Effect of Chemical and Enzymatic Degradation on the Functional Properties of Fraction-1-protein

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Chemical and enzymatic treatments for 30 min degraded F-1-p and altered its functional properties. A decrease in solubility and fat-binding capacity but an increase in emulsion viscosity were observed with proteins from hydrolysis at pH 11 and 110 °C. Selective cleavage of tryptophanyl peptide bonds by *N*-chlorosuccinimide/urea reagent lowered water absorption, fat-binding capacity, and emulsion viscosity. Acid- (0.015 N HCl, 110 °C) and pepsin-treated proteins had respectively low and high emulsion viscosities, whereas both showed poor foaming capacity and stability. Protease digestion increased foaming properties but totally lost its emulsion viscosity. All degradations negligibly modified emulsion capacity. Prolonged treatments with acid and protease rendered the hydrolyzed proteins completely soluble at pH 3 and pH 9, respectively, while their water and fat absorption progressively reduced. The native F-1-p is virtually superior in all functional properties measured over its degradation products; however, the latter remained comparable to or better in functionality than food proteins.

Fraction-1-protein (F-1-p) identified as the enzyme D-ribulose 1,5-biphosphate carboxylase/oxygenase is responsible for photosynthesis and photorespiration in green plants (Kung, 1976) and is the most abundant protein on earth. This protein is evolutionarily conserved and thus exhibits a high degree of homogeneity among higher plants (Miziorko and Lorimer, 1983). F-1-p's from young tobacco (Sheen and Sheen, 1985), alfalfa (Knuckles and Kohler, 1982), and soybean (Sheen, 1986) plants have recently been isolated in large quantities and demonstrated to be nutritionally and functionally superior over egg white, casein, and soy protein isolates. The leafy residues from the protein process are quality animal feeds (Kohler et al., 1978; Sheen, 1983, 1986). In tobacco, these residues can also be processed into a potentially safer smoking product due to the low quantity of smoking and health-related chemical constituents in young leaves (Wildman and Sheen, 1981). The quality of life in terms of human health and illness could be improved in industrial and developing countries alike when F-1-p and leafy residues are commercially accepted as agricultural products.

F-1-p contains no carbohydrate and lipid moieties and is pure in amino acid composition (Kawashima and Wildman, 1970). Isolation of protein to high purity often encounters solubility problems. Proteins can be rendered more soluble by controlled acid or alkali hydrolysis. It is well-known that alkali treatment of soybean seed protein results in an increase of solubility to facilitate texturization. In addition, a partial degradation of protein by proteolytic enzyme could improve its functional properties to meet the needs of the food industry. A good example is the formation of cheese by enzymatic hydrolysis of casein. A recent study showed that F-1-p lost its quaternary structure soon after exposure to acid, alkali, and proteolytic enzymes (Sheen and Sheen, 1987). The large subunits of the native protein were subsequently degraded into polypeptides, while the small subunits remained intact for a long time. Protein denaturation and degradation resulted in a mixture of polypeptides whose composition varied depending upon the treatment method and duration. It is anticipated that F-1-p could be subjected to similar harsh conditions during processing and reprocessing in the food industry. How the resultant degradation of the protein alters its functional properties is a natural concern for the food industry. On the other hand, any change in functionality bears scientific interest in that it would reflect a result of interactions within and between polypeptides without the interference of nonproteinaceous macromolecules. This could give insight into the F-1-p structure and functionality relationship. The present study, therefore, examines the functional properties of F-1-p partially degraded by a variety of chemical and enzymatic treatments and treatment duration. Tobacco F-1-p was used as the model protein.

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MATERIALS AND METHODS

F-1-p Isolation. Leaves from young plants of tobacco (*Nicotiana tabacum* L.) cultivar Ky 14 grown in the greenhouse were processed by the procedure recently described (Sheen, 1986) to yield crystalline F-1-p, which was solubilized at pH 8.5 prior to freeze-drying. Three separate batches were prepared for the present study. The purity of the preparations was judged by the analysis of subunit pattern on SDS-PAGE gel (Sheen and Sheen, 1987) and the absorption ratio of 280 nm/260 nm being greater than 1.8. The latter criterion was based on the UV spectra of crystalline F-1-p purified by gel permeation (Lowe, 1977) and collodion bag dialysis (Chan et al., 1972).

