Thermal Modification of the Structural and Functional Properties of Fraction-1-protein

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When tobacco crystalline fraction-1-protein was solubilized at pH 8.5 and 3.0 and heated to boiling for 2 h, it remained soluble. Electrophoretic analyses revealed that the oligomeric structure was denatured at 60 °C. As a result, the subunits formed soluble aggregates that appeared as a film upon dehydration. Studies of chemicals interfering with molecular bondings indicated that heating weakens electrostatic forces between the large and small subunits to facilitate disulfide bonding and/or hydrophobic association. After cooling, the pH 3.0 preparation reestablished hydrogen and electrostatic bonds to form a transparent gel that was thermally reversible, whereas the pH 8.5 sample showed irreversible heat-setting only in the presence of salt. Protein powders from the heat treatment had decreased foaming capacity and stability, fat-binding capacity, emulsion viscosity, and solubility index. However, the preheated pH 3.0 protein can be completely solubilized by reheating, suggesting that minor protein denaturation at low acidity may stabilize conformation due to increasing hydrophobic association that limits disulfide linkage upon heating.

Crystalline fraction-1-protein (F-1-p; D-ribulose-1,5-bisphosphate carboxylase-oxygenase) from tobacco leaf is exclusively composed of amino acids (Kawashima and Wildman, 1970) and has a protein efficiency ratio better than that of casein (Ershoff et al., 1978). In addition, it exhibits excellent functionality at either a native or a partially denatured state (Sheen and Sheen, 1985, 1988). Similar results were obtained from F-1-p of alfalfa (Knuckles and Kohler, 1982), spinach (Barbeau and Kinsella, 1986), and soybean (Sheen, 1986). These biological and physicochemical properties suggest the potential of F-1-p in formulated foods for the health-conscious society. Since tobacco F-1-p crystals contain a large amount of water, dehydration is necessary to obtain a protein powder for easy shipping, storage, and food application. In the production of food proteins such as egg white, soy isolate, milk whey concentrate, and beef plasma, dehydration is accomplished by spray-drying. High inlet and outlet temperatures of a spray-dryer could modify the structural integrity of protein molecule and in turn its functional properties. Such modifications would also be expected in food systems during cooking, roasting, and steaming.

Thermal stability during processing is an important functional attribute for a food protein. In the case of casein, heating facilitates pasteurization and sterilization for food preservation. On the other hand, stability to heating without aggregation is an obstacle for soy protein mixtures to be utilized as a functional food protein. Thermal dissociation and association behaviors of casein and soy protein reflect molecular interactions in a heterogeneous system consisting of not only different protein molecules but also carbohydrates and possibly lipids, phytate, minerals, and others. Such complexities make the elucidation of thermal properties of proteins on a molecular level more difficult. An understanding of the nature of molecular interactions is a prerequisite for protein modification or engineering to successfully improve thermal properties that meet the requirements of the food industry. This is better approached with a homogeneous protein that subsequently can be extended to a complex food system.

F-1-p, which is most abundant on earth, can be isolated as a homogeneous product in the form of crystal from different plant species (Johal et al., 1980). It is an ideal protein to study the effect of temperature on the relationship of structural and functional properties. The present study, therefore, employed tobacco crystalline F-1-p as a model protein to examine the molecular interactions among the subunits at two pHs as they relate to the alteration of some functional properties upon heating.

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MATERIALS AND METHODS

Preparation of F-1-p. Tobacco (*Nicotiana tabacum* L.) cultivar Ky 14 was grown on the Kentucky Agricultural Experiment Station Farm in Lexington, KY, according to the conventional burley tobacco practices. Leaves from plants at knee height in three 30-ft rows were harvested on a row basis to constitute three replications. Crystalline F-1-p was isolated from fresh leaves of each replication by a recently described procedure (Sheen, 1986). All F-1-p preparations gave an absorbance ratio (280 nm/260 nm) greater than 1.9, which indicates high purity (Kawashima and Wildman, 1971). The protein crystals were stored in distilled water at 4 °C prior to treatments.

