts were centrifuged at 10000*g* for 10 min. Each step was repeated twice. Then, globulins were separated from albumins by dialysis, with tubes with a molecular weight (MW) cutoff of 2000, against H_2O at 4 °C for 72 h and then freeze-dried. These freeze-dried proteins were used in all our studies. The details have been described previously (Gorinstein et al., 1996).

Solid freeze-dried globulins (A-G and γ-G) were denaturated in unsealed conditions at 100 °C for different time periods (overnight and for 5 days) to determine the percentage of thermal denaturation. After denaturation, globulin samples were dissolved in 0.01 M phosphate buffer, 0.4 M NaCl, and

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0.02% sodium azide (pH 7.2) and filtered from undissolved material. Protein concentration was adjusted with a Uvikon 930 spectrophotometer (Kontron AG Instruments, Zürich, Switzerland), and fluorescence emission was measured as described in detail in the following section.

Fluorescence. Fluorescence was measured with a model FP-770, Jasco-Spectrofluorometer (Japan Spectroscopic Co., Ltd., Hachioji City, Japan). Sample temperature was 30 °C at a concentration of 0.015% protein, which corresponded to an absorbance of <0.1 in a 1-cm path length to receive a linear increase in a relative fluorescence intensity. Fluorescence emission spectra of all proteins were measured at excitation wavelengths of 274 and 295 nm and recorded over the frequency range from the excitation wavelength to a wavelength of 450 nm. The percentage of protein denaturation (%D) was estimated as follows:

$$\%D = (I_0 - I_1)/(I_0) \times 100 \qquad (1)$$

[In eq 1, I_0 and I_1 are the intensities of native and denaturated proteins, respectively. The %D was measured after incubation of G held at 100 °C for varying time intervals (overnight and 5 days). All data were determined in triplicate for each experimental conditions (Zemser et al., 1994; Gorinstein et al., 1996).

DSC. The thermal denaturation of globulins was assessed with a Perkin Elmer DSC System 4 (Perkin-Elmer Limited, Beaconsfield, Buckinghamshire, England). Samples of ~1 mg of freeze-dried native salt-soluble proteins and globulins were weighed in a DSC pan and 10 μL of pH 7.2 buffer (0.01 M phosphate buffer and 0.4 M NaCl) were added without mixing. Samples of proteins denatured at 100 °C at various heating times (overnight and for 5 days) were prepared in the same way (1 mg and 10 μL buffer). One milligram of freeze-dried native protein was also denatured with 10 μL of 3 M urea, which was added to the pan. Then, the samples were sealed in aluminum pans (Ma and Harwalkar, 1988). An empty pan was used as a reference. The scanning temperature was 30–120 °C at a heating rate of 10 °/min. Indium standards were used for temperature and energy calibrations. T_d and ΔH were calculated from the thermograms (Gorinstein et al., 1995).

FT-IR Measurements. A Perkin Elmer 2000 FTIR spectrometer (Perkin-Elmer) was used to record IR spectra. The samples for measurements were prepared from native freeze-dried granulated amaranth (A-G) and bovine (γ-G) globulins: A-G and γ-G were denatured overnight at 100 °C and were also denatured by mixing with GuHCl (1:1) in the dry state. Then, the prepared samples were mixed with KBr (4:100), and the pellets were pressed by applying 10 000 kg/cm^2 pressure for 15 s.

