Effect of pH and Ionic Strength Modifications on Thermal Denaturation of the 11S Globulin of Sunflower (*Helianthus annuus*)

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Helianthinin, the main storage protein of sunflowers, has low water solubility and does not form a gel when heated; this behavior is different from other 11S globulins and limits its food applications. To understand this particular behavior, changes on helianthinin association−dissociation state induced by modifications in pH and ionic strength were analyzed. The influence of these different medium conditions on its thermal stability and tendency to form aggregates was also studied. Helianthinin behavior at different pH values and ionic strengths is similar to other 11S globulins except that it remains in a trimeric form at pH 11. Helianthinin thermal stability is higher than other 11S globulins but is lower than oat 11S globulin. Alkaline pH produces a 10 °C decrease of its denaturation temperature and also of the cooperativity of denaturation process, but it does not affect the denaturation activation energy. The decrease in thermal stability with the pH increase is also manifested by its tendency to form aggregates by SH/SS interchange reactions. When thermal treatments at alkaline pH are performed, all helianthinin subunits form aggregates, characterized by a higher proportion of β-polypeptides than α-polypeptides, which is an indication that aggregation is accompanied by dissociation. Treatments at 80 °C are sufficient to induce aggregation but not to produce denaturation, and in these conditions hexameric forms remain after the treatment.

INTRODUCTION

Helianthinin, the main storage protein of sunflower seed, belongs to the 11S globulin or legumin-like family of proteins, and similar to other legumins has a polymorphic subunit composition and a hexameric structure (*1*, *2*). Subunit composition varies among different cultivars, but at least six types of subunits with molecular weights ranging from 40000 to 64000 have been described, consisting in a large acidic polypeptide (α) and a small basic (β) polypeptide linked by a disulfide bond (*3*, *4*). Commonly, 11S globulins have good physicochemical properties, such as emulsifying and gel forming abilities (*2*, *5*), and are used in many food products. In contrast, sunflower proteins are mainly used in animal feed because most sunflower protein isolates are scarcely soluble, as solubility is a prerequisite for the properties mentioned previously (*6*, *7*). The low solubility of sunflower proteins has been attributed to protein denaturation caused by solvent extraction during oil production and to the presence of phenolic compounds that interact with the proteins, reducing their solubility (*6*, *8*, *9*). However, even native and phenolic-free sunflower 11S globulins are not able to form gels when subjected to heat (*10*). This behavior differs from that of other 11S globulins such as soybean and legumin and illustrates the particular structural characteristics of helianthinin.

Plant protein structure, and therefore, functionality depend largely on the ionic strength and pH of the medium. The variation in these conditions may disturb or even disrupt protein quaternary, tertiary, and secondary structure (*11*−*13*) and may lead to new and different functional properties (*13*, *14*). The behavior of helianthinins at acid pH has been reported to be similar to that of other 11S globulins such as those obtained from soybean, sesame, and mustard (*6*, *15*−*17*). However, there are no reports about changes in their association−dissociation state induced by alkaline pH and ionic strength or about the effect of thermal treatment on helianthinin structure in different environmental conditions. Knowledge of the structural behavior of sunflower 11S globulins in different media will be valuable to design treatments to improve its functional properties and food applications.

The aim of this work was to explore the influence of pH and ionic strength on the association−dissociation state and thermal stability of sunflower 11S globulins. Denaturation kinetic parameters were determined, and their relationship with sunflower protein structure was analyzed. The capability of helianthinin subunits to form aggregates in different conditions was also studied.

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MATERIALS AND METHODS

Materials. Seeds of *Helianthus annuus* cultivar ATAR TC 3003 were dehulled and defatted for 4 h with hexane in Soxhlet equipment. Flour was obtained by grinding defatted seeds in a laboratory mill.

Protein Isolation. Sunflower meal was treated with ethanol 20% v/v to remove phenolic compounds (*18*). Albumins were extracted by treating the defatted flour three times with 10 mM 2-mercaptoethanol (2-ME) in a ratio of 10:1 (mL of solvent/g of meal). After each treatment, the extraction residue was separated by centrifugation at 9000g for 20 min at 4 °C. The 11S globulin was then extracted by treating the last residue three times with buffer A (32.5 mM K_2HPO_4, 2.6 mM KH_2PO_4, 0.4 M NaCl, pH 7.5). Supernatants containing soluble and dispersed globulin protein were dialyzed against 10 mM 2-ME. Once dialysis was complete, the suspension was freeze-dried.

