DOI:10.1021/jf100554d

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Structural Stability and Surface Activity of Sunflower 2S Albumins and Nonspecific Lipid Transfer Protein

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The structural and interfacial properties of five different fractions of sunflower (*Helianthus annuus* L.) seed storage proteins were studied. The fractions comprised lipid transfer protein (LTP), the methionine-rich 2S albumin SFA8 (sunflower albumin 8), and three mixtures of non-methionine-rich 2S albumins called Alb1 and Alb2 proteins (sunflower albumins 1 and 2). Heating affected all of the proteins studied, with SFA8 and LTP becoming more surface active than the native proteins after heating and cooling. LTP appeared to be less thermostable than homologous LTPs from other plant species. SFA8 generated the greatest elastic modulus and formed the most stable emulsions, whereas LTP showed poorer emulsification properties. The mixed 2S albumin fractions showed moderate levels of surface activity but had the poorest emulsification properties among the proteins studied.

KEYWORDS: *Helianthus annuus* L.; sunflower; 2S albumin; nsLTP; SFA8; Alb1; Alb2; interfacial rheology; emulsion; circular dichroism spectroscopy; heating

INTRODUCTION

Proteins are widely used in the food industry to stabilize oil/water interfaces and are the most important surface-active agents in many food colloids, such as emulsions and foams (*1*). They stabilize emulsions by diffusing to the oil/water interface, where they absorb and form new hydrophobic and electrostatic interactions with both the oil and water phases as well as with adjacent protein molecules. When sufficient protein molecules have adsorbed to the interface, they can form a viscoelastic film around the oil droplet, preventing coalescence and leading to phase separation. Proteins adsorbed to the oil/water interface have been shown to adopt a different, unfolded, conformation from that found in aqueous solution (*2*). Many surface-active proteins used in foods are of animal origin, with few plant-derived alternatives. Consequently, there is interest in developing food ingredients from plant sources, in particular from protein-rich waste streams, such as the protein-rich waste from oil seeds after oil extraction.

One particular source of interest is the seed proteins of sunflower (*Helianthus annuus* L.), which comprise a mixture of 2S albumins and 11S globulins (helianthinins) in a ratio of about 2:1. The 2S albumins are cysteine-rich, water-soluble proteins (*3*), with masses of about 12000−15000 Da and sedimentation coefficients of about 2 (*4*), encoded by multigene families. They occur in seeds from a wide range of dicotyledonous plants (*5*), and their abundance in seeds means they provide a source of carbon, nitrogen, and sulfur during germination and the development of the seedling (*4*). Apart from sunflower albumin 8 (SFA8), all sunflower 2S albumins are synthesized as precursors that are post-translationally cleaved into two polypeptides (*6*−*9*), with mature sunflower albumins 1 and 2 (Alb1 and Alb2), being approximately the same size (14500 and 15300 Da, respectively). Each of these corresponds to a mature 2S albumin protein but, unlike the 2S albumins of all other species, they are not processed further to give pairs of small and large subunits linked by interchain disulfide bonds (*3*, *9*). The mature SFA8 protein also consists of a single polypeptide chain, but it is unusually rich in methionine (16 of its 103 residues) (*8*). Nonspecific lipid transfer proteins (nsLTPs) form a well-characterized family of less abundant proteins that bind phospholipids and fatty acids in vitro (*10*, *11*) and may participate in the formation of cutin and suberin present on the outer surfaces of aerial plant organs (*11*, *12*) and in the defense of plants from different pathogens and environmental stresses (*13*−*15*). Sunflower LTP (*16*) and the 2S albumins encoded by the HaG5 gene (described in ref *6*) have also been shown to have antifungal activity in vitro.

Both 2S albumins and LTPs belong to the prolamin superfamily, which also includes α-amylase/trypsin inhibitors, puroindolines, and grain softness proteins from cereal seeds, soybean hydrophobic protein, nonspecific lipid transfer proteins from a range of species and tissues, and some cell wall glycoproteins (*5*). The nsLTPs, 2S albumins, and other small S-rich proteins of the prolamin superfamily all possess a highly conserved skeleton of eight cysteine residues: C−X_n−CX_n−CC−X_n−CXC−X_n−C−X_n−C (*17*). These cysteines form intrachain disulfide bonds,

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 Published on Web 04/29/2010

which result in compact and stable structures (*18*). Indeed, it has been suggested that the thermostability of the LTP family (*19*) is related to their more general stability properties and may be important for their biological function, such as their role in stress responses in flowering plants (*20*).

Previous studies have shown that the whole sunflower albumin fraction (*21*, *22*) has excellent emulsification properties, SFA8 being the most effective individual component (*21*, *23*). The interfacial properties of sunflower LTP and SFA8 have also been reported, with the latter being studied in particular detail (*23*−*27*). The ability of SFA8 to stabilize emulsions may result from the presence of four hydrophobic patches on its surface (*24*). In contrast, the other albumins present in the Alb1 and Alb2 fractions and the LTP contain proportionately fewer hydrophobic amino acid residues, as indicated by their retention times on reversed phase high-performance liquid chromatography (RP-HPLC). However, studies of cereal nsLTPs suggest thermal processing plays an important role in allowing these proteins to stabilize foams in brewing (*10*) and may be required to render them effective, in common with many other proteinaceous emulsifiers.

