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Stability of Some Cactaceae Proteins Based on Fluorescence, Circular Dichroism, and Differential Scanning Calorimetry Measurements

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Characterization of three cactus proteins (native and denatured) from *Machaerocereus gummosus* (Pitahaya agria), *Lophocereu schottii* (Garambullo), and *Cholla opuntia* (Cholla), was based on electrophoretic, fluorescence, CD (circular dichroism), DSC (differential scanning calorimetry), and FT-IR (Fourier transform infrared) measurements. The obtained results of intrinsic fluorescence, DSC, and CD were dissimilar for the three species of cactus, providing evidence of differences in secondary and tertiary structures. Cactus proteins may be situated in the following order corresponding to their relative stability: *Machaerocereus gummosus* (Pitahaya agria) > *Cholla opuntia* (Cholla) > *Lophocereu schottii* (Garambullo). Thermodynamic properties of proteins and their changes upon denaturation (temperature of denaturation, enthalphy, and the number of ruptured hydrogen bonds) were correlated with the secondary structure of proteins and disappearance of α-helix.

KEY WORDS: Cactaceae; proteins; electrophoresis; fluorescence; calorimetry; denaturation; spectroscopy.

1. INTRODUCTION

For a long time Mexican Cactaceae have been used as food, based on their crude protein, fiber content, and digestibility. In traditional medicine cactus has been employed as hallucinogenic, anticancer, and antiinflammatory agents (Bruhn, 1973; Brazner *et al.*, 1984; Velez Boza and Chavez, 1980; Valencia *et al.*, 1985; Keeley and Keeley 1989; Teles *et al.*, 1984; Rodriguez-Felix and Cantwell, 1988; Sirohi *et al.*, 1997). Recent pharmacologic investigations have observed the effect of purified cactus extract on induced diabetes rats (Trejo-Gonzalez *et al.*, 1996), and on non-insulin-dependent diabetes mellitus patients (Noel *et al.*, 1997). Limited data on protein composition analysis have been reported. Somers *et al.* (1991) employed changes in protein composition to monitor the stress in the roots of cactus. Zenteno *et al.* (1988, 1991) studied the purification and characterization of lectins and isolectins.

Recent studies (Gorinstein *et al.*, 1996, 1995*a*) have been focused on the denaturant-induced secondary and tertiary structural changes of proteins as followed by measurements of fluorescence intensity, wavelength of the peak output response, and circular dichroism. In previous research (Gorinstein *et al.*, 1995*b*) seven species of Cactaceae were classified into three groups based on

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their chemical (elemental analysis) and biochemical properties: (I) *Machaerocereus gummosus* (Pitahaya agria), *Stenocereus thurberi* (Pitahaya dulce), *Machaerocereus eruca* (Chirinola), and *Echinocereus engelmannii* (Viejito), (II) *Lophocereu schottii* (Garambullo) and *Pachycereus pringlei* (Cardon), and (III) *Cholla opuntia* (Cholla). Each of these groups differed in polyphenol composition as well as in electrophoretic and chromatographic patterns. However, information on the denaturation of cactus proteins in the dry state is not available. Present work reports results of DSC, circular dichroism, fluorescence spectra, and FT-IR spectroscopy to characterize cactus proteins during denaturation.

2. MATERIALS AND METHODS

2.1. Plant Material

Three common species of wild Cactaceae were collected in different areas of the Baja California desert near Comitan, Puerto San Carlos, and Los Divisaderos, Mexico. These species included *Cholla opuntia* (Cholla), *Lophocereus schottii* (Garambullo), and *Machaerocereus gummosus* (Pitahaya agria).

2.2. Sample Preparation

About 1 kg of each sample of three cactus species was cut into small pieces, homogenized in a Retsch ultracentrifugal mill Type ZM 1 (5 min at 15,000 × *g*), and filtered through cloth. The supernatant, about 200 ml, was then clarified by centrifugation (20 min at 7000 × *g*) and filtered through a Whatman No. 1 filter. This fraction is the crude extract. Solid ammonium sulfate was added to the supernatant to obtain 80% relative saturation, and the precipitate formed during 48 hr at room temperature was collected by centrifugation (20 min at 7000 × *g*). The pellet was resuspended in 40 ml of 20 mM Tris buffer, pH 7.4. The insoluble material was removed by further centrifugation (20 min at 7000 × *g*). The clear supernatant was dialyzed extensively against 20 mM Tris buffer, pH 7.4, using cellulose dialysis tubing (Sigma) with a MW cutoff of 2000. Then the liquid was lyophilized and kept at 4°C until use. This fraction was designated as ammonium sulfate protein precipitate (ASPP).