Differential Scanning Calorimetry (DSC). Measurements were carried out in a Polymer Laboratories (Rheometric Scientific) calorimeter driven with the Plus V 5.41 software. Calibration was carried out at a heating rate of 10 °C/min by using (Rheometric Scientific Ltd.) indium proanalysis (p.a.), lauric acid p.a., and stearic acid p.a. as standards. To analyze the effect of pH, hermetically sealed aluminum pans were prepared to contain 12−14 mg of protein suspension (25% w/v) in 0.2 M sodium salt buffers (citric acid-citrate, pH 2.3−5.1; phosphate, pH 6.3−7.5; boric acid-borate, pH 8.8−9.7, 12; and carbonate, pH 10−11), an empty pan being employed as a reference. (a) 0.17 M $C_6H_8O_7$, 0.03 M $C_6H_7O_7^-$ (pH 2.3); (b) 0.08 M $C_6H_8O_7$, 0.12 M $C_6H_7O_7^-$ (pH 3.1); (c) 0.015 M $C_6H_8O_7$, 0.15 M $C_6H_7O_7^-$, 0.035 M $C_6H_6O_7^{2-}$ (pH 4.1); (d) 0.11 M $C_6H_7O_7^-$, 0.09 M $C_6H_6O_7^{2-}$ (pH 4.7); (e) 0.06 M $C_6H_7O_7^-$, 0.14 M $C_6H_6O_7^{2-}$ (pH 5.1); (f) 0.18 M $H_2PO_4^-$, 0.02 M HPO_4^{2-} (pH 6.3); (g) 0.12 M $H_2PO_4^-$, 0.08 M HPO_4^{2-} (pH 7.5); (h) 0.132 M $H_2BO_3^-$, 0.068 M $H_1BO_3^{2-}$ (pH 8.8); (i) 0.046 M $H_2BO_3^-$, 0.154 M $H_1BO_3^{2-}$ (pH 9.7); (j) 0.128 M HCO_3^-, 0.072 M CO_3^{2-} (pH 10.1); (k) 0.03 M HCO_3^-, 0.17 M CO_3^{2-} (pH 11.0); and (l) 0.163 M $H_2BO_3^-$, 0.037 M HBO_3^{2-} (pH 12.2). NaCl was added as required for each solution to reach 0.54 ionic strength (μ).

To test the effect of ionic strength, 25% w/w suspensions (pH 6) of dialyzed globulin-11S were prepared in NaCl solutions of increasing molarity (0.005, 0.01, 0.010, 0.030 0.050, 0.100, 0.500, 1.000, and 2.000 M).

After each DSC run, the dry matter content was determined. The denaturation parameters were calculated using the aforementioned software: the denaturation temperature (T_d) was taken as the value corresponding to the maximum transition peak; $\Delta T_{1/2}$ was the peak width corresponding to half peak height, and the transition enthalpy (ΔH) was obtained from the area below the transition peaks. At the least, duplicate runs were done for each sample to obtain the mean value and standard deviation of each parameter.

Denaturation Kinetic Parameters. The denaturation kinetics of helianthinin was also studied by DSC using the heat evolution method of Borchardt and Daniels (*19*) and activation energies by means of the Arrhenius equation.

Thermal Treatments. A 14 mg/mL suspension of helianthinin was subjected to different treatments with varying temperature and pH conditions. The pH of the protein suspension was adjusted to pH 4.1, 4.7, 5.1, 6.3, 7.5, 8.8, 10.1, and 12.2 with 2 N HCl or 2 N NaOH. Each sample was fractionated in 1 mL aliquots, which were placed in 1.5 mL Eppendorf tubes. Thermal treatments at 80, 90, and 100 °C were carried out in a constant-temperature bath. The temperature was measured with a thin Cu-constant thermocouple, which was immersed in the protein suspension. An ice−water bath was used as reference. The Eppendorf tubes were removed from the thermostatic bath after 5 min of heating and immediately cooled in an ice bath. An aliquot of each sample was adjusted to a concentration of 5 mg/mL with 0.1 M phosphate buffer (pH 7) containing 1% SDS. All the collected samples were extracted at room temperature for 1 h, with periodic vortex agitation. The samples were then diluted with the suitable sample buffer for electrophoresis.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). Linear gradient separating gels (7−20% in polyacrylamide) were run in minilabs (Bio-Rad Mini Protean II model). Runs
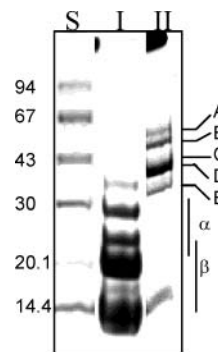

Figure 1. SDS−PAGE of sunflower 11S fractions with (lane I) and without (lane II) 2-mercaptoethanol. Helianthinin intermediate ($\alpha\beta$) subunits of molecular 57 kDa (**A**), 52 kDa (**B**), 40 kDa (**C**), 39 kDa (**D**), and 35 kDa (**E**) are labeled. In reducing conditions, these subunits dissociate in polypeptides of 40, 32, 25, 20, and 17 kDa. Low molecular weight standard is shown in the first line (**S**).

were carried out according to the method of Laemmli (*20*) as modified by Petruccelli and Añón (*21*).