Heat Treatments. Protein crystals were solubilized in distilled water at pH 8.5 and 3.0 by use of 0.1 N NaOH and HCl solutions. A 2% protein solution as determined by Lowry's method (Lowry et al., 1951) was placed in a heated water bath. Aliquots were taken at 25, 40, 50, 60, 80, and 100 °C and thereafter at 15-, 30-, and 60-, and 120-min intervals to evaluate the effect of prolonged heating on protein stability. In a separate experiment, large volumes of 2% protein solutions at both pHs in three replications were divided into two treatments: control and boiled for 1 min. The treated samples were freeze-dried and stored in bottles until analysis.

Light and Electron Microscopy. Aliquots of the pH 8.5 and 3.0 F-1-p solutions from the heat treatment were spotted onto glass slides for dehydration at ambient temperature. The resultant films were examined under a light microscope. Separately, F-1-p solutions from both pHs and with or without preheating were mounted on carbon-stabilized Formvar-coated grids and stained with 2% phosphotungstic acid for 30 s. The preparations were observed and photographed in a JEOL JEM 100-C electron microscope operated at 80 kV.

Chemical Treatments. *N,N*-Dimethylformamide, NaCl, and 2-mercaptoethanol are, respectively, capable of disrupting hydrogen bonding and electrostatic and disulfide bonds of protein, while detergents such as Triton X-100, BRIJ 35, and sodium glycolate interfere with hydrophobic association (Wall and Huebner, 1981). Sodium dodecyl sulfate (SDS), an anionic detergent, exerts effects on both hydrophobic and electrostatic forces in protein. Various concentrations of the listed chemicals, by themselves or in combination, and other chemicals known to disrupt physical and covalent bonds were mixed in 1% native and preheated F-1-p solutions at ambient temperature for 1 h. Similarly, the protein solutions were adjusted with 0.1 N NaOH and HCl to pH 12.5 and 1.5. In addition to visual observation, the

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treatments were evaluated with polyacrylamide gel electrophoresis (PAGE).

Polyacrylamide Gel Electrophoresis. The heated and chemically treated F-1-ps, along with the native form as experimental control, were analyzed with nondissociating PAGE (Davis, 1964) as well as SDS-PAGE (Laemmli, 1970). The respective PAGE analyses were performed with gel slabs having a linear gradient of 3–17.5% and 7.5–17.5% acrylamide. Electrophoretic procedures including molecular weight markers and protein band visualization with Coomassie Brilliant Blue R-250 are described elsewhere (Sheen and Sheen, 1987).

Functional Properties. The method of Voutsinas and Nakai (1983) for solubility index determination was modified for the present study. The modified procedure employing the Bio-Rad protein assay method has been discussed in a recent paper (Sheen and Sheen, 1988). In addition to a range of pH 3.0–9.0, the solubility of the native and preheated protein in distilled water was also examined and their gelation upon heating was recorded. Protein samples of all treatments were analyzed for foaming capacity and stability by the method of Lawhon and Cater (1971) and water and fat absorption by the methods of Fleming et al. (1974) and Lin and Humbert (1974), respectively. Voutsinas and Nakai's (1983) turbidimetric method was used to measure fat-binding capacity.

Absolute emulsion viscosity of the pH- and heat-treated F-1-ps in 2% and 4% solutions containing 20% and 40% vegetable oil was determined with a Stormer visocometer at 25 °C. The preparation of protein–oil emulsion and the measurement of viscosity were previously described (Sheen and Sheen, 1985). The same samples were measured for kinematic viscosity with a set of Cannon-Fenske opaque viscometers (from Industrial Research Glassware, Ltd., Roselle, NJ) held upright in a circulating water bath at 25 °C. Kinematic viscosity was calculated by multiplying the efflux time in seconds of the emulsion by the viscometer constant derived from glycerol standards.