X-ray Diffraction. X-ray diffractograms of native and denatured samples were recorded by a Rigaku (MAX-IIIA, Rigaku Keisoku Co., Japan) powder X-ray diffractometer equipment. The X-ray, Cu Kα irradiation was performed with a monochrometer. The operating conditions were as follows: voltage, 35 kV; current 25 mA; angles, 2Θ-3° to 2Θ-40°; scanning speed, 1°/min; chart speed 5 mm/min; time constant 1 s; and count rate 2 Kpcs. Freeze-dried native and denatured [at 100 °C overnight and denatured with urea (1:1) in dry state] samples were densely packed on two glass plates with an aluminum frame. Values of intensities were read from the curves over the angular range 4–30°, which includes most of the crystalline peaks. Percent of crystallinity was determined by an integral method. The "d" spacings were computed by Bragg's law using $\lambda = 2d \sin \Theta$, where λ is the wavelength of the X-ray beam (1.5405 Å), d is the spacing between unit cell edges of the specific crystal to be studied, and Θ is the angle of diffraction. The quantitative measurement of crystallinity was undertaken according to Hizukuri (1978) and Nara et al. (1978). Each point of minimum intensity on the X-ray diffractograms of proteins was joined by a smooth curve. The upper region under the most prominent peaks in proteins was the area of 100% crystalline fraction.

Electrophoresis. Nondenaturing gradient polyacrylamide gel electrophoresis (ND-PAGE) was done with native and denatured globulins with 8–25% acrylamide gradients on Phast System (Pharmacia LKB, Biotech. AB, Uppsala, Sweden). The ND-PAGE conditions were applied according to Pharmacia LKB (1985). For 8–25%, ND-PAGE running conditions were as follows: prerun (400 v; 10 mA; 2.5 w; 15 °C; 10 vh), sample application (400 v; 1 mA; 2.5 w; 15 °C; 2 vh); and run (400 v; 10 mA; 2.5 w; 15 °C; 270 vh). Molecular weight (MW) markers (Sigma) ranged between 14.2 and 545.0 kDa. Freeze-dried native and denatured at 100 °C globulins were dissolved in a sample buffer consisted of 10 mM Tris-HCl, 1 mM ethylene-diaminetetraacetic acid (EDTA), and 0.01% bromophenol blue (pH 8.0) in a concentration of 1 mg/mL. A prefocusing step of 30 min was carried out before applying the 30-ng/mL protein samples and ND-MW markers. Time for electrophoresis and staining with Coomassie Brilliant Blue R-250 was ~2 h, similar to conditions of Van-Seuningen and Davril (1992).

Proteolytic Susceptibility. Protease digestibility was carried out at 38 °C for 20 min, according to the procedure of Kato et al. (1990). To 3 mL of 0.1% protein solution (freeze-dried native globulin, denatured at 100 °C overnight and for 5 days) in 50 mM Tris-HCl buffer (pH 8.0) was added 200 μL of 0.1% α-chymotrypsin solution (with a protein:enzyme ratio of 16:1), and proteolysis was monitored by the trichloroacetic acid (TCA) precipitation method. A 3-mL portion of 4.0% TCA was added to terminate the enzymatic reaction and to precipitate the undigested protein. The extent of digestion was expressed as the percentage of absorbance of 0.05% untreated sample. Absorbance values for proteolytic susceptibility were measured with a Uvikon 930 spectrophotometer.

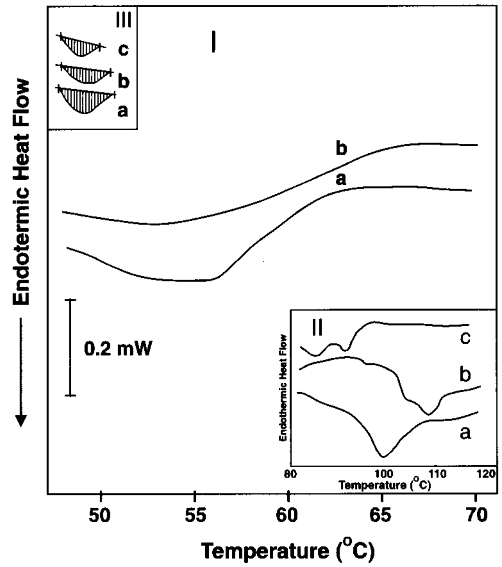

Figure 1. DSC thermograms of native and denatured proteins: (section I) amaranth (A-S) and quinoa (Q-S) salt-soluble proteins, where a = A-S and b = Q-S; (section II) a = A-G with an average MW of 40 kDa, b = γ-G with a MW of 150 kDa, and c = A-G + 3 M urea; (section III) a = γ-G; b = γ-G at 100 °C; c = γ-G at 100 °C for 5 days.