No studies have so far been reported of the surface activity of the sunflower albumins (Alb1 and Alb2) that elute between LTP and SFA8 on RP-HPLC or on the effects of thermal treatment on these properties. The sunflower 2S albumin fraction contains about 11−13 components, and it is difficult to prepare sufficient amounts of single homogeneous components (with the exception of SFA8) for detailed analyses. We therefore decided to compare the well-characterized SFA8 with three mixed fractions comprising non-methionine-rich 2S albumin components, fraction A, comprising mainly Alb1 components, and fractions B and C, both containing mainly Alb2 components, and with sunflower LTP. A range of approaches was used to compare their stability to thermal treatments, emulsification properties, and their behavior at oil/water interfaces.

MATERIALS AND METHODS

Purification of 2S Albumins from Sunflower Seed. Seeds of sunflower (*H. annuus* L.) line PR A381 were obtained from Pioneer Ltd., Hungary. A total sunflower albumin fraction (*26*) was prepared by RP-HPLC, and five fractions (LTP, SFA8, and three mixed 2S albumin fractions, called fractions A, B, and C) were collected and analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) (*28*). Preparative separation was carried out on a Phenomenex Jupiter 10 μm C18 300 Å (250 × 10 mm) column with peak fractions being collected, freeze-dried, and stored at −20 °C over silica gel.

LTP stock solutions were prepared in a 10 mM phosphate buffer at pH 8.0, a pH unit lower than the calculated isoelectric point of the SF-LTP (pI_{LTP} = 9.0). The p*I* values of the mature proteins, after the post-translational modifications, were calculated by using the Expasy server Computing pI application. The SFA8 stock solutions were prepared accordingly in citrate buffer at pH 5.0, as its calculated pI_{SFA8} = 5.9. All solutions were prepared with ultrapure water purified to a resistance of > 18.2 MΩ and a surface tension of > 72.6 mN m^{-1}. Stock solutions of albumin fractions A, B, and C were prepared in 10 mM phosphate buffer, pH 7.0.

Peptide Analysis. HPLC-purified fractions were reduced, pyridylethylated, and subjected to N-terminal sequencing by automated Edman degradation (*29*) using a model 494 Applied Biosystems protein sequencer.

Interfacial Rheology. All experiments were conducted in the buffers that the proteins were dissolved in. Protein solutions were heated at 100 °C for 20 min, followed by standing on ice for 10 min. Solutions were then allowed to equilibrate to room temperature for 10 min before surface tension measurements were undertaken. Both control (native) and heated solutions were measured at room temperature. The interfacial tension and interfacial dilatational rheological properties of five different concentrations of LTP, fractions A, B, and C, and SFA8 were measured at the *n*-hexadecane/water interface using the pulsating drop technique (*30*). The interfacial tension was calculated from the shape of the solution drop hanging from a hydrophobic needle in *n*-hexadecane, using the selected-plane method (*31*) on an FTA32 apparatus (First Ten Ångstroms, Portsmouth, VA). Equilibrium surface tensions were taken 15 min after drop formation as only a minimal change was observable after this period. Each data point is the average of three independent parallels on separate drops.

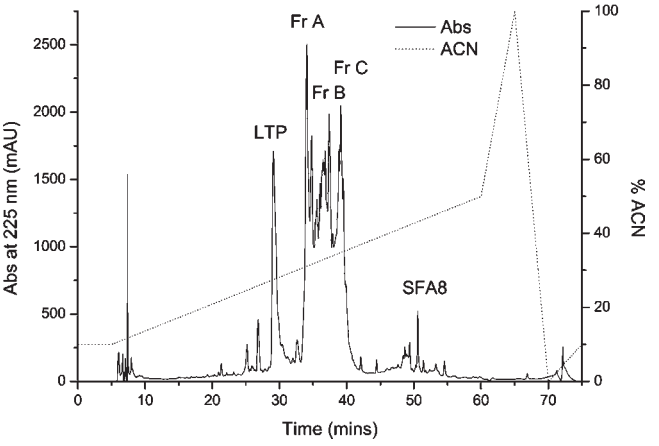

Figure 1. Chromatograph of the 2S albumin fraction of sunflower. The peaks of LTP, fractions A, B, and C, and LTP are marked. The concentration of acetonitrile in the eluting solvent is shown on the right-hand axis.