Ultracentrifugation. Ultracentrifugation was performed in an Optima TL, Beckman Ultracentrifuge, using the TLS-55 swinging bucket rotor at 50 000 rpm for 5 h at 10 °C. Samples were layered on top of 10−30% (w/w) sucrose linear gradients. Calibration was carried out using soy 7S and 11S purified globulins as standards. After each run, gradients were carefully fractionated in 150 μL aliquots, and UV absorbance at 280 nm was measured (Beckman DU 650 Spectrophotometer).

Statistical Analysis. A variance analysis (ANOVA) was carried out; significant differences between treatments were determined by means of the Fisher test, with a level of significance of 0.05. Both analyses were carried out using the SYSTAT statistical software (*22*).

RESULTS AND DISCUSSION

Effect of pH and Ionic Strength on the Association− Dissociation State of Helianthinin. Sunflower 11S globulins differ from other 11S proteins in that they are scarcely soluble. How this low solubility affects other physicochemical properties of heliathinins has not been well-characterized. In this work, a phenolic-free globulin fraction from the *H. annuus* cultivar ATAR TC 3003 was prepared, and the protein structure was studied in different conditions. In agreement with the results reported by Dalgalarrondo et al. (*23*), five different intermediate ($\alpha\beta$) subunits of molecular weight 57 kDa (A), 52 kDa (B), 40 kDa (C), 39 kDa (D), and 35 kDa were present in this cultivar (**Figure 1**), the D subunit being predominant. Analysis by sucrose gradients of this fraction showed only one band with a sedimentation coefficient of 11S at pH values ranging from 6.3 to 9.7, which corresponds to the hexameric form of helianthinin (**Figure 2**).

When the medium pH was increased to 10−11, helianthinin dissociated into a trimeric form. At pH 5, there was equilibrium between 7S, 11S, and 15S forms, while extremely low (pH 3) or extremely high (pH 12) pH values produced complete and partial dissociation into monomeric forms, respectively (**Figure 2**). Glycinin and pea legumin show hexameric forms at pH 6−9, but at a pH above 10, they dissociate into monomeric forms (*24*). Therefore, the oligomeric behavior of helianthinin was slightly different, while according to Osborne, storage proteins are classified into albumin, globulin, and glutelin, structural features indicate that 11S globulins and glutelins belong to the same family (*2*). The primary sequence of helianthinin reveals an equal number of acidic and basic amino acids, differing from glycinin or other legumins, which are rich in acidic amino acids,
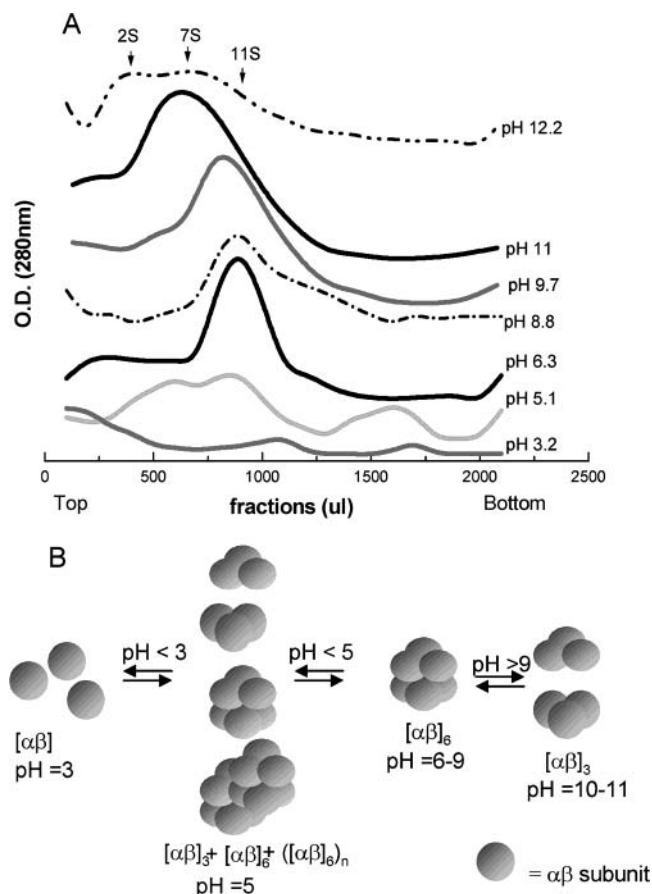
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Figure 2. (**A**) Sedimentation patterns of helianthinin at pH values ranging from 3.1 to 12.2 and constant ionic strenght ($\mu = 0.54$). (**B**) Schematic representation of the changes in helianthinin oligomeric state.