RESULTS AND DISCUSSION

DSC Measurements of Globulins. The DSC method has been extensively used to study protein unfolding in a liquid state. This method is highly sensitive to conformational changes. The DSC scans for native and denatured globulins are shown in Figure 1. The native structure of globulins from quinoa and amaranth was stable up to a critical temperature and then disrupted with intense heat absorption (Figure 1; Table 1). Urea destabilized the protein conformation of A-G with average MWs of ~40 kDa, as reflected by the marked decrease in enthalpy (ΔH) and temperature of denaturation (T_d) values (Table 1; Figure 1, IIc). These results suggest changes in molecular conforma-

Table 1. Thermodynamic Properties[a] of Native and Denatured Proteins from Amaranth and Quinoa

protein	T_d, °C[b]	ΔH, J/g[c]	ΔS, kcal/mol K[d]	n[e]	%D[f]
A-S[g]	59.0 ± 0.8	7.00 ± 1.0	0.227	19	30
A-S + urea (1:1)	47.5 ± 0.7	2.00 ± 0.8	0.067	6	
A-G[h]	101.0 ± 1.0	4.25 ± 0.9	0.123	10	50
A-G + 3 M urea	84.0 ± 1.3	2.08 ± 0.9	0.063	5	
γ-G[i]	112.0 ± 2.5	4.61 ± 1.3	0.129	11	
Q-S[j]	58.0 ± 1.5	10.00 ± 1.6	0.325	27	44
Q-S + urea (1:1)	46.3 ± 1.7	4.30 ± 1.1	0.145	12	

[a] Mean values of triplicates ± standard deviation. [b] Temperature of denaturation. [c] Enthalpy. [d] Entropy. [e] Number of broken hydrogen bonds. [f] Denaturation. [g] Salt-soluble amaranth proteins. [h] Amaranth globulins. [i] Bovine γ-globulins. [j] Salt-soluble quinoa proteins.

tion of globulins and are consistent with results of other authors (Kato et al., 1990). Disordering of the system takes place upon heating. A considerable number of globulin molecules shift to a state that contributes much less to the unfolding transition, thus causing a significant decrease in the calorimetric *H*. The ΔH of the initial and remaining DSC endotherm were measured and used for calculation of percentage of globulin denaturation. The entropy (*S*) values, which are associated with state transition and affirmed the disordering of protein structure, were also calculated (Table 1). Comparison of the thermograms of native globulins from amaranth (Figure 1, section I, curve a) and quinoa (Figure 1, section I, curve b) showed not only differences in T_d and ΔH but also the broadening of the peak. The decrease in ΔH indicates denaturation, a less stable structure, and that the conformation of the protein molecule has shifted towards the unfolded state. It has been well documented (Biliaderis, 1983; Wang and Damodaran, 1991; Nagano et al., 1994) that broadening of peaks indicates the existence of intermediate forms different from the native ones. A half of band width was calculated where broadening of peaks was recorded.

The influence of hydrogen bond disruption on ΔH in DSC was reported by Wagner and Añon (1985). According to these authors, thermal protein denaturation involves the rupture of one disulfide bond, contributing a ΔH of 25 kcal/mol and a negligible ΔS, and the rupture of *n* hydrogen bonds, corresponding to ΔH 4 of kcal/mol and ΔS of 0.012 kcal/mol/per protein molecule. Thus, the number of broken hydrogen bonds can be calculated as $n = \Delta S/0.012$ and $n = (\Delta H - 25)/4$, where *n* is the number of broken hydrogen bonds and ΔS is the entropy and ΔH is the enthalpy of denaturation. Our calculations have shown that during denaturation, 19 hydrogen bonds ruptured in native A-G compared with 27 in Q-G. We assume that during thermal denaturation, only the rupture of hydrogen bonds is involved. Our previous data showed that during urea-induced denaturation, the number of hydrogen bonds was reduced to ~30−50% (Gorinstein et al., 1995). This trend is associated with the disruption of hydrogen bonds during heat denaturation and reflects a decrease in the α-helix content of denatured protein (Kato et al., 1987). Hence, hydrogen bonding is the main stabilizing force in protein stability.