Emulsion Preparation and Characterization. Emulsions were prepared with 20% *n*-hexadecane (*23*), using 2 mg mL^{-1} solutions of LTP, SFA8, and the albumin fractions A, B, and C prepared as described above. Emulsion (3 mL) was prepared by homogenizing *n*-hexadecane with aqueous protein solution using a Status 200 sonicator. The sonication procedure consisted of a 0.1 s pulse (10% power) followed by a 0.9 s relaxation repeated 20 times. The emulsion was then allowed to stand for 2 min, and then the 20 pulses were repeated. This procedure ensured that the emulsion temperature never exceeded 25 °C, thereby minimizing heating effects on protein structure. The size of the particles formed by the proteins was measured by an LS-230 (Coulter, USA) light scattering instrument. Measurements of size were carried out in independent triplicates and found to be in good agreement.

Far-Ultraviolet Circular Dichroism (Far-UV CD) Spectroscopy. The same stock solutions with the same buffers and pH values were used for the UV CD spectroscopy experiments as for the interfacial measurements described above. CD spectra were recorded using a JASCO J-700 spectropolarimeter (Jasco Corp., Tokyo, Japan) under the following conditions: 100 nm min^{-1} scan speed, 0.2 nm bandwidth, 0.2 nm resolution, 100 mdeg sensitivity, and a response time of 2 s. Samples were measured in quartz demountable cells with a path length of 0.5 mm. Spectra (190−260 nm) are the averages of three accumulations, with the background of the cell and buffer subtracted. The samples were scanned at 20 °C and heated to 80 °C for 20 min inside the spectropolarimeter, rescanned, and then cooled to 20 °C for a further 20 min and allowed to equilibrate for 20 min at 20 °C before being scanned for a third time in situ. Analysis was carried out using the Contin method (*32*) with a basis set containing 29 proteins. The unsmoothed spectra are represented as molar CD (with respect to moles of amide bonds), based on the average molecular weight of an amino acid of 115.

RESULTS

Identification of Proteins. A 2S albumin fraction extracted from seeds of sunflower was separated by RP-HPLC (**Figure 1**). Five fractions corresponding to LTP, SFA8, and three mixed albumin fractions (A, B, and C) were initially identified by SDS-PAGE (**Figure 2**) and collected. SDS-PAGE showed that fraction A contained components between 7000 and 18000 Da, fraction B components between 4000 and 20000 Da, and fraction C components between 4000 and 19000 Da. The identities of LTP and SFA8 were confirmed by N-terminal sequencing and by electrospray mass spectrometry, their molecular masses being 9000 and 12000 Da, respectively. MALDI-TOF analysis (results not

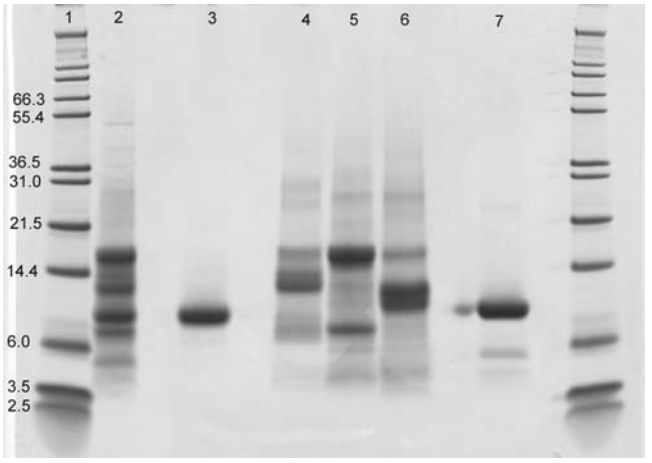

Figure 2. SDS-PAGE analysis of albumin fractions under reduced conditions. Lanes: 1, molecular weight markers, molecular weights are shown on the left (M_r, kDa); 2, total albumin fraction; 3, LTP; 4, fraction A; 5, fraction B; 6, fraction C; 7, SFA8.

shown) showed that the major bands in fraction A corresponded to Alb2, the N-terminal polypeptide encoded by the 2S albumin gene, whereas those in fractions B and C corresponded to Alb1, the C-terminal polypeptide. All of the 2S albumins in these fractions comprise single-subunit chains with intrachain disulfide bonds, with molecular weights ranging from about 10000 to 18000 (*9*). Swiss Prot accession numbers for the studied proteins are as follows: LTP, Q39950; non-methionine-rich sunflower albumin precursor (containing Alb1 and Alb2), Q8GUD8; SFA8, P23110.