Statistics. The data were subjected to the analysis of variance according to a randomized plot design with three replications. Means of the different treatments were compared with the least significant difference (lsd) at the 1% and 5% levels of probability.

RESULTS

General Observations. Crystalline F-1-p was readily soluble at pH 8.5 and 3.0, and the protein solution showed birefringence like fresh egg white. The solution was colorless, odorless, and tasteless. Upon boiling, the protein solution became as transparent as water. Prolonged boiling for up to 2 h neither altered the watery appearance nor resulted in insoluble protein aggregates. The ultraviolet spectrum of F-1-p solution with absorption maximum at 279 nm and minimum at 250 nm was identical with that reported by Kawashima and Wildman (1971). The specific activity of D-ribulose-1,5-bisphosphate carboxylase of the F-1-p crystals in the present preparation was also in a good agreement with their results. Boiling did not alter the ultraviolet spectrum but totally abolished the carboxylase activity.

Light and Electron Microscopy. Dehydration of the unheated pH 8.5 F-1-p solution on glass slides formed a thin transparent film with large airspaces. Airspaces were small on the film of the protein solubilized at pH 3.0. The size of airspace decreased further as the protein solution was heated to boiling. However, 2-h boiling resulted in the reappearance of large airspaces in the film, especially for the pH 3.0 sample. The light microscopic observation was consistent with electron micrographs. The unheated pH 8.5 protein solution showed coarse aggregates with large spacing (Figure 1A,D), while the aggregation appeared as a uniform film with small airspaces for the pH 3.0 preparation (Figure 1C,F). Heating transformed the coarse aggregates of the pH 8.5 sample to that similar to the low-pH one except with the airspaces being smaller (Figure 1B,E).

Heat Treatments. Nondissociating PAGE of both pH 8.5 and 3.0 preparations at different temperatures and heating duration showed the same pattern where F-1-p formed soluble aggregates at temperatures above 60 °C (Figure 2A,B). The aggregates were excluded from 3% acrylamide gel matrix. However, SDS-PAGE revealed that both large and small subunits in aggregation remained relatively intact during the high-temperature treatment (Figure 2C,D). As heating prolonged, protein aggregation occurred, resulting in the appearance of bands of 62, 146, and 163 kDa in the pH 8.5 F-1-p solution. This did not alter the intensity of the small subunits but coincided with a slight loss of the large subunits. In contrast, the acidity at pH 3.0 caused minor degradation of the large subunits as previously reported (Sheen and Sheen, 1987), and the prolonged heating accelerated its disappearance but caused little effect on the small subunits. Although heating aggregated protein in the form of streaking on the gel, no discrete bands were identifiable.

Chemical Treatments. With the unheated and heated F-1-p solubilized at pH 3.0, precipitation occurred upon addition of 1–2% NaCl or in the 1% SDS treatments during the nondissociating PAGE. Nondissociating PAGE with the unheated pH 8.5 protein showed the stability of quaternary conformation as evidenced by the 550-kDa band (Figure 3). A minor band of approximately 1000 kDa may be the aggregates of F-1-p. Treatments of the protein solution with 1–5% *N,N*-dimethylformamide and 2-mercaptoethanol or 1–2% NaCl did not change the 550-kDa band. SDS (0.1–2%) alone or in combination with NaCl and/or *N,N*-dimethylformamide dissociated F-1-p to large and small subunits along with a major band of about 100 kDa. This band disappeared upon addition of 2-mercaptoethanol with a corresponding increase in banding intensity of the large subunit.

The pH 8.5 F-1-p solution at 60 °C could maintain protein conformation as a 550-kDa band if NaCl or KCl was added to a 0.2% concentration. This phenomenon was not reproducible at the same temperature with other chemicals mentioned above. At temperatures above 60 °C, none of the chemicals tested for up to a 5% concentration could preserve the integrity of protein conformation. With the preheated protein solution, the addition of the same chemicals in the concentration of 0.5–5% resulted in precipitation with the exception of SDS. SDS partially dissociated the preheated protein into subunits along with numerous bands and streaking in high molecular weights (Figure 3). This pattern was not changed irrespective of the presence of NaCl and *N,N*-dimethylformamide. When SDS and 2-mercaptoethanol were used in combination, subunits were recovered in a greater quantity.