Table 1. Protein Sequence Analysis of Different 11S Globulins[a]

	helianthinin	glycinin	oat 11S
no. of amino acids	473	465	528
theor pI[b]	7.85	5.67	9.02
total no. of negatively charged residues (Asp + Glu)[b]	47	57	41
total no. of positively charged residues (Arg + Lys)[b]	48	49	49
aliphatic index	70.11	68.58	73.66
denaturation temp (°C)	99.7[c]	91.5[d]	114.2[e]

[a] Gene Bank Accession: heliathinin, P19084; glycinin, P04776; oat 11S globulin, CAA52763. [b] Calculated using ProtParam tool from ExPASy Molecular Biology Server (http://us.expasy.org). [c] Reference *9*. [d] Reference *38*. [e] Reference *29*.

and from oat globulin or glutelins, which are rich in basic amino acids (**Table 1**). This difference in amino acid composition might explain why helianthinin retains a trimeric assembly at pH 10.

Helianthinin is hexameric at ionic strength (μ) 0.5, but it dissociates into a trimeric form at μ above 1. In addition, sunflower globulin converts to a 15S form when the ionic strength falls below 0.1 (**Figure 3**). This behavior is similar to that reported for glycinin (*25*).

Thermal Properties of Helianthinin at Different pH and Ionic Strength. Helianthinin thermal stability at ionic strength 0.54 and pH between 3.1 and 12.2 was analyzed by DSC. The representative thermograms obtained are shown in **Figure 4A**. A single denaturation endotherm was detected between pHs 4.5 and 12. No endothermic transition was observed at pH 4.1 or lower, indicating that helianthinin was completely denatured.
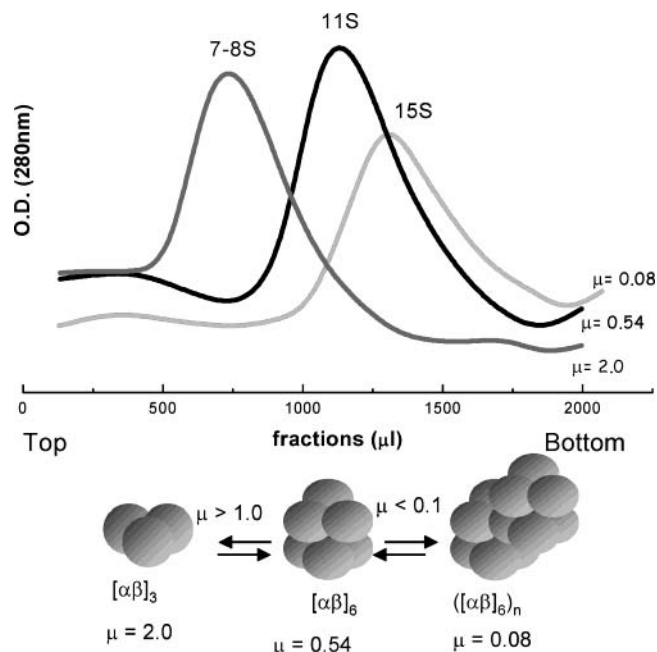

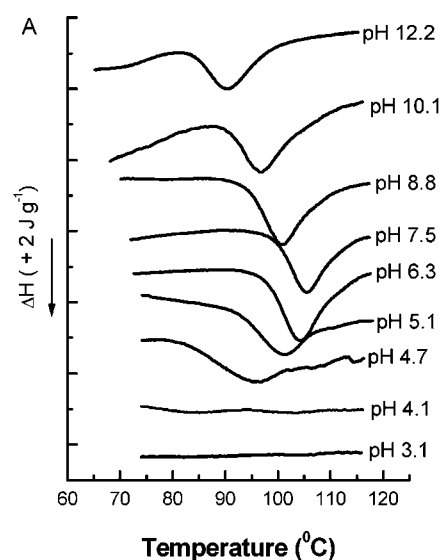**Figure 3.** Variation in helianthinin sedimentation coefficient with the ionic strength at pH 8.5.

Figure 4. (**A**) DSC thermograms of helianthinin obtained at pH values ranging from 3.1 to 12.2 and constant ionic strength ($\mu = 0.54$). Heating rate is 10 °C/min. (**B**) Changes in Td and ΔH as a function of pH. Points are means of three independent experiments, and vertical bars represent the standard deviation.