One-half g samples of dialyzed and lyophilized crude cactus were dissolved in 5 ml of THA (10 mM Tris-HCl + sodium azide 0.02%; pH 8.0) and dialyzed against 500 ml of the same buffer (three times, 12–16 hr, 10°C). The sample was put on a DEAE-Sepharose (Pharmacia Fine Chem.) column (53 ml) equilibrated with THA, and washed with the same buffer until the absorbance (280 nm) was near zero. The absorbed proteins were eluted with NaCl–THA (0.5 M NaCl dissolved in THA) by washing the column until the absorbance was near zero. The 2-ml fractions were collected and the absorbance at 280 nm was measured.

2.3. Protein Content

The protein content was measured by the procedure of Bradford (1976) and Lowry *et al.* (1951) and bovine serum albumin (BSA) was used as a standard.

2.4. Electrophoretic Separation

The proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli (1970). The composition of the gels was the following: stacking (acrylamide, 4% T, 2.67% C) and separation (acrylamide, 15.2%T, 1.32 %C) gels. SDS–PAGE system MiniProtean II (BioRad) was used. The samples were diluted 1:30 in 125 mM Tris/HCl, pH 6.8, 4% (w/w) SDS, 20% (w/w) glycerin, 10% mercaptoethanol, and 0.002% (w/w) bromophenolblue and heated for 3 min at 95°C. The samples of 1 µl were applied to the top of stacking gel. As molecular markers the SeeBlueTM prestained standard from Novex was applied: bovine serum albumin (98 kDa), glutamic dehydrogenase (64 kDa), alcohol dehydrogenase (50 kDa), carbonic anhydrase (36 kDa), myoglobin (30 kDa), lysozyme (16 kDa), and aprotinin (6 kDa).

Two-dimensional electrophoresis (2DE) was done following the procedure of Otto *et al.* (1996). The protein sample was dissolved in lysine buffer containing 9 M urea, 70 mM DTT, and 2% ampholyte mixture (Servalyte 2–4), 25 mM Tris/HCl, pH 7.1, 50 mM KCl, 3 mM EDTA, 2.9 mM benzamidin, and 2.1 µM leupeptin. Then the sample in concentration of 33µg/µl was applied to isoelectrofocusing (IEF) in the first dimension. The IEF gels contained 2% of an ampholyte mixture at pH 2–11. In the second dimension SDS–PAGE was used with 15% SDS–PAGE gels. The 2DE gel size was about 7–8 cm.

2.5. Fluorescence Spectra

Fluorescence measurements were done using a Model FP-770 Jasco spectrofluorimeter. The temperature of the samples was maintained at 30°C with a thermostatically controlled circulating water bath. Fluorescence emission spectra for all native and denatured samples were taken at excitation wavelengths (nm) of 274 and 295 and recorded over the frequency range from the excitation wavelength to a wavelength of 500 nm.

2.6. Differential Scanning Calorimetry (DSC)

The denaturation of proteins was assessed with a Perkin Elmer DSC System 4. Lyophilized samples of about 1 mg were sealed in aluminum pans (Ma and Harwalkar, 1988). Denatured samples were prepared by homogeneous mixture of native protein and denaturant in the dry state. Then the mixed sample of 1 mg was sealed in aluminum pan in the same way as the native one. As reference an empty pan was used. The scanning temperature was 30–120°C at a heating rate of 10°C/min. Indium standards were used for temperature and energy calibrations. T_d and ΔH were calculated from the thermograms (Gorinstein *et al.*, 1995a).

2.7. Fourier Transform Infrared (FT-IR) Spectra

A Perkin-Elmer 2000 FT-IR spectrometer was used to record IR spectra. Lyophilized material was mixed with KBr, and the pellet was pressed at 10,000 kg/cm^2 for 15 sec.

2.8. Circular Dichroism (CD) Spectra

CD spectra were measured with a Jasco J-600 spectropolarimeter (Japan Spectroscopic Co., Ltd., Japan) at room temperature under constant nitrogen purge. Solutions (0.03 mg/ml) of proteins were prepared by dissolving the lyophilized powder in 0.01 M phosphate buffer, pH 7.2. The absorbancies of all solutions were kept below 1.0 (Matsuura and Manning, 1994; Zemser *et al.*, 1994). Denaturation of proteins was performed with 6 M GuHCl. CD spectra represent an average of eight scans collected in 0.2-nm steps at average rate of 20 nm/min over the wavelength range 180–250 nm of far-UV (FUV). CD spectra were baseline-corrected, and the data are presented as the mean residue ellipticities (θ). The CD spectra were evaluated with Contin software according to Provencher's algorithm, allowing the comparison of secondary structures from different cactuses.