Adjustment of the solution of preheated F-1-p to pH 1.5 and 12.5 did not dissociate the soluble aggregates. Neither Triton X-100, BRIJ 35, and sodium glycolate at a 1% concentration nor ethanol up to 50% could disrupt the aggregates as judged by nondissociating PAGE pattern. In these treatments, protein appeared as precipitates at the origin of the gel. Combinations of these chemicals with *N,N*-dimethylformamide and 2-mercaptoethanol failed to modify the electrophoretic pattern but with NaCl resulted in an instant precipitation. The addition of cysteine hydrochloride also changed the soluble aggregates to insoluble precipitates.

Functional Properties. Both freeze-dried pH 8.5 and 3.0 protein powders were readily soluble in distilled water, whereas preheating prior to freeze-drying reduced the solubility to less than 20% for the pH 3.0 preparation and even lower for the pH 8.5 sample (Table I). When the solutions were heated to boiling, the protein became com-

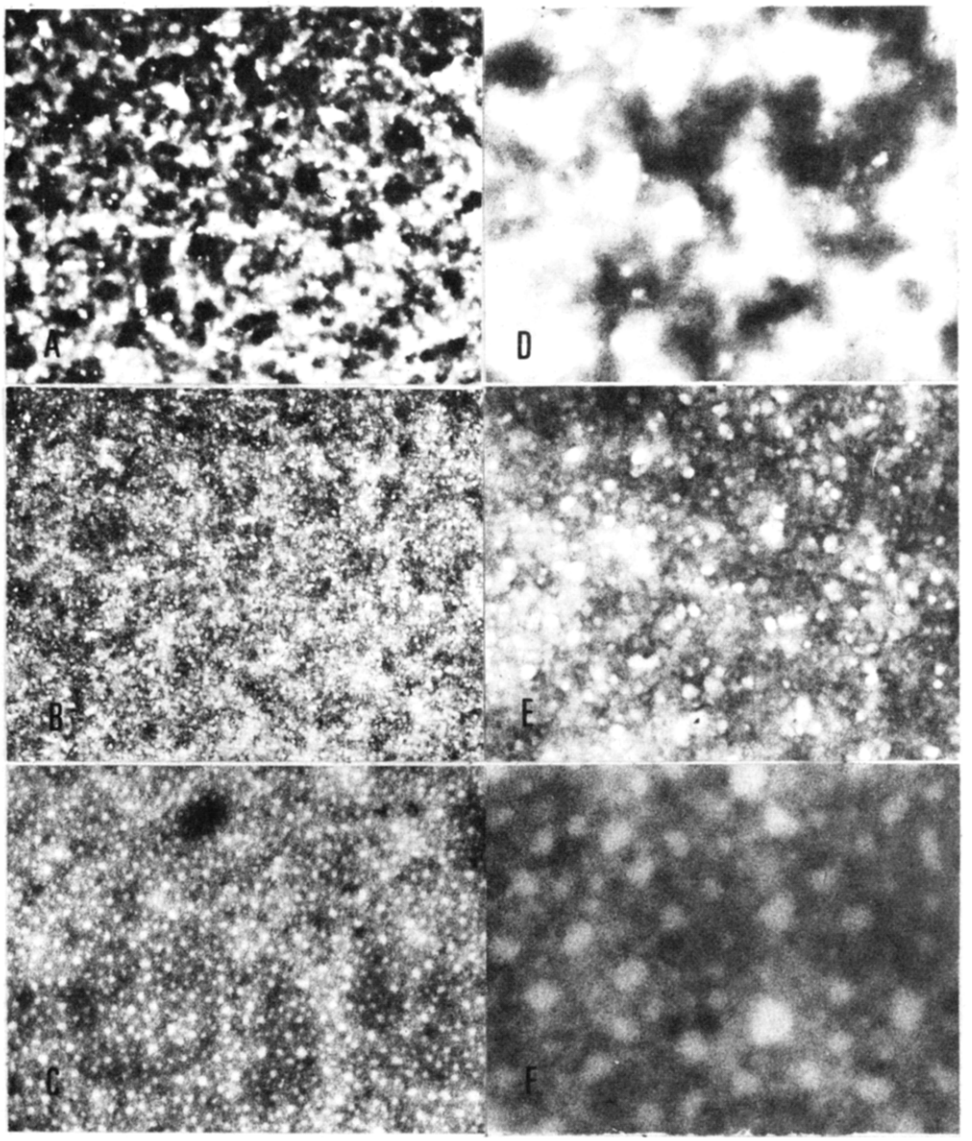

Figure 1. Electron micrographs of F-1-p: (A) unheated, pH 8.5, (B) heated, pH 8.5, and (C) unheated, pH 3.0, at 8000× magnification. (D)–(F) are 30000× magnification of the respective samples.

pletely soluble, except for the preheated pH 8.5 sample whose solubility was only slightly improved. With different pH buffers, the solubility index of all samples was least at pH 5.0 and increased toward either direction of acidity and alkalinity. In all cases, the preheated protein showed a lower solubility index than the corresponding counterpart.