Hydrophobic interactions also play an important role in the thermal stability of A-G. Addition of protein denaturants, such as urea, led to a decrease in ΔH and T_d, indicating protein denaturation and loss of cooperativity. The presence of reducing agents, such as 2-ME, did not affect DSC characteristics (results not shown), suggesting that disulfide bonds present in globulins do not contribute to the thermal response of the protein.

Preheating treatments at 100 °C resulted in a progressive decrease in ΔH, indicating partial denaturation of globulins. There was a marked increase in T_d, suggesting that the preheated globulins may aggregate to form a more compact structure with higher thermal stability and cooperativity (Figure 1, section III, curves b and c). The DSC measurements of pea mixed globulins (vicilin and legumin) showed one transition between 74 and 95 °C, with a maximum at 86.2 °C (Arntfield and Murray, 1981; Bora et al., 1994). The A-G exhibited a T_d of 101 °C; however, with urea, the T_d decreased to 84 °C (Table 1; Figure 1: section I, curve a; section II, curves a and c). Bovine γ-G, with a MW of 150 kDa (which was higher than MW of A-G) and used as a reference, also showed a higher T_d of ~113 °C (Table 1; Figure 1: section II, curve b). Wheat gluten proteins showed a lower T_d (88.4 °C) than amaranth; the 7S and 11S soybean globulins exhibited 76 and 97 °C, respectively. Thus, soybean 11S globulins have a similar T_d as those from amaranth (Saio and Watanabe, 1978; Nagano et al., 1994). The thermal stabilities of soybean globulins and A-G may be explained by the hydrophobic type of interaction between the subunits (Nagano et al., 1994). The strength of such hydrophobic type forces increases with temperature (Biliaderis, 1983; Konishi and Yoshimoto, 1989). Low T_d values (Figure 1: section I, curve a; Table 1) probably characterize the behavior of crude salt-soluble proteins; these proteins are mostly mixture of albumins and globulins. Globulins have a more stable structure than albumins as judged by the higher T_d; oat albumin denatures at 87 °C and globulin at 110 °C (Ma and Harwalkar, 1988).

Our data show that DSC can be used to study the effect of medium composition and heating on protein tertiary and quaternary structures. These treatments (heating, pH adjustment, salt addition) are often required in the production of foods containing proteins as major ingredients. Because the functional properties of proteins are greatly influenced by their conformation, DSC is a valuable tool in assessing the potential of globulins as a functional ingredient in different food systems (Bora et al., 1994).

FT-IR Spectrometry. Kaiden et al. (1987) applied IR spectroscopy to the study of the secondary structure of proteins. On the basis of this investigation, IR spectra of A-G were analyzed to determine the protein conformation. The changes induced by heat or denaturant treatment resulted in the alterations of amide I, II, and III bands (Figure 2, section I, curves a and b). The intensities of the amide I and II bands decreased in the denatured samples in comparison with those of the native globulins. Also, the intensity ratio of the amide II band to the amide I band increased in both denatured samples in comparison to the native ones (Figure 2: section I, curves a−c). In the denatured proteins, this ratio was higher. This observation indicates a decrease in the α-helix content of the treated globulins in comparison with the native ones (Kato et al., 1987). GuHCl also induced alterations in protein conformation (Figure 2: section I, curve c).