Effect of Thermal Treatments on Secondary Structure. Figure 3 shows the wavelengths of the minima and maxima together with the intensities of the far-UV CD spectra of LTP, SFA8, and fractions A, B, and C at room temperature, at 80 °C, and after cooling to 20 °C after heating to 80 °C for 20 min. The estimated secondary structure contents of the proteins from Contin analysis of the CD spectra (**Table 1**) indicated significant proportions of α-helical structure in all of the proteins studied. In general, as might be expected for such highly disulfide bonded proteins, all were thermostable, showing minimal changes in secondary structure following heating to 80 °C. The spectra of fractions A, B, and C were very similar, with positive maxima at 190 nm and double minima, with that at 209 nm being slightly more intense than that at 225 nm. Fraction A gave a much more intense positive peak than the other albumin fractions, which may reflect differences in secondary structure. In particular, Contin analysis indicated a much lower content of β-sheet (8%) compared with fractions B and C (about 23%) (**Table 1**). Although broadly similar to the other albumins, the spectrum of SFA8 was shifted slightly to longer wavelengths, with a distinct peak at 193 nm and double minima of equal intensity at 210 and 220 nm. The far-UV CD spectrum of the LTP fraction is typical of a protein rich in α-helix with a maximum at around 192 nm and with a broad minimum between 206 and 221 nm. When heated to 80 °C, the maximum decreased, and it also changed on cooling when both the maximum and minima became more intense. Contin analysis indicated a decrease in α-helix and a corresponding increase in β-sheet structure upon cooling after heating to 80 °C, suggesting the LTP adopted a different conformational state, although most of the native-like α-helix and β-sheet structure may have been retained. The minima and maxima of fractions A, B, and C and SFA8 did not change greatly upon heating and after cooling in either intensity or wavelength. However, the intensities of both the maximum and minima decreased slightly at 80 °C for fractions A and C and SFA8, but returned close to the initial values after cooling.

Surface Properties. *Interfacial Tension and Dilatational Rheology*. **Figure 4** shows the interfacial tension of protein solutions after 15 min as a function of concentration. All of the native proteins showed at least moderate capacity to lower interfacial tension at a relatively high concentration (0.1 mg cm^{-3}). SFA8 was the most surface active and LTP the least, yielding a surface tension some 7 mN m^{-1} higher at the highest concentration (**Figure 4a**). Fractions A, B, and C showed very similar surface activities at concentrations above 0.01 mg cm^{-3}. However, considerable differences were observed at lower protein concentrations. Fraction C and LTP showed the lowest surface activity, whereas SFA8 and fraction B showed the highest.

The surface activities of all of the proteins increased after heating, at all concentrations (**Figure 4b**). The LTP showed the greatest change upon heating, with the interfacial tension at the concentration of 0.1 mg cm^{-3} decreasing by 4 mN m^{-1}, whereas that of SFA8 decreased by only 1.3 mN m^{-1}. SFA8 was less surface active than fractions A, B, and C at the lowest concentration of 0.001 mg cm^{-3}. However, at higher concentrations (0.005 mg cm^{-3}) SFA8 had the highest surface activity.

Analyses of the interfacial rheological properties of the five protein fractions (**Figure 5**) showed interesting differences. To take into account the differences in the surface tension and surface concentration caused by differences in the surface activity of the different samples, the elastic moduli are shown as a function of interfacial pressure and not bulk concentration. SFA8 (**Figure 5a**) gave the most elastic films, and the elastic modulus increased as a function of interfacial pressure. Fractions A and C showed similar behavior but with lower elastic moduli. However, fraction B and the LTP showed quite different behavior in which the elastic modulus essentially did not change with the interfacial tension. **Figure 5b** shows the effect of heating, with all of the curves looking rather different from those for the unheated samples (**Figure 5a**). However, the curve for heated SFA8 is hardly changed compared with that for the native sample, except at low interfacial pressure. With the exception of SFA8, the samples showed a maximum in elastic modulus at an interfacial pressure of around 20 mN m^{-1}. The elastic modulus increased until the interfacial pressure reached a certain point, at which the moduli decreased. Again, the LTP gave the lowest elastic modulus at the higher interfacial pressures.

Emulsion Sizing. The emulsifying capacity of the five sunflower storage protein preparations was also determined. The emulsion produced using SFA8 contained droplets with an average diameter of 1.43 μm (**Table 2**), and the emulsion remained stable for at least 2 days (data not shown), with no significant change in the droplet size, which is in agreement with results of previous studies (*23*). The emulsion formed by the LTP had a larger average diameter of 3.84 μm and also produced stable emulsion. In contrast, none of the fractions A, B, and C gave stable emulsions, with a significant proportion of the oil remaining free on top of the emulsion (**Figure 6**). Some large oil drops were observed just after formation, showing that not all of the oil was emulsified; the emulsions then formed a separate oil phase. Fraction A contained a significant number of very large drops that creamed too quickly to be measured in the LS-230 light-scattering instrument. Thus, although fraction A appears to show a small size, this has been skewed by the removal of many larger drops and flocs. This sample showed some separation of oil after a comparatively short time. Thus, the size distribution is not an accurate guide to the properties of these systems as the very large droplets creamed and coalesced too rapidly to be measured.