Chemical Treatments. Acid (0.015 N HCl) and alkali (10 mM glycine–NaOH buffer, pH 11) hydrolysis of F-1-p at 110 °C yielded more polypeptides in the range of 5–50 kDa on SDS-PAGE gel when treated for 30 min (Sheen and Sheen, 1987). A 30-min treatment was therefore chosen for the partial degradation of F-1-p under these acidic and alkaline conditions. A protein solution (10 mg/mL) was heated at boiling temperature on a Soxhlet apparatus to accommodate each 1-L volume. Another method of chemical degradation employed the *N*-chlorosuccinimide (NCS)/urea reagent, which selectively cleaves tryptophanyl peptide bonds (Lischwe and Sung, 1977). In practice, F-1-p at a concentration of 0.018 mM (10 mg/mL) was mixed in a 10-fold excess molar ratio of NCS in a cleavage buffer composed of 27.5% acetic acid and 4.68 M urea. The protein solution and NCS/urea buffer were kept at a 4 to 10 ratio in volume. The reaction was carried out at room temperature for 30 min and stopped by adding *N*-acetyl-L-methionine. The reaction mixture was subsequently dialyzed against water at 4 °C in dialysis tubing of 1-kDa cutoff.

To ascertain the dependency of functional modification to treatment duration, the acid hydrolysis of F-1-p in the above specified conditions was conducted at time intervals

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of 0, 15, 30, 60, 90, 120, and 150 min. The protein solutions either from chemical treatment or with varying treatment duration were freeze-dried.

Enzymatic Degradation. Immobilized protease (type XVI from *Bacillus subtilis*) and pepsin (1:10 000 powder from porcine stomach mucosa) on porous glass were prepared according to the procedure described by Bliss and Hultin (1977). The amount of enzyme bound to porous glass, the volume of porous glass employed in relation to that of pH 8 and pH 3 buffers containing F-1-p (10 mg/mL), and the incubation condition at 40 °C were identical with that detailed in a recent paper (Sheen and Sheen, 1987). The protein was digested for 30 min, and the end solution was freeze-dried. A time course experiment, similar to the acid hydrolysis mentioned earlier, was also performed with the immobilized protease in order to study the change of functionality as a function of digestion duration.

Polyacrylamide Gel Electrophoresis. F-1-p preparation and chemically and enzymatically degraded proteins were analyzed with SDS-PAGE on a gel slab having a linear gradient of 7.5–17.5% acrylamide (Laemmli, 1970). Freeze-dried protein powder was solubilized in 62.5 mM Tris-HCl buffer, pH 6.8, containing 10% glycerol, 5% 2-mercaptoethanol, and 2.3% SDS. An aliquot containing 30–50 μg of protein for each treatment was electrophoresced along with molecular weight markers (from Bio-Rad Laboratories). Coomassie brilliant blue R was used to visualize the protein bands.

Functional Properties. Solubility index was determined by the method of Voutsinas and Nakai (1983) with modifications. Protein samples of 1% concentration in 0.1 M each of citrate–phosphate buffer, pH 3 and 5, phosphate buffer, pH 7.4, and boric acid–borax buffer, pH 9, were blended in a Sorvall homogenizer at a speed of 5000 rpm for 1 min. Aliquots in duplicate were taken from the suspension of each sample, and one drop of 0.1 N NaOH was added to solubilize the protein. The remaining homogenate was centrifuged at 20000*g* for 30 min. Again, aliquots were taken from the supernatant in the same manner. Aliquots of both suspension and supernatant were quantified for protein content by the Bio-Rad protein assay method (Bradford, 1976). The solubility index expressed in percentage was the ratio of protein content in the supernatant to that in the suspension.