Differences in the peak position and peak intensity ratios (*R*) of native and denatured samples are shown in Table 2. The *R* values were obtained as the ratio of band intensity to the CH_2 deformation vibration (1450 cm^{-1}) by the baseline method [Figure 2: section I, curve a (dotted line)]. As can be seen, the frequency of the

Table 2. Amides I, II, and III Band Positions[a] and Relative Intensity Ratios (*R*) to 1450 cm^{-1} Band[b] of Amaranth (A-G) and Bovine γ (γ-G) Globulins

sample	amide I		amide II		amide III	
	band position, cm^{-1}	R	band position, cm^{-1}	R	band position, cm^{-1}	R
native A-G	1656	10.5	1535	4	1313	1
					1235	5
denatured A-G	1660	7	1535	3	1314	1
					1235	4
native γ-G	1646	10	1534	4.5	1235	3
denatured γ-G	1655	8	1535	4	1235	2.5

[a,b] Mean values of triplicates.

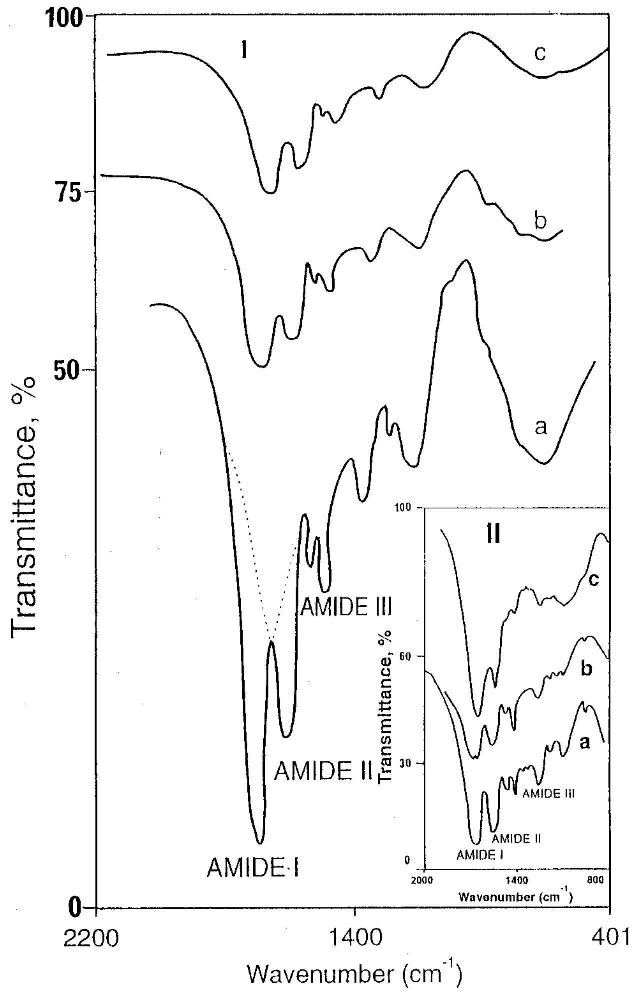

Figure 2. FT-IR spectra of A-G and γ-G: (section I) a = A-G, b = A-G at 100 °C, and c = A-G + GuHCl (1:1); (section II) a = γ-G, b = γ-G at 100 °C, and c = γ-G + GuHCl (1:1).

amide I band (1660 cm^{-1}) shifted 4 cm^{-1} towards the high frequency side (Table 2; Figure 2: section I, curve b) in comparison with the native (1656 cm^{-1}) state (Table 2; Figure 2: section I, curve a). Globulin spectra showed alterations in the 1313−1235 cm^{-1} region (i.e., the amide III band; Table 2); namely, the difference in intensity ratio between the 1313 and 1450 cm^{-1} bands decreased and the distinction between 1313 and 1233 cm^{-1} peaks increased (Figure 2: section I, curve b). The peak at 1233 cm^{-1} was converted from a sharp intensive band to a broad one, and the intensity of 1313 cm^{-1} band decreased, suggesting that the α-helix and β-sheet were disordered upon heat treatment. The broad band in the 1300−1250 cm^{-1} region was identified as belonging to α-helix, the relatively sharp band in the 1240−1230 cm^{-1} region to β-sheet, and the broad, medium intensity band in the 1270−1240 cm^{-1} region to a disordered structure. The band at 1515 cm^{-1}, which is associated