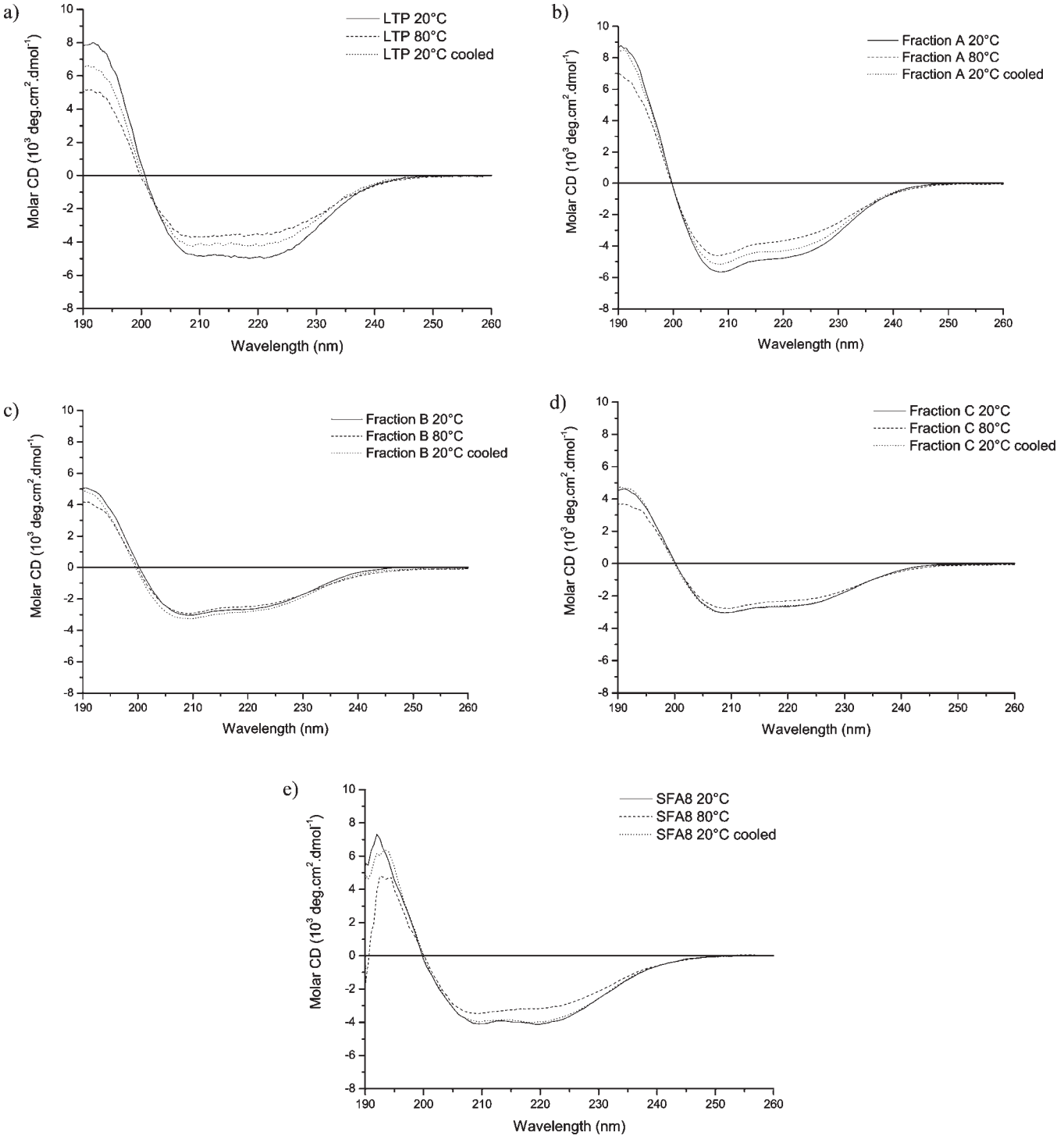

Figure 3. Effect of heating on protein secondary structure of (**a**) LTP, (**b**) albumin fraction A, (**c**) albumin fraction B, (**d**) albumin fraction C, and (**e**) SFA8. Far-UV CD spectra were collected at 20 °C, after heating to 80 °C and then cooling back to 20 °C.

DISCUSSION

Using far-UV CD spectroscopy we confirmed that all of the proteins used in this study showed a significant proportion of α-helix in nature, consistent with the α-helix bundle structures of the prolamin superfamily. Fraction A (corresponding to predominantly Alb 2) had a secondary structure with 45% α-helix, thus resembling the low-methionine 2S albumin isoform from Brazil nut (*33*), whereas fractions B and C (comprising predominantly Alb 1) had lower α-helical contents of 27% and thus were more like the high-methionine 2S albumin variants from Brazil nut and the 2S albumin from sesame (*33*, *34*). Our estimate of the α-helical content (35%) of SFA8 is in accordance with the findings of others (*25*). The Alb 1 and Alb 2 fractions together with SFA8 all proved to be resistant to thermal denaturation, showing only small conformational changes on heating. This is consistent with observations of the heat stability of SFA8 (*25*) and of napin, a closely related 2S albumin from rapeseed (*Brassica napus*), which has a three-dimensional structure very similar to that of SFA8 (*35*) and a transition temperature above 100 °C at pH 6 (*36*). Microcalorimetry measurements (data not shown) also indicated that the thermal transition for SFA8 was above 100 °C. The LTP was also thermostable but showed structural changes on

Table 1. Protein Secondary Structure Estimates from Contin Analysis of CD Spectra