Foaming capacity and stability up to 2 h were determined according to Lawhon and Cater's procedure (1971). Water and fat absorption capacity was measured by the methods of Fleming et al. (1974) and Lin and Humbert (1974), respectively. To distinguish the amount of oil truly bound to protein from that due to entrapment, the turbidimetric method of Voutsinas and Nakai (1983) was followed for determination of fat-binding capacity. Fat-binding capacity is supposedly related to protein surface hydrophobicity. Hydrophobicity of the native and partially degraded F-1-p was determined by a method using *cis*-parinaric acid as a fluorescence probe and is expressed as S_0 which is the initial slope of the fluorescence intensity vs protein concentration plot (Kato and Nakai, 1980).

Protein samples in 1% solution adjusted to pH 5, 7, 9, and 11 with 0.1 N HCl or NaOH were blended at the low-speed setting of a two-speed Waring blender while vegetable oil was added. Emulsion capacity of the protein was determined by colloidal phase change by monitoring for infinite resistance with a volt–ohm meter (Regenstein and Regenstein, 1984). Viscosity of the emulsion with various combinations of protein and oil concentration was measured with a Stormer viscometer at 25 °C by the method previously reported (Sheen and Sheen, 1985).

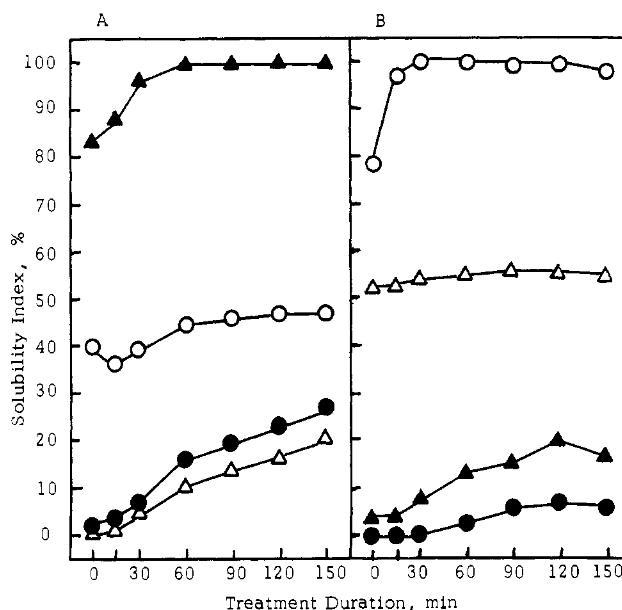

Figure 1. Change of solubility index of F-1-p hydrolyzed with (A) acidic condition and (B) immobilized protease as a function of treatment duration. Protein hydrolysates were tested at pH 3 (▲), pH 5 (●), pH 7.4 (△), and pH 9 (○).

For comparison, egg white, casein (from Sigma Chemical Co.), and soy protein isolates Ardex F and Ardex R (from Archer Daniel Midland Co.) were included in all functionality determinations. The experiments were separately performed with three protein preparations unless otherwise stated. The results are presented as an average of the experiments.

RESULTS

Functionality Change as a Function of Degradation Duration. Analyses of SDS-PAGE on the time course of acid hydrolysis and protease digestion of F-1-p revealed gel patterns identical with those published in a recent paper (Sheen and Sheen, 1987). The results confirm that the large subunits of native protein were rapidly degraded to many polypeptides within 30 min and the number and size of polypeptides were reduced after 150 min. Small subunits remained relatively stable throughout the treatments. This demonstrates the change of polypeptide composition with respect to treatment duration.

In general, both acid hydrolysis and protease digestion increased the solubility index of F-1-p as a function of time (Figure 1). At pH 3, the acid-treated protein showed initially as being over 80% soluble and after 1 h hydrolysis became completely soluble. The same preparation was partially soluble at pH 9, less soluble at pH 5, and least soluble at pH 7.4. A 30-min protease digestion rendered the protein completely soluble at pH 9. At pH 7.4, the digested samples maintained about 50% solubility, while they were less than 20% soluble in the acidic pHs.