3. RESULTS AND DISCUSSION

3.1. Electrophoretic Separation

The results of chromatography on DEAE-Sepharose showed that part of the Pitahaya agria crude extract was not absorbed to the column (peak A), while the main protein content is retained and could be eluted with NaCl (peak B), and the yield was about 91.3%. The peaks A and B contain the color of the crude extract, but peak A was more intense in color than peak B. Peak C, which was eluted from the column by 0.1 M HCl, was colorless. All ion-exchange chromatography patterns of Cactaceae from the three crude extracts varied from those, which are shown on Fig. 1 with slightly different intensities of colors in peaks A and B (data not shown).

Figure 2 shows sharp bands for Pitahaya agria (lane 2) and Cholla (lane 4) and diffuse ones for Garambullo (lane 3). Pitahaya agria has pronounced bands at ca. 38–40 kDa and ca. 27–29 kDa with minor components at higher and lower molecular weight ranges. Garambullo shows three prominent bands at ca. 20, 26, and 33 kDa (the appearance of a strong background stain in this sample is probably due to the presence of high amounts of polyphenols in this plant, which could not be removed). Cholla, on the other hand, possesses a main protein band at ca. 37 kDa with minor ones at higher molecular weights. In order to clarify the composition of the main protein bands additional resolution was achieved by isoelectrofocusing on 2DE minigels.

Two-dimensional electrophoretic separation of the proteins (peak B, Fig. 1) from Pitahaya agria, Garambullo, and Cholla is shown in Figs. 3 a-c, respectively. Better resolution was obtained in the sample of Pitahaya agria. Pitahaya agria samples migrated in several bands with an apparent molecular weight of about 24, 29, 36, and 43. The main band of ca. 38–40 kDa resolved here into a pearl-chain-like series of proteins with pI's of ca. 4–7, whereas the lower protein band consisted of one pronounced, strongly acidic protein of ca. pI 2 and additional components spreading over the entire pH range. In addition, some small polypeptides in the basic region (pI ca. 8–10) and a few high-molecular-weight proteins in the acidic region (pI ca. 3–5) were visible. In all gels the majority of proteins migrated with a molecular mass of ca. 38–45 kDa differing in their isoelectric point over a broad range of pI 2.0–11.0. In addition, there are a few spots in the acidic region with distinctly lower molecular

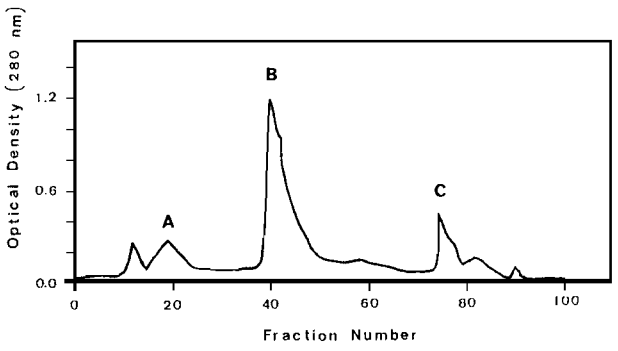

Fig. 1. DEAE-Sepharose chromatographic patterns of crude cactus from Pitahaya agria.

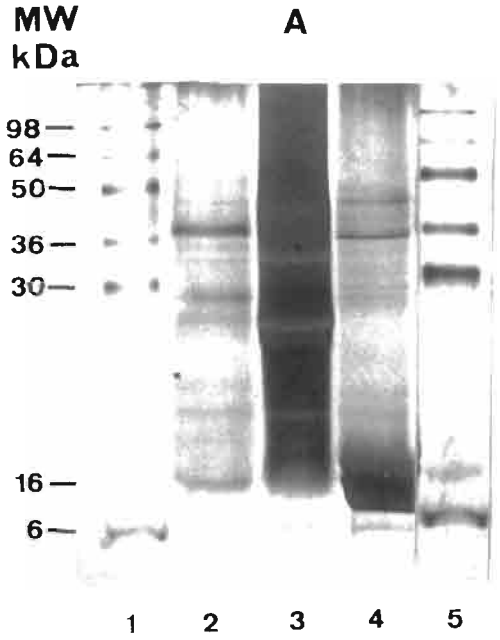

Fig. 2. Electrophoretic separation of cactus proteins (peak B in Fig. 1) in SDS-gradient gel 10–15% PAAG. Molecular weight marker (lane 1), Pitahaya agria (lane 2), Garambullo (lane 3), Cholla (lane 4), molecular weight marker (lane 5). Proteins were detected by silver staining.

masses. A protein of ca. 37 kDa with a pI 4 and three polypeptides of ca. 10 kDa with pI values of about 2.5, 4.0, and 5.5, respectively, were found for Cholla. Pitahaya agria, Cholla, and Garambullo demonstrated some similar bands. Garambullo had aggregated subunits mostly of low molecular weight around 14–25 kDa. Electrophoretic separation of cactus proteins does not give the estimation for their classification or stability, but it is an additional index for their partial characterization.