Maximun thermal stability was observed at pH 7.5 ($T_d = 105.4 \pm 0.1$ °C), coinciding with the helianthinin isoelectric point. The denaturation temperature of helianthinin reported in this

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paper is higher than that reported by Gonzalez-Perez et al. (*9*). Helianthinin T_d is also higher than that of other 11S globulins such as legumin, glycinin, and amaranth 11S globulin (*26–28*) and lower than that of oat 11S globulin (*29*). High denaturation temperatures are usually attributed to a higher number of hydrophobic bonds stabilizing the protein molecule (*26, 30*). **Table 1** shows also the aliphatic index, which constitutes a measure of the number of hydrophobic amino acids of different 11S globulins (*31*). Similar to that observed for the denaturation temperature, the aliphatic index of helianthinin lay between the indices of glycinin and oat 11S globulin.

There was a 10 °C decrease in the thermal stability when the pH was increased to 12, which may be due to dissociation of hexamers into trimers, an increase of electrostatic repulsions and/or reactions of SH/SS interchange (*27, 32*). These results are different from those published by Sanchez and Burgos (*10*) who found no changes in helianthinin T_d at pH 5–11. Thermal stability of glycinin and amaranth globulin P also depends on pH (*27, 33*).

Denaturation enthalpy (**Figure 4B**) followed the same trend that thermal stability did, reaching a maximum at a pH between 6 and 8. The helianthinin behavior was slightly different at extreme pHs. While the protein was totally denatured at acidic pH, it was only partially unfolded at a pH higher than 10 (20% of denaturation at pH 12 with respect to the maximum value obtained at pH 7; $p < 0.05$).

Privalov et al. (*34*) have suggested the use of a $\Delta T_{1/2}$ value as an indication of the cooperativity of protein unfolding. Progressive sharpening of the endothermic peak is indicative of an increase in cooperativity. The greatest cooperativity for thermal denaturation of helianthinin was observed at pH 6.3 ($\Delta T_{1/2} = 7.2$ °C). At extreme pHs (lower than 6 and higher than 7.5), a widening of peaks was detected ($\Delta T_{1/2} = 9.0$– 12.4 for pH 5.1–3.1, respectively, and $\Delta T_{1/2} = 8.0$–9.14 for pH 7.5–12.1, respectively), indicating that pH-induced changes in protein conformation cause a loss of cooperativity ($p < 0.05$).

Thermal Properties of Helianthinin at Different Ionic Strengths. The thermal behavior of dialyzed globulin was analyzed at pH 6.5 in NaCl concentrations ranging from 0.005 to 2 M. A unique endothermic transition was present in all samples (**Figure 5A**). Thermal stability of helianthinin, T_d, remained constant at very low ionic strength ($\mu = 0.005$–0.05) and then increased up to $\mu = 2.0$ ($p < 0.05$) (**Figure 5B**). On the other hand, ΔH did not show significant variations over the whole range of ionic strength analyzed ($p < 0.05$), suggesting that the presence of a higher ionic strength during thermal treatment has little effect on the forces that stabilize helianthinin conformation. Nevertheless, the exothermic protein aggregation is likely to take place during the DSC run and so would oppose to the increase of ΔH, keeping constant the resulting enthalpy change.

When μ decreased from 2.0 to 0.005, a gradual loss of cooperativity ($p < 0.05$) was observed ($\Delta T_{1/2} = 8.2 \pm 0.1$ at $\mu = 2.0$ and $\Delta T_{1/2} = 10.4 \pm 0.1$ at $\mu = 0.005$). The loss of cooperativity can result from changes in the helianthinin conformation that does not modify its denaturation enthalpy. Similar results have been observed in other storage globulins (*29, 33, 35–37*).

Denaturation Kinetics and E_a. Kinetic parameters of the thermal denaturation of helianthinin at different pH and ionic strengths were obtained by analyzing DSC thermograms as indicated by Borchardt and Daniels (*19*). Application of this method allows the determination of the activation energy, E_a, and the apparent reaction order, n. The E_a calculated for the
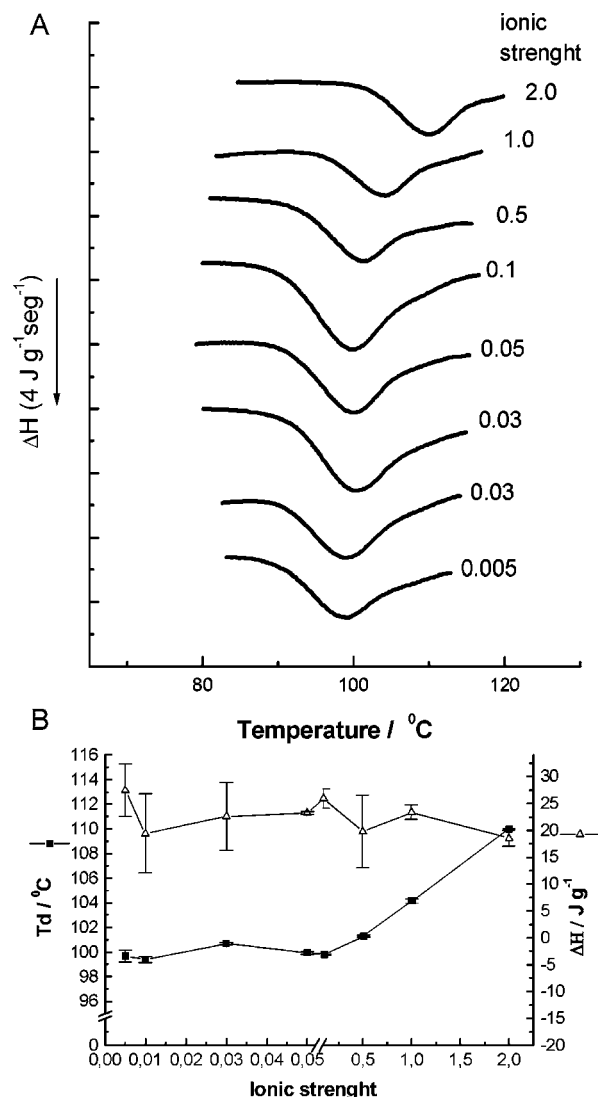