Changes in foaming capacity and stability as well as water and fat absorption during the course of F-1-p degradation are given in Table I. Acid hydrolysis enhanced foaming capacity only if the treatment was longer than 1 h. This enhancement appeared to coincide with an increase in foam stability. Of interest is the loss of foam stability but not foaming capacity for the 30-min sample. In comparison, the 30-min sample of protease digestion exhibited the highest foaming capacity and stability. A prolonged digestion like the case of 150 min deteriorated the foaming properties. In both acid and protease treatments, the percentage of water absorption of F-1-p de-

Table I. Changes as a Function of Time in Some Functional Properties of Fraction-1-protein Subjected to Chemical and Enzymatic Treatments[a]

treatment time, min	foaming capacity,[b] mL/0.5 min	foam stability,[b] mL				water absorption,[c] %	fat absorption,[d] %
		10 min	30 min	1 h	2 h		
Acid Treatment							
0	66	22	19	18	17	411	319
15	68	22	18	18	16	388	328
30	66	18	13	6	3	358	270
60	71	25	20	17	15	330	261
90	86	36	32	24	18	330	235
120	82	37	33	23	21	303	235
150	90	40	38	35	28	305	237
Protease Digestion							
0	86	36	34	32	32	441	312
15	81	31	29	28	26	445	313
30	96	46	42	40	38	378	311
60	90	38	37	35	33	365	312
90	89	36	35	33	32	360	313
120	88	36	35	33	31	314	296
150	79	29	25	23	17	297	231

[a] Average of two experiments. [b] Lawhon and Cater (1971). [c] Determined according to the method of Flemming et al. (1974). [d] Lin and Humbert (1974).

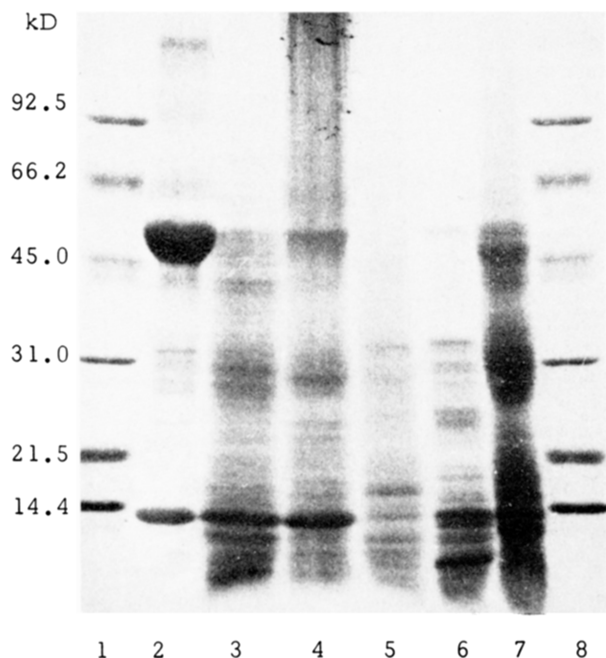

Figure 2. Gradient SDS-PAGE for the degradation pattern of F-1-p subjected to 30-min chemical and enzymatic treatments. Lanes 1 and 8 are molecular weight standards, whereas lanes 2–7 are acid, alkali, and NCS/urea treatments as well as pepsin and protease digestion, respectively.

creased progressively as the treatments prolonged. Progressive decrease of fat absorption was also evident for acid-treated protein. Protease digestion, however, did not modify fat absorption until the treatment lasted longer than 2 h.

Functionality Change in Relation to Degradation Methods. Thirty-minute treatments with chemical and proteolytic enzyme employed in the present study fragmented F-1-p. Polypeptide pattern varied according to the degradation methods (Figure 2). Acid treatment and the digestion with protease and pepsin yielded distinct polypeptide bands in the range 5–50 kDa, whereas alkali and NCS/urea treatments showed fewer bands and a broad streak.