The pH 3.0 preparation, either preheated or control, at 2% concentration formed a transparent gel upon cooling after boiling. This sol–gel transition is thermally reversible and unaffected by the addition of sucrose. At a higher concentration, the gel was heat-set upon heating. In either case, gelation was not inhibited by 1% *N*,*N*-dimethylformamide or 2-mercaptoethanol but by 1% NaCl. The latter caused protein precipitation. In contrast, salt induced heat-setting for the unheated pH 8.5 sample at boiling temperature when the protein concentration is higher than 2%. The texture of heat-set gel resembled coagulated egg white and did not vary within the range of 0.5-5% salt concentration.

Unheated proteins had significantly higher foaming capacity than the preheated ones, and the pH 3.0 preparation possessed the best foaming capacity and stability as shown in Table II. In general, preheating reduced foaming stability especially for the pH 8.5 sample. The same table shows that protein powder of either pH and with or without preheating did not modify water and fat absorption. Fat-binding capacity, however, was lowered by preheating and was slightly reduced for the unheated pH 3.0 sample.

Absolute and kinematic viscosity measured with two different apparatus was in a good agreement for all samples tested (Table III). Preheated pH 8.5 protein showed poor

Table I. Solubility Index of Fraction-1-protein Pretreated at Different pHs and Temperature

	solubility index,[a] %					
treatment	pH 3.0	pH 5.0	pH 7.4	pH 9.0	water	water, heated
unheated, pH 8.5	24.7	2.6	56.6	96.2	97.3	100.0
heated, pH 8.5	1.8	1.5	2.8	9.1	4.3	10.8
unheated, pH 3.0	95.0	6.7	8.3	31.4	100.0	100.0
heated, pH 3.0	20.1	3.0	3.4	8.4	19.7	100.0
lsd 0.05	2.8	1.5	2.1	4.5	7.4	0.8
lsd 0.01	4.2	2.2	3.2	6.8	11.1	1.1

[a] Determined by the modified method of Voutsinas and Nakai (1983) as described in Materials and Methods. Protein samples in 1% concentration were solubilized with water and 0.1 M each of citrate phosphate buffer, pH 3.0 and 5.0, phosphate buffer, pH 7.4, and boric acid–borax buffer, pH 9.0.