		α-helix (%)	β-sheet (%)	β-turn (%)	random coil (%)
LTP	at 20 °C	44.8	8.9	18.5	27.7
	at 80 °C	35.8	13.2	22.2	28.8
	at 20 °C cooled	38.6	12.1	20.3	28.9
fraction A	at 20 °C	45.6	8.7	18.5	27.2
	at 80 °C	36.6	15.6	22.0	25.8
	at 20 °C cooled	42.4	12.0	18.5	27.1
fraction B	at 20 °C	27.3	23.4	21.9	27.4
	at 80 °C	25.7	24.6	23.3	26.4
	at 20 °C cooled	28.7	20.9	22.4	28.0
fraction C	at 20 °C	27.2	22.8	22.6	27.4
	at 80 °C	24.0	25.7	23.6	26.7
	at 20 °C cooled	27.6	22.5	22.5	27.4
SFA8	at 20 °C	35.4	13.4	21.1	30.1
	at 80 °C	42.2	8.5	32.3	17.0
	at 20 °C cooled	39.0	8.3	22.9	29.8

cooling to 20 °C. The extent to which heating induced unfolding in LTPs is dependent upon the time and temperature combinations employed. Thus, in situ heating of LTP from apple shows that although the protein unfolds slightly at elevated temperature, it will refold on cooling (*19*), and no major changes were found in the structure of LTP1 from barley in the temperature range of 20−90 °C (*20*). However, more extensive, prolonged (> 1 h) heating causes modification of the protein (*19*, *37*). In contrast, LTP from peach is thermolabile on heating to 95 °C at pH 7 but appears more stable at pH 3 (*38*).

It appears that sunflower LTP shows properties intermediate between those of the highly stable wheat and apple LTPs and the more labile peach LTP. A factor that may also contribute to the apparent differences in thermostabilities shown by various LTPs is the type of heating cycle employed, with more extensive changes in structure likely to occur during the extended cooling cycle employed in the CD experiment in this study. These data are consistent with observations made regarding the thermostability of many members of the prolamin superfamily; the presence of intramolecular disulfide bonds has been implicated in their thermostability (*18*−*20*).

Such thermal treatments are often employed in processing to modify the surface properties of protein ingredients. We therefore investigated the impact of heating on the interfacial properties of the sunflower fractions. Although a number of factors determine the interfacial properties of a protein, they predominantly depend on the ability to adsorb to an interface and thus decrease surface tension. This is largely governed by a combination of surface hydrophobicity and molecular flexibility. Thus, a protein with a more hydrophobic surface is likely to be more surface active. However, a balance is required between surface activity and aggregation, which occurs if the surface of the molecule becomes too hydrophobic. Measurements of interfacial tension can also be sensitive to very small changes in protein structure, and measurements of interfacial rheology can also be useful in this regard (*39*). The results presented are consistent with these principles. The LTP had the shortest retention time on RP-HPLC, indicating the lowest surface hydrophobicity of the sunflower proteins in this study, and was also the least surface active. It also gave the least elastic interfacial film, and as a result it had limited emulsifying capacity. The proportion of α-helix in the heated-cooled LTP decreased, whereas the proportions of β-sheet and β-turn increased, and it only partially refolded to its native structure when cooled to 20 °C. These changes in the secondary structure of the LTP were reflected in the marked increase in the surface activity, as demonstrated by the interfacial tension measurements and by the low elastic modulus of the interfacial films, which decreased even further. The loss of α-helical content, due to the heat treatment, has also been reported previously (*27*). Conversely, SFA8, having the longest retention time on RP-HPLC, proved to be the most surface-active. It generated interfacial films with the greatest elastic modulus, indicating it had the highest packing density on the interface, and formed emulsions with the smallest droplet size. Previous studies have shown that SFA8 contains a single tryptophan residue, which is exposed on the surface and becomes buried in the oil matrix following adsorption to the oil/water interface (*23*). The very small heat-induced structural changes clearly demonstrated by the far-UV CD are also in good agreement with the small increase in surface activity upon heating and the fact that the elastic modulus of the films formed was almost identical to those formed by the unheated protein.

The surface activities of fractions A, B, and C containing Alb 1 and Alb 2 were between those of the LTP and SFA8. Heating