In comparison to native protein, acid treatment drastically increased protein solubility at pH 3, while the others resulted in either a limited increase or decrease of solubility at the tested pHs (Table II). In most cases, the degraded

Table II. Solubility Index of Chemically and Enzymatically Cleaved Fraction-1-protein

protein	solubility index,[a] %			
	pH 3	pH 5	pH 7.4	pH 9
fraction-1-protein				
control	16.8	13.4	32.2	85.5
acid treatment	98.6	11.7	9.2	55.2
alkali treatment	6.1	2.1	7.0	7.3
NCS/urea treatment	38.3	13.3	34.0	39.6
protease digestion	9.4	5.5	56.9	97.3
pepsin digestion	20.4	13.0	1.4	15.8
egg white	95.7	89.4	95.5	93.9
casein	44.0	2.5	81.8	86.7
soy protein (Ardex F)	13.3	2.0	15.2	29.4
soy protein (Ardex R)	17.8	2.1	22.2	30.2

[a] The modified method of Voutsinas and Nakai (1983) for determination of solubility index was described in Materials and Methods.

Table III. Foaming Capacity and Stability of Chemically and Enzymatically Cleaved Fraction-1-protein

protein	foaming capacity,[a] mL/0.5 min	foam stability,[a] mL			
		10 min	30 min	1 h	2 h
fraction-1-protein					
control	88	35	31	30	29
acid treatment	67	12	9	9	7
alkali treatment	84	28	26	26	25
NCS/urea treatment	77	27	25	24	24
protease digestion	94	40	38	36	34
pepsin digestion	62	11	10	10	8
egg white	70	18	13	11	10
casein	78	26	21	17	9
soy protein (Ardex F)	56	5	5	5	4
soy protein (Ardex R)	54	2	1	1	0

[a] Determined by the method of Lawhon and Cater (1971).

F-1-p was more soluble than soy protein isolates but less so than egg white and casein. Acid treatment and pepsin digestion significantly lowered foaming capacity and stability (Table III). Foaming properties were affected only little by the alkali and NCS/urea treatments but were enhanced by protease digestion. With the exception of acid- and pepsin-treated samples, the others maintained better foaming properties than the food proteins tested.

Native F-1-p was superior in most cases over its partially degraded products and food proteins in water and fat absorption, fat-binding capacity, and hydrophobicity (Table IV). Although soy protein isolates and NCS/

Table IV. Water Absorption, Fat Absorption, Fat-Binding Capacity, and Hydrophobicity of Fraction-1-protein Partially Cleaved by Chemical and Enzymatic Treatments

protein	water absorption,[a] %	fat absorption,[b] %	fat-binding capacity,[c] %	hydrophobicity[d] (S_0)
fraction-1-protein				
control	399	298	107	171
acid treatment	359	280	99	117
alkali treatment	396	283	23	132
NCS/urea treatment	253	281	14	215
protease digestion	371	319	62	170
pepsin digestion	378	243	80	75
egg white	*e*	145	54	40
casein	414	139	12	137
soy protein (Ardex F)	306	208	46	201
soy protein (Ardex R)	219	128	45	268

[a] Flemming et al. (1974). [b] Lin and Humbert (1974). [c] Turbidimetric method of Voutsinas and Nakai (1983). [d] Kato and Nakai (1980). [e] Water soluble.

Table V. Emulsion Capacity of Chemically and Enzymatically Cleaved Fraction-1-protein

protein	emulsion capacity,[a] mL oil emulsified/100 mg protein			
	pH 3	pH 7	pH 9	pH 11
fraction-1-protein				
control	52	39	38	88
acid treatment	40	39	46	61
alkali treatment	41	40	41	69
NCS/urea treatment	38	40	45	74
protease digestion	53	40	41	76
pepsin digestion	46	38	52	87
egg white	44	41	40	50
casein	34	42	42	95
soy protein (Ardex F)	30	32	38	83
soy protein (Ardex R)	35	40	42	88

[a] Determined according to the method of Regenstein and Regenstein (1984).

urea-treated protein had greater hydrophobicity than others including native F-1-p, there was a lack of correlation among fat absorption, fat-binding capacity, and hydrophobicity. Among the degradation products, those from alkali and NCS/urea treatments were lowest in fat-binding capacity. Pepsin digestion caused a decrease in fat absorption and hydrophobicity.