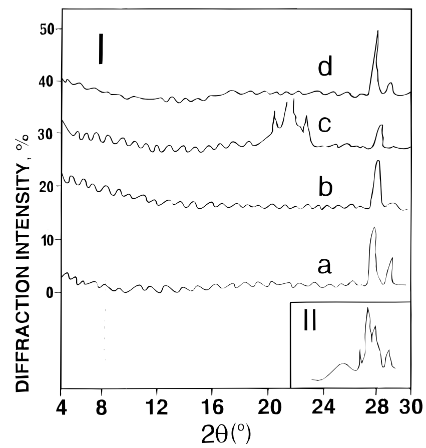

Figure 3. X-ray diffraction patterns of A-G and Q-G (section I) a = A-G, b = A-G at 100 °C, c = A-G + urea (1:1) and d = Q-G; (section II) urea.

with β-sheet or random structure, is not shown in the globulin spectrum (Figure 2: section I, curve a), indicating that A-G contain α-helix as the main structure. These results are in agreement with our recent data obtained by CD (Gorinstein et al., 1996). For comparison of the changes of A-G, the spectra of bovine γ-G are shown in Figure 2: section II, curves a−c) and in Table 2. The clear sharp band (amide III, Table 2) at 1235 cm^{-1} (Figure 2: section II, curves b and c) corresponds to β-sheet and becomes broader and smaller after denaturation compared with native γ-G (Figure 2: section II, curve a). This result reflects a decrease in β-conformation. Also, the intensity ratio of peak at 1450 cm^{-1} to peak at 1401 cm^{-1} increased after denaturation. However, the intensity ratio of amide II to amide I band (for native γ-G, 2.2; for the denatured form was 2.0; Table 2) did not change drastically. Such a difference can be attributed to the fact that β-sheet is the main ordered structure of γ-G.

X-ray Diffractometry. Native A-G and Q-G showed two peaks at 3.12 and 3.23 Å, but the intensity of these peaks differed (Figure 3). The relative crystallinity was determined taking into consideration these two peaks. The crystallinity of samples a, b, c, and d (Table 3) was, respectively, 100, 82, 68, and 85%. From the results shown in Table 3 and Figure 3 it may be concluded that the crystallinity of globulins is correlated with the degree of denaturation. A larger crystallinity of the sample corresponded to a larger ΔH of denaturation as determined by DSC.

Native PAGE. Differences in the structure of native and denatured globulins are shown in Figure 4. Native protein samples in PAGE demonstrated a smaller number and faster mobilities of subunits than denatured ones at 100 °C, showing some aggregated forms in the region of 400 kDa (Figure 4, lane 2). Therefore, the denaturation was marked by outstanding changes

Table 3. X-ray Diffraction Spacings[a] in Amaranth (A-G) and Quinoa (Q-G) Globulins

number[b]	protein	interplanar spacings (*d*), Å[c]	relative crystallinity, %
a	A-G	3.23 (vs); 3.12 (w)	100
b	A-G + 100 °C	3.08 (m); 3.08 (w)	82
c	A-G + urea (1:1)	3.29 (w)	68
d	Q-G	3.23 (s); 3.12 (m)	85

[a] Mean values of triplicates. [b] Corresponds to the peaks in Figure 3. [c] Intensity shown in parentheses: (vs) very strong; (s)

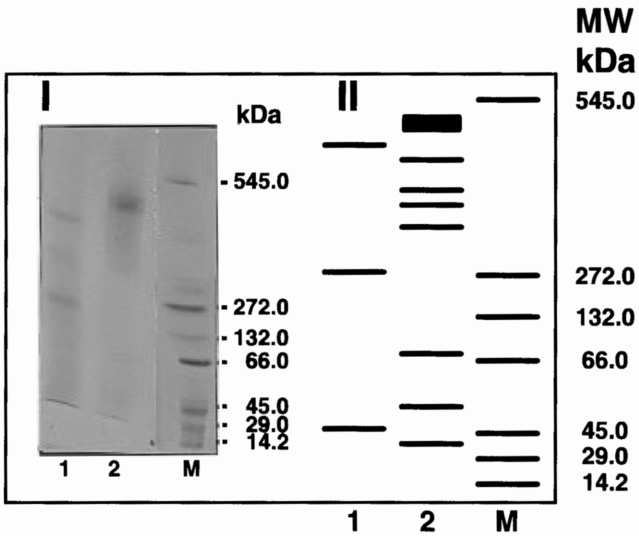

Figure 4. PAGE of native A-G in 8−25% PAAG: (section I) 1 = A-G; 2 = A-G at 100 °C; and M = marker; (section II) same conditions presented schematically.