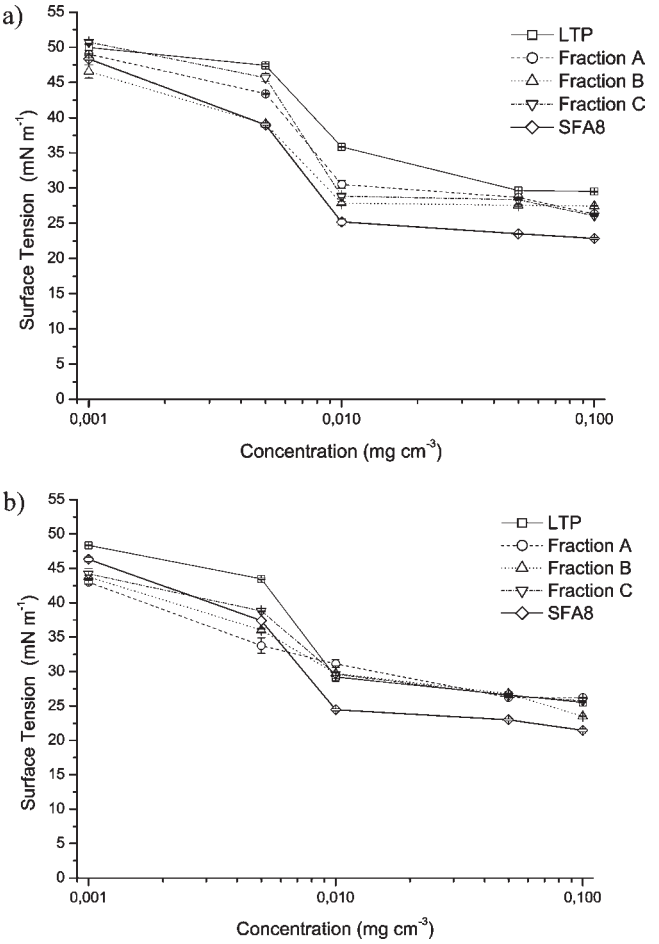

Figure 4. Plot of interfacial tension (**a**) between native aqueous sunflower protein solutions and *n*-hexadecane as a function of concentration before heat treatment and (**b**) between heated aqueous sunflower protein solutions and *n*-hexadecane as a function of concentration after heat treatment. Error bars indicate standard error (SE) and are shown on the graphs.

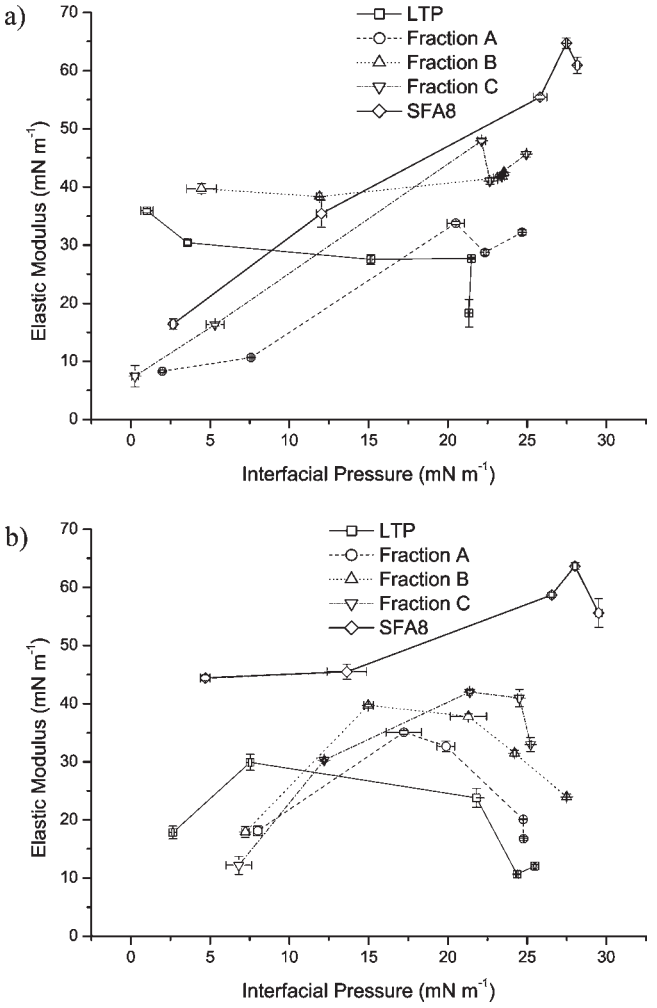

Figure 5. Plot of elastic modulus as a function of interfacial pressure (**a**) between native aqueous sunflower protein solutions and *n*-hexadecane before heat treatment and (**b**) between heated aqueous sunflower protein solutions and *n*-hexadecane after heat treatment. Error bars indicate standard error (SE) and are shown on the graphs.

Table 2. Average Diameter of the Droplets and $D_{3,2}$ Values of the Emulsions

	$D_{3,2}$[a] (μm)
LTP	3.84 (0.040)
fraction A	0.96 (0.042)
fraction B	2.19 (0.104)
fraction C	3.24 (0.532)
SFA8	1.43 (0.093)

[a] Standard error (SE) values are in parentheses.

decreased the surface tension of all three fractions, but the behavior of fraction B differed from those of fractions A and C. The elastic modulus of fraction B, like that of the LTPs, remained constant regardless of the interfacial tension. This suggests that the rigidity of the molecules made it impossible for intra- and intermolecular interactions to increase, even when the surface concentration was increased. Fractions A, B, and C also showed increases in their elastic moduli as the interfacial pressure increased. However, above a certain threshold, which equates to a threshold interfacial concentration, the moduli decreased, indicating a propensity of the interfacial film to collapse rather than compress further.