Modification of emulsion capacity due to chemical and enzymatic treatments was minimal within the range of pH 3 to pH 11 (Table V). In general, the emulsion capacity of F-1-p and its degradation products was comparable to that of food proteins. However, the emulsifying property of viscosity varied tremendously among the degradation products and between F-1-p and food proteins (Table VI). Alkali treatment and pepsin digestion did not alter emulsion viscosity as compared with the native protein. Almost a total loss of viscosity was observed for the samples of acid and NCS/urea treatment and protease digestion. The viscosity of food proteins ranged from nil to a fraction of that of native F-1-p.

DISCUSSION

F-1-p in higher plants is an oligomeric globular protein consisting of eight large subunits and eight small subunits (Baker et al., 1975). It contains no inter- or intra-disulfide bonds as deduced from the number of titratable SH groups (Kawashima and Wildman, 1970). The physicochemical property of globular protein rests on its native conformation having a predominance in hydrophilic residues on the surface and on denaturation, which exposes the hydrophobic residues inside. The large and small subunits of F-1-p contain 51% and 45% apolar amino acids with hydrophobic groups, respectively (Miziorko and Lorimer, 1983). The denaturation of F-1-p at the level of quaternary and tertiary structures could be induced readily under adverse conditions such as extreme pH and temperature. Such a flexibility in protein conformation and surface chemistry could underlie the chemical basis of excellent functionality for native F-1-p observed in the past and present studies (Sheen and Sheen, 1985). When F-1-p denaturation is followed by degradation in the present study, functionality became less desirable in most cases. This may be attributed to the fragmentation and random annealing of polypeptides that affect the surface distribution of hydrophilic and hydrophobic groups. In addition, dehydration could make the inter- and intra-polypeptide chain linkages irreversible and thus lower the surface activity of degradation products.

Prolonged treatments with mild acid and protease on F-1-p progressively reduced the size of polypeptides but did little to affect the protein solubility (Figure 1). However, polypeptide size appeared to be positively correlated

Table VI. Emulsion Viscosity of Chemically and Enzymatically Cleaved Fraction-1-protein[a]

protein	emulsion viscosity,[b] cP					
	1%[c]		2%[c]		4%[c]	
	20%[d]	40%[d]	20%[d]	40%[d]	20%[d]	40%[d]
fraction-1-protein						
control	5	845	18	6302	411	>20000
acid treatment	2	16	5	32	120	428
alkali treatment	7	291	121	10943	959	>20000
NCS/urea treatment	1	2	3	8	8	28
protease digestion	1	8	2	8	5	36
pepsin digestion	8	613	39	8669	325	>20000
egg white	2	18	3	686	9	1060
casein	4	8	28	33	135	460
soy protein (Ardex F)	5	117	33	1100	276	2561
soy protein (Ardex R)	1	2	1	2	1	2

[a] Proteins were pooled from three preparations. Results are averages of duplicate analyses. [b] Measured with a Stormer viscometer as described by Sheen and Sheen (1985). [c] Protein concentration. [d] Oil concentration.

with water and fat absorption (Table I). This may be explained in that the annealing of small polypeptides with minimal steric hindrance could decrease the number of hydrophilic and hydrophobic groups available for binding water and oil. Acid hydrolysis but not protease digestion lowered the foaming capacity and stability of F-1-p. This suggests that acidic condition could destabilize electrostatic and hydrophobic interactions and hydrogen bonding essential for foaming property. This result is consistent with the poor foaming property from pepsin-digested protein (Table III) since digestion was carried out under acidic condition. On the other hand, emulsion viscosity was high in pepsin-digested protein but low in acid-treated ones (Table VI), indicating that this functional property is independent of acidic condition. Since the preferred cleavage sites for pepsin involve phenylalanine and tryptophan (Fruton, 1975), one may question whether pepsin digestion exposed more hydrophobic groups on the surface to enhance the viscosity in oil–water emulsion.