in mobility pattern as well. These data are in agreement with reported values of A-G of 440, 398, and 260 kDa (Konishi et al., 1985; Barba de la Rosa et al., 1992) plus 398 and 337 kDa (Marcone and Yada, 1992).

Proteolytic Susceptibility. The susceptibility to α-chymotrypsin of A-G heated in the dry state for various time periods are shown in Figure 5. Susceptibility to proteolysis gradually increases (Figure 5: section I, curves a, b, and c) with the time of heat treatment, indicating the changes in protein conformation towards a less stable structure during heating. The treatment with ME (Figure 5: section I, curve d) for the same period of heating (Figure 5: section I, curve c) increased the susceptibility to proteolysis. These results correspond to the conclusions of Marcone and Yada (1992) and are in agreement with our findings that were generated by CD, DSC, and intrinsic fluorescence; that is, susceptibility to proteases is proportional to the flexibility of protein structure (Gorinstein et al., 1995). In our previous report (Gorinstein et al., 1996), intrinsic fluorescence was used to study the unfolding of globulins with different denaturants. Denaturants and temperature changes cause the displacement of the tryptophan residues to a more polar environment during folding (Arntfield et al., 1987; Gorinstein et al., 1995, 1996). Globulins denatured at 100 °C gave a small shift in the emission wavelength maximum and a decrease in fluorescence intensity (Figure 5: section II, curves a and b). Denaturation was calculated by the two methods proteolytic susceptibility and intrinsic fluorescence. Both methods showed a %*D* of 52%.

In summary, thermal denaturation parameters, FT-IR spectra, X-ray diffraction, electrophoretic mobility, and proteolytic susceptibility were measured to elucidate the conformational changes and degree of globulin denaturation in solid and liquid states.

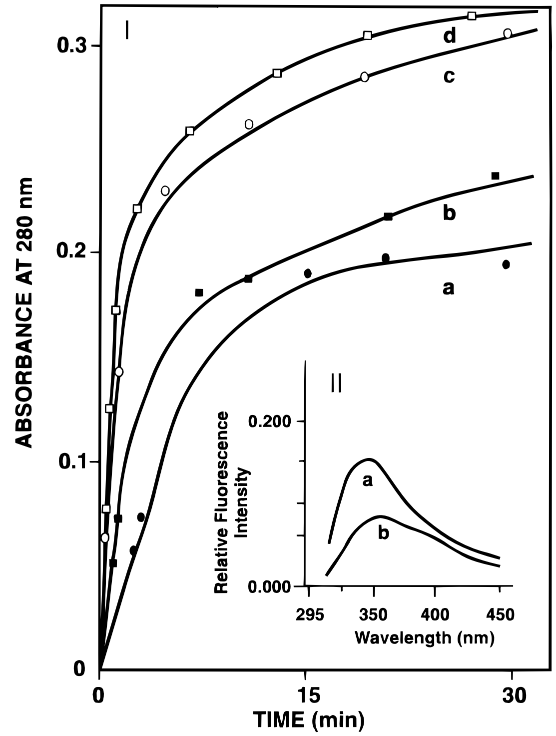

Figure 5. Proteolysis of A-G with α-chymotrypsin: (section I) a = A-G, b = A-G at 100 °C, c = A-G at 100 °C for 5 days, and d = A-G + 2ME at 100 °C for 5 days; (section II) fluorescence emission of a = A-G, and b = A-G at 100 °C for 5 days.

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