3.2. Intrinsic Fluorescence Properties of Proteins

Lyophilized material of ASPP was used for the fluorescence studies. Fluorescence spectra of the extracts obtained from the three cactus species studied are shown in Fig. 4. At the excitation wavelength of 295 nm (Table I, Fig. 4) the fluorescent spectra of Pitahaya agria and Cholla demonstrated typical emission for tryptophan-containing proteins in the interval 331–342 nm (Arntfield *et al.*, 1987). The emission peak of Garambullo was displaced to a longer wavelength (356.5 nm). This displacement shows that the tryptophan is in a more polar environment, possibly implying that the protein is in a more opened or relaxed state. The emission spectrum for Pitahaya agria showed the maximum at 335.5 nm and for Cholla at 344 nm (Fig. 4). Pitahaya agria proteins show high contents of phenylalanine plus tyrosine in comparison with Cholla. At an excitation wavelength of 274 nm slight shoulders, corresponded to tyrosine emission, were seen in Pitahaya agria and Garambullo proteins at 307 and 304 nm, respectively. At an excitation wavelength of 295 nm tryptophan appears to be the only aromatic amino acid that absorbs light (Khan *et al.*, 1980). The wavelength of emission for tryptophan, tyrosine, and phenylalanine was situated at 348, 303, and 282 nm, respectively. The maximum at 344.0 nm for Cholla protein may be the contribution of tryptophan and tyrosine or mostly tryptophan itself. No data are available in the literature for fluorescent properties of cactus proteins; the results may be compared with other plant proteins. Arntfield *et al.* (1987) and Gorinstein *et al.* (1996) reported a maximum of tryptophan emission for vicilin and other plant globulins around 347 nm, which is in agreement with obtained results on cactus proteins (344 nm).

3.3. Urea-Induced Denaturation

Denaturation of cactus proteins with urea was examined at both excitation wavelengths of 295 and 274 nm.

Table I shows a decrease of relative fluorescence intensity with increasing urea concentration. The percentage of denaturation for Pitahaya agria proteins with 2, 3, and 8 M urea was 7.7%, 12.8%, and 23.0%, respectively (Table I). When the concentration of urea increased, the fluorescence intensity gradually decreased. At the urea concentration of 2 M a small shift in the wavelength was fixed. At 3 M urea a shoulder appeared at 309 nm, which is characteristic of tyrosine fluorescence. At the same time the tryptophan emission peak shifted from 335.5 to 343.5 nm for Pitahaya agria, from 356.5 to 362.0 nm for Garambullo, and from 344 to 350 nm for Cholla. These wavelengths slightly changed till the addition of 8 M urea. The appearance of a shoulder was a result of ceasing energy transfer from excited tyrosine to tryptophan residues in proteins during unfolding. Probably the distance between the tryptophan and tyrosine residues was increased. A shift of tryptophan residues was found. It was toward an increase in polarity of the microenvironment around the aromatic amino acids (Khan *et al.*, 1980). This was attributed to a decrease in energy in the maximum of emission and of fluorescence intensity. Another explanation might be that this shift was not due to quenching of fluorescence by urea. There is a linear increase of tryptophan fluorescence with urea concentration in the model compound N-acetyltryptophanamide (Chen *et al.*, 1969).