Alkaline treatment hydrolyzes proteins in a nonspecific manner and often induces cross-linkage of polypeptides, especially at high temperature. Extensive cross-links of polypeptides in alkali treatment can be a factor for high emulsion viscosity. Upon dehydration, cross-links could hinder protein solubility as is the case of alkali-hydrolyzed F-1-p. It is generally recognized that the more soluble the protein, the lesser its fat-binding capacity. In other words, high protein solubility exerts an adverse effect on fat-binding capacity. This generalization, however, does not hold true for F-1-p and its degradation products, which can be explained by the possible variations in particle size and reversibility of polypeptide aggregations due to the degradation methods. Fat-binding capacity is positively correlated with hydrophobicity, both of which are expectedly high for proteins with β-pleated-sheet conformation (Voutsinas and Nakai, 1983). Because of the lack of correlation between these functional properties among F-1-p products, one may suspect that the α-helical conformation predominates. The result that the percentage of fat absorption is much greater than that of fat-binding capacity for all proteins tested (Table IV) substantiates the physical entrapment of oil according to the analytical procedure of Lin and Humbert (1974).

It has been reported that the NCS/urea reagent selectively cleaves tryptophanyl peptide bonds with about 50% efficiency (Lischwe and Sung, 1977). The large subunits of F-1-p have eight tryptophan residues and expectedly yield nine polypeptides (Miziorko and Lorimer, 1983). Two large polypeptides would have 153 and 144 amino acids each with molecular mass about 18 kDa. The corresponding band is identifiable on the SDS-PAGE gel (Figure 2). However, the absence of large subunits on the gel suggests that cleavage had occurred at other sites as well. Cleaved products showed poor fat-binding capacity but high hydrophobicity. In the case of the latter, if there is residual urea in the protein preparation, the association of the carbonyl and amide groups of urea with the hydroxyl and carbonyl counterparts of *cis*-parinaric acid through hydrogen bonds could overestimate hydrophobicity.

The present study indicates that a partial degradation of F-1-p usually leads to lowering functional properties. Nevertheless, the degraded F-1-p is still comparable to or better in functionality than the food proteins tested. F-1-p degradation products from acid and protease treatments, being completely soluble at pH 3 and pH 9, respectively, offer a potential application in the beverage industry for human nutrition. Our preliminary study showed that a F-1-p polypeptide solution containing 10% sucrose in 10 mM citrate buffer, pH 3.5, which was autoclaved and kept under sterile condition at room temperature, has remained crystal clear and colorless for over 1 year. Protein-enriched beverage with long shelf life will be a novel drink for the benefit of millions of health-conscious people if F-1-p from green plants can be accepted by the public as a dietary protein.

Registry No. D-Ribulose 1,5-bisphosphate carboxylase/oxygenase, 9027-23-0.

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Studies on the Interaction of Sunflower Albumins with Chlorogenic Acid

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Acidic butanol extraction removed 90% of the phenolics from sunflower meal. The phenol-free meal contained a higher albumin level than the defatted meal. Gel permeation chromatography showed four and two protein peaks for defatted and phenol-free albumins, respectively. Considerable variation in the electrophoretic mobilities in these two albumin preparations was observed. Amino acid analyses indicated low levels of phenylalanine, tyrosine, leucine, and valine in phenol-free albumin as compared to defatted albumin. Chlorogenic acid and phenol-free albumin interaction studies indicated aggregation of low molecular weight albumins in sunflower.