Figure 5. (**A**) DSC thermograms of dialyzed helianthinin obtained at ionic strength values ranging from 0.005 to 2.0 and constant pH (pH 6.5). Heating rate is 10 °C/min. (**B**) Changes in Td and ΔH as a function of ionic strength. Points are means of three independent experiments, and vertical bars represent the standard deviation.

thermal transition at pH 6.3 and ionic strength 0.54 was 586 ± 20 kJ/mol. This value is higher than those reported for glycinin and β-conglycinin (*27, 38*) and similar to those of oat globulin and amaranth 11S globulin (*39, 40*). The highest energy barrier to be overcome by the macromolecules to reach the activated state and to denature was observed between pH 6 and 12. When ΔH and apparent E_a calculated in the pH range 3–12 were compared, the correlation between both values was observed only at acid or neutral pH (**Figure 6A** vs **Figure 4B**). At a pH higher than 9.7, the reduction of ΔH was not followed by a reduction in E_a. The E_a also increased with ionic strength, whereas ΔH associated to thermal denaturation did not vary significantly (**Figure 6B**).

The thermal denaturation of helianthinin at $\mu = 0.54$ and pH between 5.1 and 11.0 did not follow first-order kinetics; the n values calculated in this condition varied between 1.5 and 2. An apparent denaturing reaction order value higher than 2, $n = 2.5$, was obtained for helianthinin at pH 6.3 and different ionic strengths. The lowest values were obtained at extreme ionic strength (2.2 and 2.5 at $\mu = 0.005$ and 2.0, respectively), while for $\mu = 0.01$–1.0, n values ranged from 3 to 4.
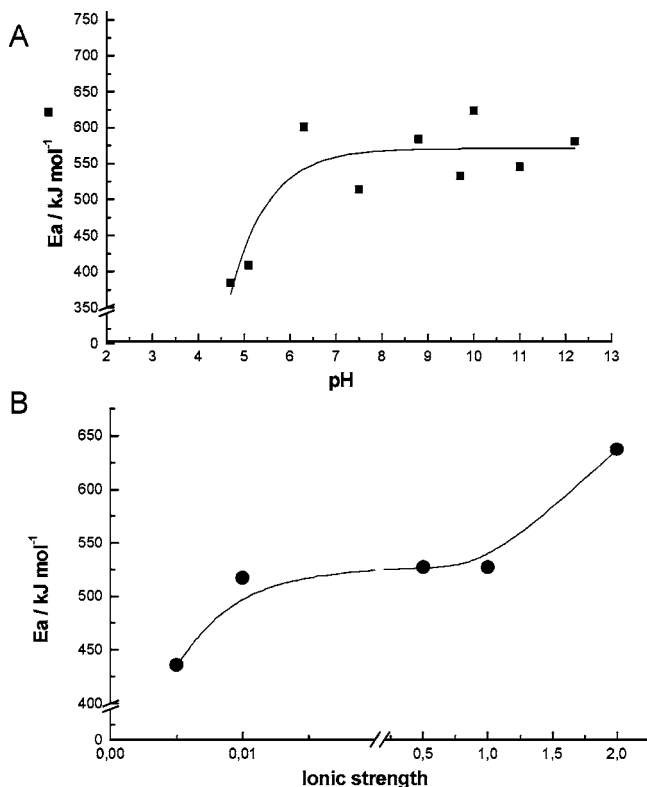
Figure 6. Activation energy, E_a, of helianthinin as a function of pH (**A**) and ionic strength (**B**).