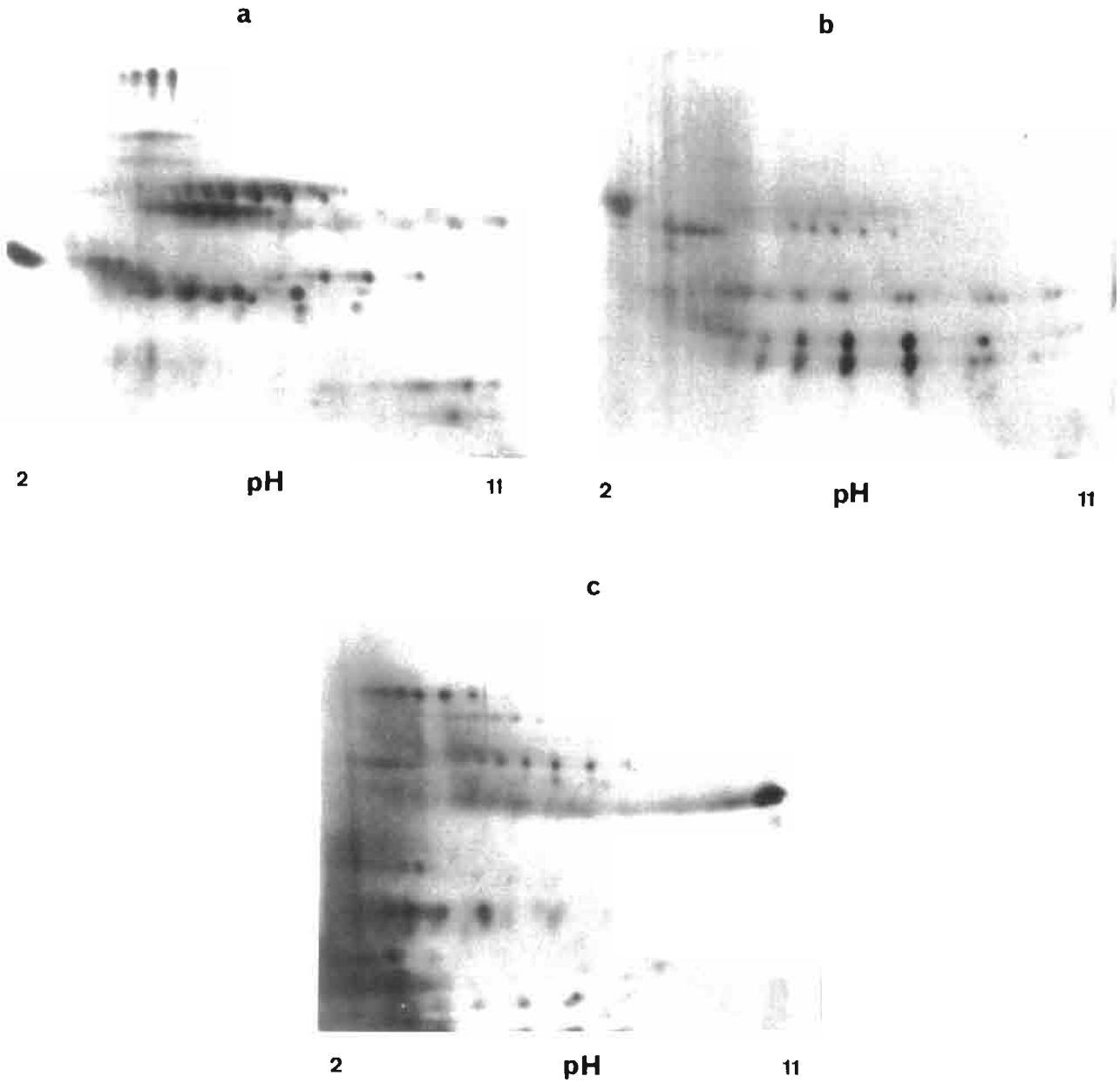

Fig. 3. Electrophoretic separation of cactus proteins (peak B in Fig. 1) in 2DE: (a) Pitahaya agria, (b) Garambullo, (c) Cholla. Samples were applied at the anodic side of the IEF gel. Proteins were detected by silver staining.

Table I shows that the maximum of denaturation was achieved at 8 M urea. The effect of 8 M urea on the degree of denaturation of Pitahaya agria, Cholla, and Garambullo is shown in Table I. The percentage of denaturation was 23.0%, 27.4%, and 33.3%, respectively. The difference in the extent of denaturation among the three species of Cactaceae may be explained by the differences in amounts of amino acids and by the sulfur bridges existing in such proteins.

3.4. GuHCl-Induced Denaturation

The relative fluorescence intensity decreased with addition of GuHCl at the excitation of 295 nm in all protein samples (results not shown). Denaturation of 14% was achieved at 3 M GuHCl and nearly did not change further up to 6 M GuHCl (15%). The difference between addition of 3 M and 6 M GuHCl was only in the shift of the peak. At 3 M GuHCl the extent of de-