Sunflower (*Helianthus annuus* L.) is one of the larger sources of vegetable oil and protein of good nutritional quality. But the presence of phenolic compounds in the seeds contribute dark color to formulated foods and prevent the use of sunflower defatted meal (Cater et al., 1972; Gheyasuddin et al., 1970a; Lusas, 1985). Rahma and Rao (1981) have characterized the total proteins from defatted and chlorogenic acid free sunflower meal by disc gel electrophoresis, gel permeation chromatography, ion-exchange chromatography, and analytical centrifugation. Further, various studies have indicated that chlorogenic acid is associated with low molecular weight proteins (Prasad, 1987; Rahma and Rao, 1981; Sabir et al., 1974). Studies on interactions of chlorogenic acid with 11S sunflower protein have showed negative cooperativity in the presence of high salt concentration, high temperature, and 8 M urea (Sastry, 1984).

Attempts have been made to remove these polyphenolic substances to obtain colorless sunflower isolates from sunflower meal (Fan et al., 1976; Kilara et al., 1972; Lawhon et al., 1982; Sosulski et al., 1972). Lawhon et al. (1982) have developed a process for preparing white sunflower protein isolate, by using alkali extraction followed by acid precipitation under vacuum, and deoxygenated water was used in this procedure. However, these methods caused denaturation of the proteins and incomplete removal of color-forming phenols. Sodini and Canella (1977) described the use of acidic butanol reagent for removing phenolics from sunflower meal without causing detectable protein denaturation. The present paper compares albumins from phenol-free and defatted sunflower meal and in vitro binding studies of chlorogenic acid with phenol-free albumins.

MATERIALS AND METHODS

Dehulled seeds of the sunflower cultivar Morden were crushed and defatted with use of petroleum ether (40–60 °C) in a Soxhlet extractor. The defatted meal (DFM) was air-dried and ground to pass through a 100-mesh sieve. The phenol-free meal (PFM) was prepared by the procedure as described by Sodini and Canella (1977).

Solubility Fractionation. Proteins from sunflower meals were fractionated by the modified method of Gheyasuddin et al. (1970b) using a meal to solvent ratio of 1:10 (w/v): (a) distilled water to extract albumins; (b) 1 M sodium chloride to extract globulins; (c) 60% (v/v) aqueous 2-propanol to extract prolamines; (d) 0.4% sodium hydroxide to extract glutelins. The samples were extensively dialyzed against distilled water at room temperature and freeze-dried.

Nitrogen content in the samples and protein fractions was determined by the micro-Kjeldahl method and multiplied by 6.25 to obtain crude protein content (AOAC, 1980). Phenolic content was estimated by the method of Swain and Hillis (1959) using Folin–Denis reagent.

Amino Acid Composition. Amino acid analyses of defatted and phenol-free albumins (DFA and PFA) were performed according to Spackman et al. (1958) using a Hitachi Model KLA-3B amino acid analyzer.

Gel Permeation Chromatography. A 50-mg portion of albumin was dissolved in 4.0 mL of 0.02 M sodium phosphate buffer (pH 7.2) containing 0.01 mM β-mercaptoethanol, 0.02% sodium azide, and 2.5% sodium chloride. The solution was centrifuged at 5000 rpm for 10 min at room temperature, 3.5 mL of the supernatant was applied on a Sephadex G-200 column (3 × 40 cm) equilibrated with the same buffer, and 4.0-mL fractions were collected with use of an LKB 2112 redirac automatic fraction collector. Absorbances of the fractions were read at 280 and 328 nm for protein and phenolics, respectively.

Polyacrylamide Gel Electrophoresis. Portions of 100 μg of each protein were separated on 7.5% polyacrylamide gels with 0.02 M Tris–borate buffer (pH 8.8) and a current of 5 mA/gel for 1.5–2.0 h by the method of Davis (1964). The protein bands were visualized with Coomassie brilliant blue.

In Vitro Binding of Chlorogenic Acid to PFA. PFA and chlorogenic acid (CA) in the ratios 10:1, 10:1.5, and

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