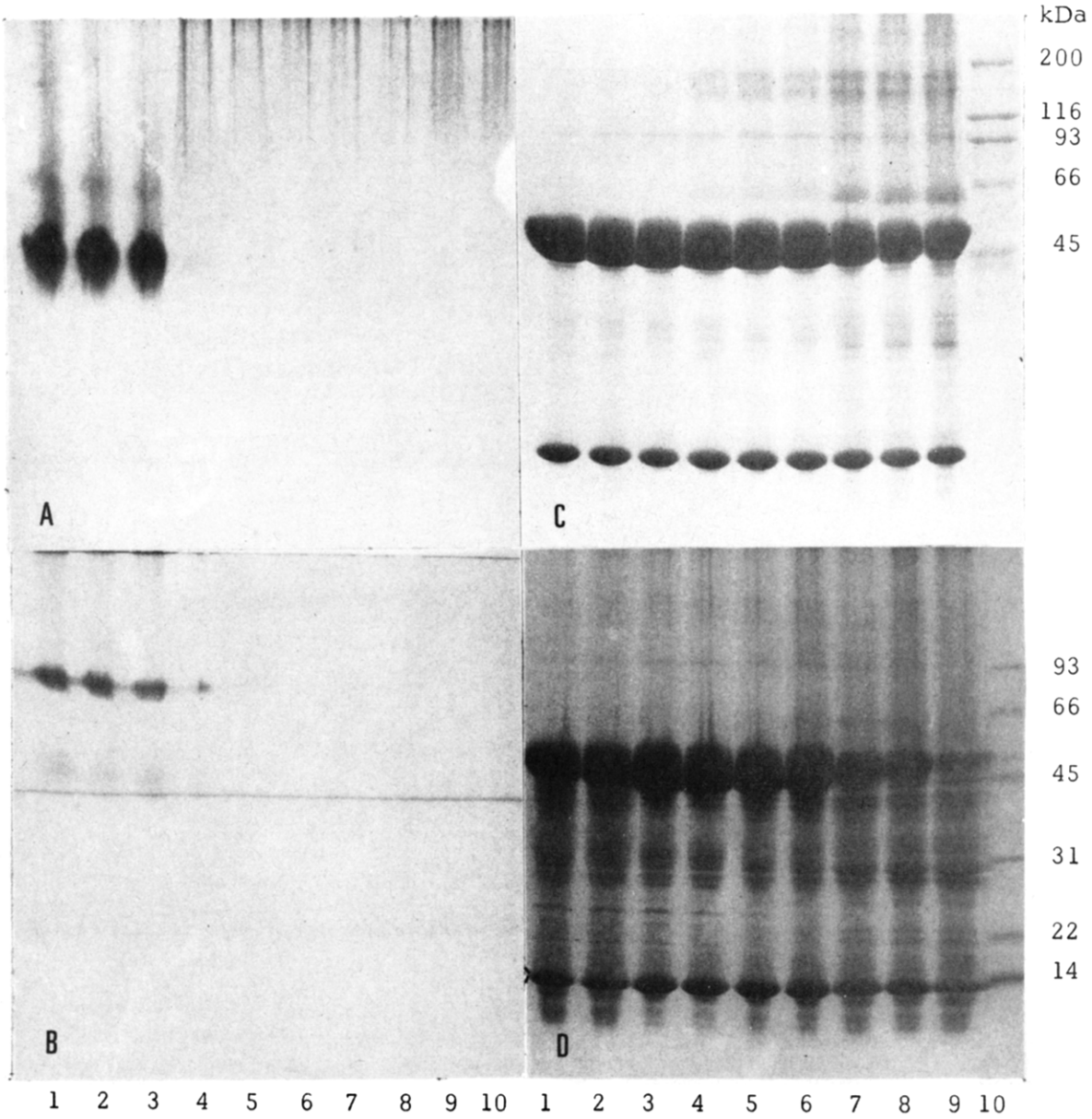

Figure 2. Effect of temperature and heat duration on F-1-p conformation and degradation. (A) and (B) are nondissociating PAGE for the pH 8.5 and pH 3.0 solubilized proteins, respectively. Lanes 1–10 are the treatments: 25, 40, 50, 60, 80, and 100 °C and 15, 30, 60, and 120 min of boiling temperature. (C) and (D) are SDS-PAGE for the same treatments except the deletion of the 15-min boiled sample and lane 10 being molecular weight markers.