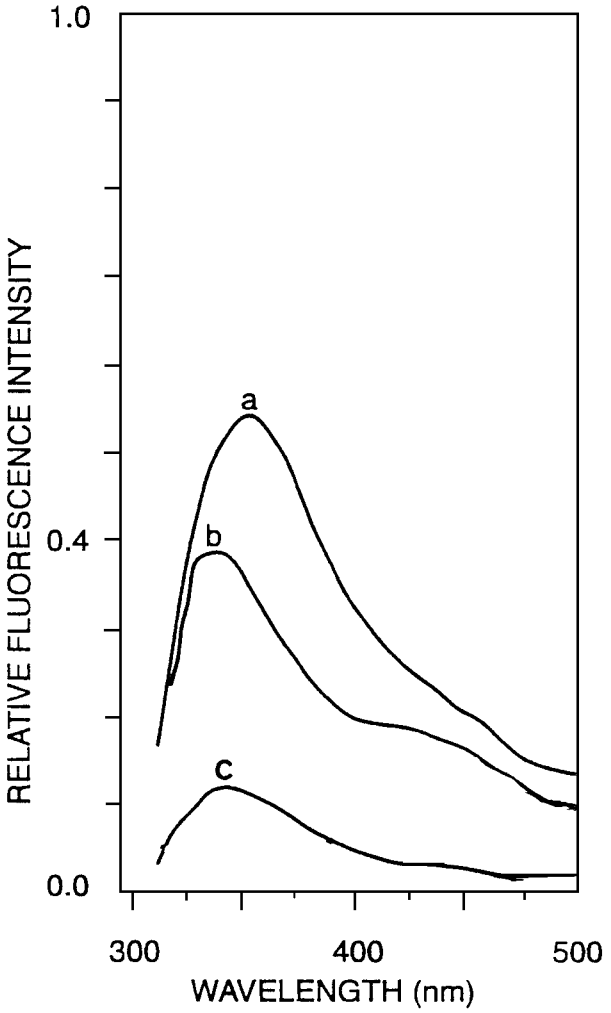

Fig. 4. Fluorescence emission spectra of proteins from Pitahaya agria (a), Garambullo (b), and Cholla (c). Excitation at 295 nm.

naturation for Pitahaya agria was similar to that obtained with 3 M urea. But a shift in the maximum of emission achieved at 6 M GuHCl (to 348.0 nm) was larger than the shift to 343.5 nm after 6 M urea treatment. This is an indication of the lower stability of the proteins for GuHCl than for urea. These changes in the tertiary structure of proteins with urea and GuHCl agree with the results of Lilley (1986) on the reactivity of free sulfhydryl groups in protein fractions. At 6 M GuHCl all SH groups of the proteins reacted; however, unfolding with urea was not sufficient to expose all free SH groups buried in the interior of the protein. Its helices resist the disruption by urea. Apparently the same phenomena can be observed with cactus proteins. GuHCl and urea induce transition by interfering with the hydrophobic regions in the interior as well with the hydrogen-bonding

Table I. Fluorescence Properties of Native and Denatured Pitahaya agria (P.a.), Garambullo (G), and Cholla (C) Proteins[a]

Protein	I_{274}	I_{295}	%D
P.a. + 0 M urea	0.77±0.031	0.39±0.014	0
P.a. + 2 M urea	0.60±0.011	0.36±0.021	7.7
P.a. + 3 M urea	0.55±0.027	0.34±0.011	12.8
P.a.+ 8 M urea	0.50±0.015	0.30±0.004	23.0
G + 0 M urea	0.55±0.021	0.54±0.016	0
G + 8 M urea	0.31±0.019	0.36±0.011	33.3
C + 0 M urea	0.27±0.005	0.11±0.034	0
C + 8 M urea	0.17±0.013	0.08±0.008	27.4

[a] Mean values of triplicates±standard deviation. I_{274}, Fluorescence intensity at excitation of 274 nm. I_{295}, Fluorescence intensity at excitation of 295 nm. *%D*, Percentage of denaturation.

pattern involved in the polar regions of the peptide chain in the protein molecule. The same picture was seen in such type of proteins from Pitahaya agria, which showed similar fluorescence spectra as other plant globulins (Gorinstein *et al.*, 1996).

3.5. DSC Measurements of Cactus Proteins

The DSC method has been extensively used to study unfolding in the liquid state. This method is highly sensitive to conformational changes and can be a measure of disorder in macromolecular systems upon heating. The native structure of proteins from cactus was stable up to a critical temperature and then became disrupted with intense heat absorption (Table II). Urea destabilized the protein conformation of cactus proteins as reflected by the marked decrease ΔH and T_d values for Pitahaya agria, Cholla, and Garambullo. These results suggest changes in molecular conformation of proteins and are consistent with those of other authors (Ma and Harwalkar, 1988; Nagano *et al.*, 1994; Wang and Damodaran, 1991). A considerable number of protein molecules shift to a state that contributes much less to the unfolding transition, thus causing a significant decrease in the calorimetric enthalpy. The enthalpy changes of the initial and remaining DSC endotherm were measured and used for calculation of percentage of denatured proteins. The entrophy S associated with state transition and affirmed disordering of protein structure was also calculated (Table II). Comparison of the thermograms of native and denatured proteins from Pitahaya agria, Garambullo, and Cholla showed the changes in the temperature of denaturation (T_d, °C) for Pitahaya agria of 53.5, for Garambullo of 43.5, and for Cholla of 48.5, and the enthalpy (ΔH, kcal/mol) for Pitahaya agria of 109.7, for Garambullo of 23.0, and for Cholla of 45.6. Broadening of the peak was also demonstrated. The decrease in ΔH indi-