Values of apparent order of reaction higher than 1 have been reported for other storage proteins. Iwabuchi et al. (*41*) studied the kinetics of the thermal denaturation of β-conglycinin using spectrophotometric methods and concluded that the reaction order was higher than 2. In our laboratory, values ranging from 2 to 2.5 for glycinin (*38*) and an apparent reaction order of 2 for amaranth 11S globulin (*40*) have been measured. On the other hand, Ma and Halwarkar (*39*) also found apparent reaction orders close to 2.5 for the oat globulin. These high values could be attributed to polymolecular denaturation reactions, complex mechanisms, the presence of intermediate products, or the existence of multiple domains in the protein molecule, which present different thermal stability.

It has been shown that the sunflower 11S globulin used in this work is formed by five different subunits whose association–dissociation state varies with pH and ionic strength. This structural characteristic would explain the possible existence of multiple domains and/or the presence of different intermediates. Another possible explanation is that helianthinin could

dissociate before thermal denaturation, as has been suggested in phaseolin (*42*).

Effect of Thermal Treatment at Different pHs on the Structure of Helianthinin. Thermal treatments are frequently used to modify protein functional properties. Depending on the condition, proteins with different degrees of denaturation and/ or aggregation, and therefore, different functional properties can be obtained. On the basis of the kinetic parameters presented previously, thermal treatments at different pH values were designed to produce proteins with different degrees of denaturation, and changes in their association dissociation state were evaluated by 1-D and 2-D SDS–PAGE and ultracentrifugation analysis. Protein solutions at pH values ranging from 4.1 to 12.2 were heated at 80, 90, or 100 °C. According to the thermograms presented in **Figure 4** and to the helianthinin kinetic parameters, treatments at 80 °C were not sufficient to produce denaturation. Treatments at 90 °C were sufficient to produce complete denaturation of the samples at pH 12.2 but not of the samples in the pH range of 5.1–8.8. Treatments at 100 °C were completely denatured for all the samples. Helianthinin was completely denatured at pH 4.1, even without thermal treatment. The effects of these treatments were analyzed by SDS-PAGE in the absence of 2-mercaptoethanol (**Figure 7**). All the thermal treatments produced the formation of SDS-resistant aggregates that could not penetrate the polyacrylamide gel (see arrow). These aggregates were more intense when samples were treated at pH 7.5 and 8.8 than in samples at pH 4.1–6.3. At alkaline pHs, ionization of free SH occurs, promoting reactions of interchange SH/SS responsible for the formation of these aggregates. When the intensity of helianthinin subunits labeled as A–E was analyzed, it was observed that these subunits were not present in the sample heated at 100 °C at pH over 7.5, indicating that they were completely aggregated. The same thermal treatment but in the pH range of 4.7–7.5 produced complete aggregation of A, B, and E subunits and partial aggregation of C and D subunits. Little aggregation of all subunits was observed at pH 4.1. While kinetic parameters suggested that treatments at pH 4.7–8.8 at temperatures 80–90 °C are not sufficient to produce denaturation, the SDS-PAGE of these samples showed both presence of helianthinin subunits A–E and high molecular weight aggregates (arrow). In contrast, glycinin AB subunits suffer a complete aggregation by SS bonds at 90 °C (*32*). This difference agrees with a higher thermal stability of helianthinin as compared with glycinin. Thermal treatments at 90 °C and pH 10.1 also produced a complete aggregation of helianthinin subunits. These results agree with the calorimetric findings presented in this work, showing a
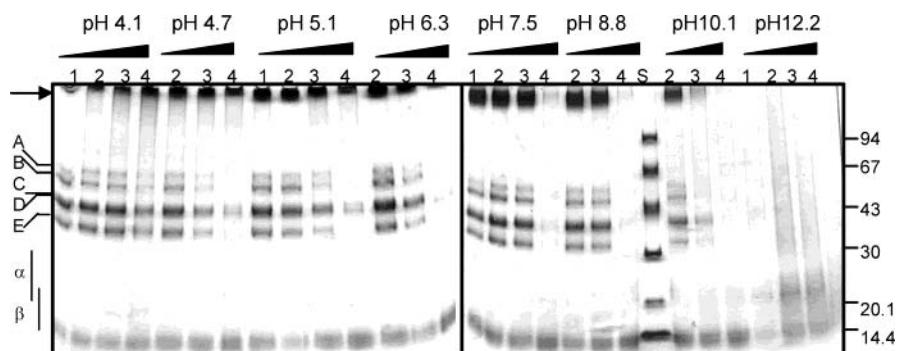