Table II. Foaming Capacity and Stability, Water and Fat Absorption, and Fat-Binding Capacity of Fraction-1-protein Pretreated at Different pHs and Temperature

treatment	foaming capacity,[a] mL/0.5 min	foam stability,[a] mL				water abs,[b] %	fat abs,[c] %	fat-binding capacity,[d] %
		10 min	30 min	1 h	2 h			
unheated, pH 8.5	92.7	45.1	42.9	39.0	35.6	428.9	281.4	98.7
heated, pH 8.5	68.9	18.7	16.5	15.3	14.1	434.8	299.4	63.0
unheated, pH 3.0	101.2	55.3	51.9	50.2	40.5	430.7	303.5	84.1
heated, pH 3.0	76.0	32.9	31.3	30.0	28.4	465.1	306.4	60.5
lsd 0.05	7.8	8.1	7.9	5.4	5.2	130.5	52.6	10.0
lsd 0.01	11.9	12.6	12.0	8.1	7.8	197.7	79.6	15.1

[a] Determined by the method of Lawhon and Cater (1971). [b] Fleming et al. (1974). [c] Lin and Humbert (1974). [d] Voutsinas and Nakai (1983).

emulsion viscosity. In contrast, viscosity was improved by a preheating treatment for the pH 3.0 preparation at 2% protein but not at the 4% level. With the exception of the preheated pH 8.5 protein, the other three preparations had extremely high viscosity with the formulation of 2% protein and 40% oil. The preheated pH 3.0 protein was emulsified to thick curd which over the time separated from water, while the emulsion of unheated protein looked like thick mayonnaise under either ambient or refrigerated conditions for a long time.

DISCUSSION

It is generally believed that the heating process leads to protein denaturation by unfolding polypeptides and

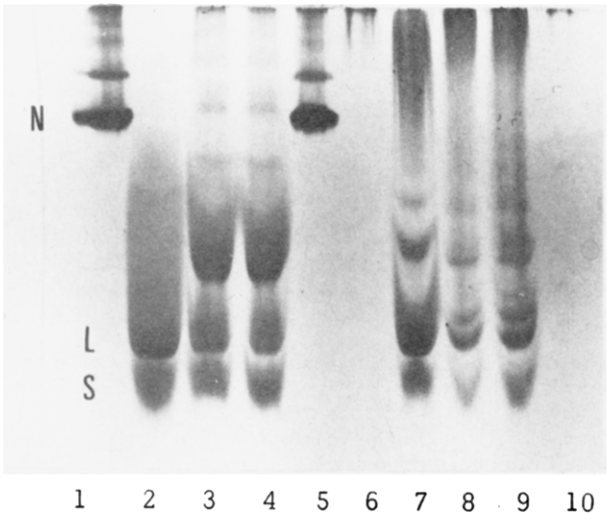

Figure 3. Effect of chemicals (1% each) on the subunit dissociation of the unheated (lanes 1–5) and heated (lanes 6–10) F-1-p solubilized at pH 8.5 analyzed with nondissociating PAGE. Lanes 1 and 6, no chemicals added; lanes 2 and 7, SDS and 2-mercaptoethanol treatment; lanes 3 and 8, SDS and NaCl treatment; lanes 4 and 9, SDS and *N,N*-dimethylformamide treatment; lanes 5 and 10, 2-mercaptoethanol and *N,N*-dimethylformamide treatment. N, L, and S denote native protein and large subunit and small subunit, respectively.