LTP is a moderately surface active protein; however, it is not able to stabilize emulsions by preventing coalescence to the same

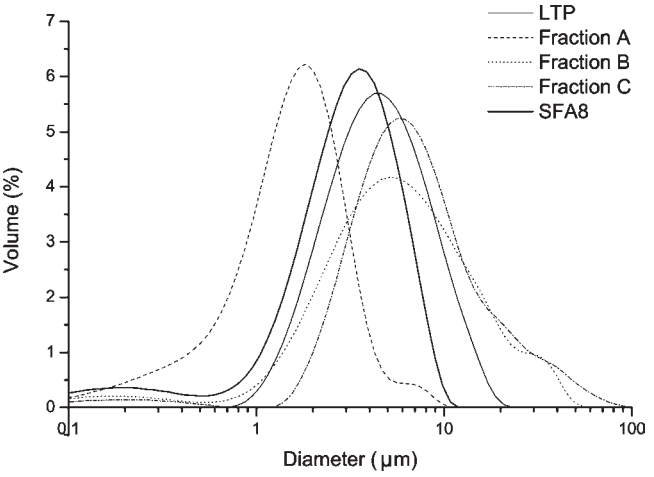

Figure 6. Size of the droplets of the emulsions formed by LTP, albumin fractions A, B, and C, and SFA8.

extent as SFA8. This is due to the weak elastic film formation of the LTP compared to SFA8. Whereas SFA8 is adsorbed to the emulsion interface, LTP is excluded from the interface, as shown by Guéguen and co-workers (*21*). Our results are consistent with their findings. The amino acid sequence of LTP shows fewer hydrophobic residues, and the strength of the SFA8 film could be a consequence of its containing an unusually high proportion of hydrophobic residues, including 16 methionines of 103 residues. The distribution of hydrophobic residues on the protein surface could explain the surface-active behavior of SFA8 (*24*) as they are clustered in four hydrophobic patches on the surface, the fourth of which is the largest and contains Trp76 (*24*). This residue locates in the oil phase on the interface, facilitating adsorption to the oil droplet and strengthening the interaction (*23*). The indole group of Trp76 is surrounded by hydrophobic residues, including four methionines, forming a crown around it (*24*).

None of the mixed albumin fractions (A, B, or C) was able to stabilize emulsions. Although fractions A, B, and C had poor surface activity and formed films with low elastic moduli, the fact that they were less effective than LTP as emulsifiers is surprising. The explanation is likely to be that the aggregation state of the protein fractions was slightly increased as a result of emulsification at higher concentrations (2 mg cm^{-3}). Certainly, the surface activity became almost identical to that of the LTP at the highest concentrations measured (0.1 mg cm^{-3}). These results suggest that although LTP and SFA8 are reasonably good emulsifiers, the other albumin fractions do not contain enough surface active proteins to form a stable emulsion under the same conditions. It has been reported earlier (*23*) that although LTP was not able to stabilize emulsions, the pooled "albumin" fraction (including all of the 2S albumins and LTP) showed reasonable emulsifying properties. Differences between the previously published data and the present results may be due to the differences in SFA8 content between the sunflower varieties used for the experiments. In particular, the hybrid variety Alphasol studied by Burnett and co-workers (*23*) contained a higher proportion of SFA8 than the PR A381 line studied here (based on comparison of the HPLC trace shown in **Figure 1** with a similar analysis reported in ref*23*. However, despite the fact that sunflower 2S albumins are highly polymorphic (*40*), a previous study showed no differences between the emulsifying properties of lines with different compositions (*21*).

In summary, we have shown that five protein fractions from sunflower seeds, including LTP and SFA8, differ in their surface activity at interfaces and that these differences may relate to

differences in their amino acid sequences and protein surfaces. The differences may also be modulated by thermal treatments, which may increase (LTP) or reduce (fractions A, B, and C containing Alb1 and Alb 2) the surface activity. It has been suggested by other authors (*22*) that these fractions would provide excellent functionality in foams and emulsions if used at low pH. In this study it was found that only SFA8 provided significant interfacial properties at higher pH values and that LTP and three mixed 2S albumin fractions (A, B, and C) showed poorer interfacial properties.

ABBREVIATIONS USED

nsLTP, nonspecific lipid transfer protein; SFAs, total fraction of sunflower albumins; SFA8, sunflower albumin 8; Alb 1 and Alb2, albumin genes and proteins; RP-HPLC, reversed phase high-performance liquid chromatography; far-UV CD, far-ultraviolet circular dichroism; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; $D_{3,2}$, diameter of droplets of emulsions.

ACKNOWLEDGMENT

We thank Roger Fido for help with the purification, Graham Moates for assistance with emulsion sizing, and Fiona Husband for assistance with CD spectrometry.

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Received for review February 9, 2010. Revised manuscript received April 14, 2010. Accepted April 16, 2010. Rothamsted Research and the Institute of Food Research receive grant-aided support from the Biotechnology and Biological Sciences Research Council of the U.K. B.B. was funded by IFR Marie Curie Training Site Fellowship QLK1-CT-2000-60030.