Table II. Thermodynamic Properties of Native and Denatured Proteins from Pitahaya agria (P.a.), Garambullo (G), and Cholla(C) Proteins[a]

Protein [b]	T_d (°C)	ΔH (kcal/mol)	ΔS (kcal/mol K)	n_b	%D
P.a.	61.2±0.8	219.4±3.7	0.656	55	0
P.a. + U	53.5±0.9	109.7±8.1	0.336	28	20
G.	52.0±1.3	75.3±7.3	0.232	19	0
G. + U	43.5±1.9	23.0±4.5	0.073	6	31
C.	59.0±1.1	121.5±4.5	0.366	31	0
C. + U	48.5±1.0	45.6±3.1	0.142	12	26

[a] Mean values of triplicates ± standard deviation. T_d, Temperature of denaturation. ΔH, Enthalpy. ΔS, Entropy. n_b, Number of broken hydrogen bonds. $\%D$, Percentage denaturation.
[b] U, Urea (1:1).

cates denaturation and less stable structure. It means that conformation of the protein molecule has shifted toward 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 one.

Wagner and Anon (1985) reported the influence of hydrogen bond disruption on enthalpy changes in DSC. According to these authors thermal protein denaturation involves the rupture of disulfide and hydrogen bonds. One disulfide bond contributes ΔH = 25 kcal/mol and a negligible ΔS. The number n of hydrogen bonds corresponds to ΔH = 4 kcal/mol and ΔS = 0.012 kcal/mol/per protein molecule. Thus, the number n_b of broken hydrogen bonds can be calculated as $n_b=\Delta S/0.012$ and $n_b=(\Delta H-25)/4$, where ΔS is the entropy and ΔH is the enthalphy of denaturation. Our calculations show that during denaturation the rupture of 55 hydrogen bonds was involved in native Pitahaya agria in comparison with 19 in Garambullo and 31 in Cholla proteins. It was assumed that during thermal denaturation only the rupture of hydrogen bonds is involved. With previous urea-induced denaturation the number of hydrogen bonds was reduced to about 30–50%. This trend is associated with the disruption of hydrogen bonds during heat denaturation and reflects a decrease in α-helix content of denatured protein (Kato *et al.*, 1987), which was shown in our CD measurements. Hence, hydrogen bonding is the main stabilizing force in protein stability. Hydrophobic interactions play an important role in the thermal stability of proteins and probably in cactus proteins. Addition of protein denaturants such as urea led to a decrease in enthalpy and T_d, indicating protein denaturation and loss of cooperatively. The presence of reducing agents such as 2-ME did not affect DSC characteristics (results not shown). Probably, disulfide bonds present in proteins do not contribute to the thermal response of the protein. Preheating treatments at 100°C resulted in a progressive decrease in enthalpy, indicating partial denaturation. There was a marked increase in T_d, suggesting that the preheated cactus proteins may aggregate to form a more compact structure with higher thermal stability and cooperativity.

Our data show that DSC can be used to study the effect of medium composition and heating on the tertiary and quaternary structures. Since the functional properties of proteins are greatly influenced by their conformation, DSC is a valuable tool in assessing the potential of cactus proteins as a functional ingredient in different systems (Bora *et al.*, 1994).

3.6. CD Studies

The optical activity of α-helix in the far-ultraviolet (FUV) permits the use of CD studies for investigation of conformational changes in protein solutions. The CD band position for various structures has been reported (Sarkar and Doty, 1966; Chen *et al.*, 1972). The CD spectra of proteins from Pitahaya agria, Garambullo, and Cholla in FUV region were compared. Proteins from Pitahaya agria showed a prominent band with minimum at wavelength 213 nm, which represents the β-structure of the protein (50%), and peak at 207 nm, corresponding to 7.5% of α-helix. In Garambullo (peaks at 206 and 218 nm) 29% of α-helix and 8% of β-sheet were calculated. Cholla proteins also contained both α-helix (12%) and β-sheet (17%) structures. The corresponding mean residue ellipticity (θ) was calculated in deg cm^2 $dmol^{-1}$. Garambullo and Cholla showed a lower percentage of β-structure than Pitahaya agria. In the presence of 6 M GuHCl a shift in the peak of 213 nm was registered in the Pitahaya agria spectrum, suggesting that a loss in β-structure has occurred (to 7%). α-Helix disappeared and disordered structure content increased to 93%. In Garambullo α-helix de-