Figure 7. SDS-PAGE of the helianthinin treated a room temperature (lanes 1), 80 °C (lanes 2), 90 °C (lanes 3), and 100 °C (lanes 4) at different pH values as is indicated at the top of the figure. The width of the line over the lanes indicates the increase in strength of the thermal treatment. Lane S corresponds to the low molecular weight standard.
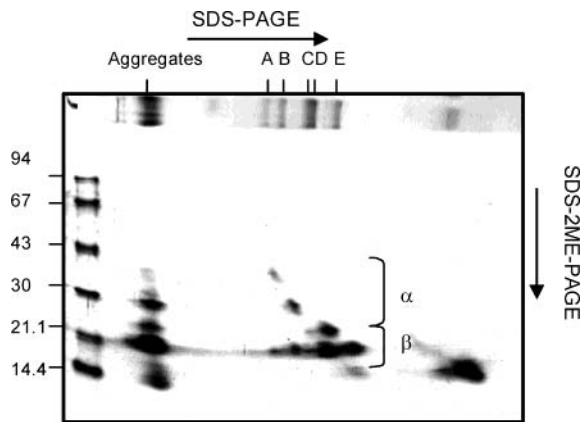
Figure 8. 2-D electrophoresis patterns of helianthinin heated at 80 °C (**A**) and 100 °C (**B**). Arrows indicate the direction of SDS-PAGE and SDS-2ME-PAGE. Low molecular weight standard is visualized in the left lane.
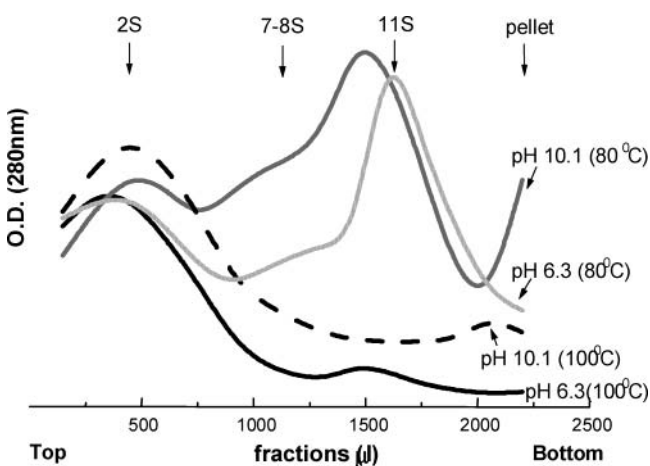

Figure 9. Sedimentation patterns of helianthinin at constant ionic strength ($\mu = 0.54$) treated at 80 and 100 °C at pH 6.3 and 10.1.

decrease in thermal stability when the pH increased over 7.5 but disagree with previous reports (*10*).

Helianthinin subunits underwent complete dissociation at pH 12 even without thermal treatment, as can be deduced from the absence of A−E subunits and the presence of α- and β-polypeptides (**Figure 7**).

The composition of high molecular aggregates produced by thermal treatment at different pH values was analyzed by 2-D electrophoresis. As similar 2-D PAGE patterns were obtained for globulins treated at different temperatures and pH, only a representative gel of these treatments is presented in **Figure 8**. When aggregates were dissociated with 2-mercaptoethanol, α- and β-polypeptides corresponding to all helianthinin subunits (A−E) were released (**Figure 8**). β-polypeptides were present in a higher proportion than the rest of the polypeptides. This result indicates that the dissociation of subunits occurs in the process of aggregation even at temperatures of 80 °C and that β-polypeptides have a higher tendency to aggregate. Glycinin B polypeptides also have a high tendency to form aggregates (*32*).

Soluble aggregates produced by thermal treatments were also analyzed by ultracentrifugation (**Figure 9**). Helianthinin 11S forms were observed with treatments performed at 80 °C, either at pH 6.3 or at pH 10.1. At the latter pH, a partial dissociation into 7S and 2S forms was also observed. In contrast, treatments at 100 °C produced complete dissociation of hexameric forms.

In summary, the sunflower 11S globulin oligomeric state at

different pH and ionic strengths is similar to other 11S globulins except that it remains in a trimeric form at pH 11. Helianthinin thermal stability is higher than other 11S globulins and is modified by extreme acidic and alkaline pH. Changes in the medium pH from 7.5 to 12 produce a 10 °C decrease on helianthinin denaturation temperature and also in the cooperativity of denaturation process, but it does not affect the denaturation activation energy. Thermal treatments at alkaline pH produce aggregations by SH/SS interchange reactions and also the dissociation of sunflower 11S subunits that is evident by the higher proportion of β-polypeptides than α-polypeptides in the aggregates produced. Treatments at 80 °C produce aggregation without previous denaturation, and in these conditions, hexameric forms (11S) remain after the treatment. In contrast, after treatments at 100 °C, which are completely denaturing, soluble proteins remain in a 2S form (monomeric).

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