creased to 3.4% and β-structure was transformed into random coil (97%) upon treatment with 6 M GuHCl. The composition of the secondary structure in Cholla indicated a decrease in both α-helical (2%) and β-structures (5%). Similar behavior for all protein samples in the presence of 6 M GuHCl was observed. With the addition of 50 mM of 2-ME and 0.6 M GuHCl only negligible α-helix remained in Garambullo proteins (the highest percentage of the three samples) after reducing action of 2-mercaptoethanol overnight (data not shown). These observations are in agreement with Marcone and Yada (1992) and Gorinstein *et al.* (1995*a*, 1996), who explained this by existing polymeric species and partly as a result of sulfhydryldisulfide interchange reaction. Our results are in correspondence with such characteristics in the gelling and functional behavior of the plant protein. Garambullo protein has a typical CD profile for an α-helix-containing protein with a minimum near 206 nm with nonstable structure due to a lack of the disulfide cross-links. The SDS–PAGE of Garambullo proteins shows four dissimilar aggregated subunits. Probably, weak secondary forces participate in subunit interaction and dissociate under denaturation.

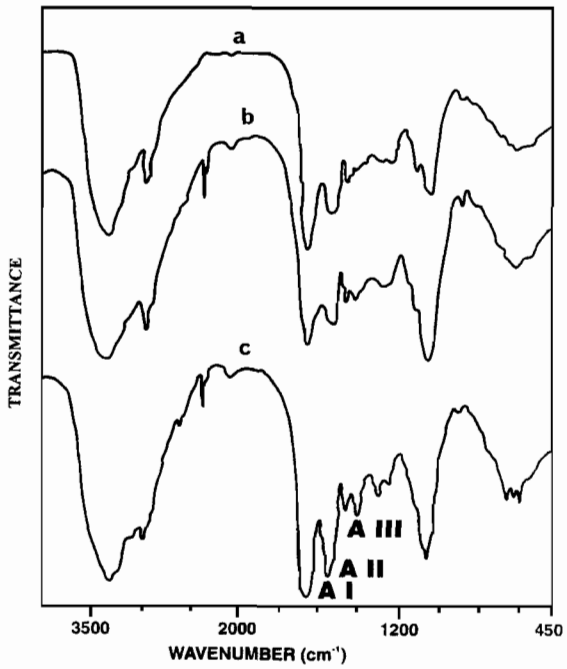

Fig. 5. FT-IR spectra of proteins from Garambullo (a), Pitahaya agria (b), and Cholla (c).

FT-IR spectra

All protein samples showed similar bands at 3300 cm^{-1} (amino acid peak) and at 2900 cm^{-1} (CH stretching). Amide I (AI), Amide II (AII), and Amide III (AIII) bands (in the range of 1650, 1530, and 1300–1250 cm^{-1}) differ for spectra of Pitahaya agria, Garambullo, and Cholla, (Kaiden *et al.*, 1987). Most notable is the absence of the 2600-cm^{-1} band in Garambullo proteins. Cholla proteins (Fig. 5, curve c) demonstrated two sharp large bands in the AI (1656 and 1544 cm^{-1}). Bands in the 1300–1200 cm^{-1} region pointed out on the presence of both α-helix and β-structure in this protein. Rather high of AII to AI bands in Garambullo can be attributed to the high content of α-helix. The broad band at 1268 cm^{-1} confirms this suggestion. The FT-IR spectrum of Pitahaya agria demonstrated a significant shift in the AI band to 1611 cm^{-1}. Such changes and the absence of a clear band in the region of 1300–1250 cm^{-1} confirm the high β-sheet content of this protein.

In this work the protein composition of three cactus species was characterized. The obtained results may be used in clarifying their structure–function properties. Differential calorimetry (DSC), circular dichroism (CD) and intrinsic fluorescence (IF) have been used to describe the conformational changes in proteins. The IF and FT-IR spectra of studied proteins were measured to compare their structure. Differences in the emission peak response and fluorescence intensity, as well as changes in amide band content, were found. It is tempting to speculate that the changes in the protein structure of cactus species observed by fluorescence measurements can result from the partial unfolding of α-helix found in the CD and FT-IR spectra.

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