Table III. Emulsion Viscosity of Fraction-1-protein Pretreated at Different pHs and Temperature[a]

	2%[b]		4%[b]	
treatment	20%[c]	40%[c]	20%[c]	40%[c]
	Absolute Viscosity,[d] cP			
unheated, pH 8.5	15	5787	374	>20000
heated, pH 8.5	5	57	6	260
unheated, pH 3.0	75	1662	1593	>20000
heated, pH 3.0	105	4813	1374	>20000
	Kinematic Viscosity,[e] cS			
unheated, pH 8.5	36	>20000	614	>20000
heated, pH 8.5	6	96	7	259
unheated, pH 3.0	98	1209	2178	>20000
heated, pH 3.0	221	8614	1818	>20000

[a] Proteins were pooled from three replications. Results are averages of duplicate determinations. [b] Protein concentration. [c] Oil concentration. [d] Measured with a Stormer viscometer as described by Sheen and Sheen (1985). [e] Determined with a set of Cannon-Fenske opaque viscometers.

destroying their secondary and tertiary structures. Denaturation facilitates protein precipitation, especially at boiling temperature. Crystalline F-1-p from tobacco leaf is soluble in water at pH 8.5 and 3.0 and remains so under boiling for 2 h. This heat stability has obvious implications with regard to the usability of this protein in the food industry. Soluble F-1-p can be gelled in the presence and absence of salt, an asset that ensures its versatility in formulated foods. Nevertheless, some changes of functionality due to heating are inevitable.

In elucidation of the physicochemical properties of a protein upon heating and chemical treatments, it is essential to know its molecular structure. Tobacco F-1-p is a globular oligomer with eight large subunits elongatedly arranged along a 4-fold axis and a cluster of four small subunits at both top and bottom of a *z*-axis (Chapman et al., 1987). Its quaternary and tertiary structures are held together by electrostatic, hydrophobic, and hydrogen bonds without disulfide linkage (Chapman et al., 1988). When the native protein is treated with detergents and other chemicals, only SDS dissociated the small subunits and destabilized the structure of the large subunits. SDS binds excessively onto the positively charge sites of peptides and renders them into solution. This disruption of quaternary and tertiary structures facilitated disulfide cross-link between cysteines of neighboring large subunits. This is supported by a complete recovery of large subunits upon the addition of 2-mercaptoethanol but not with NaCl and *N,N*-dimethylformamide (Figure 3). The SDS effect points to the importance of hydrophobic and electrostatic forces for the stable conformation of native F-1-p.

Elevated temperatures usually weaken electrostatic and hydrogen-bonding forces in native protein. Fluorescent emission spectra revealed conformational changes of oligomeric F-1-p at temperatures above 30 °C (Barbeau and Kinsella, 1983). Such changes may not involve subunit dissociation and major unfolding of peptides. The present study showed that the quaternary conformation of tobacco F-1-p remained intact at 50 °C but began modifications at 60 °C in favor of oligomeric aggregation. The structural modification at 60 °C is likely due to peptide unfolding that facilitates electrostatic attraction and repulsion in a random manner. The addition of salt slowed the aggregation by possibly preventing the random association of ionic moieties from neighboring oligomers. Kawashima and Wildman (1970) reported that at alkaline pH greater than 11, tobacco F-1-p dissociated into large and small subunits. This suggests that the association between the subunits are mainly electrostatic forces.

Higher temperatures result in more random configurations of unfolding peptides, which exposes the sulfhyryl groups for the formation of disulfide bonds. Unfolding of peptides also results in exposure of nonpolar groups capable of hydrophobic association, so that the surface of the aggregates arrives at a maximal hydrophilicity. These heat-induced events taking place among the dissociated large and small subunits may confer the solubility and heat stability of F-1-p aggregates. A similar conclusion on the basis of a complex formation between dissociated 7S subunits and 11S basic subunits of soy proteins was given for the failure of protein aggregation on boiling (German et al., 1982). Nonionic detergents and cysteine hydrochloride precipitated the heat-denatured F-1-p, which substantiates the involvement of hydrophobic association and disulfide bonds in its denatured state. The unfolding of the heated F-1-p to maximize inter- and intramolecular interactions is consistent with its appearance as a fine matrix observed under the electron microscope (Figure 1B,E). Covalent bonds other than disulfide linkage are not formed in the heated F-1-p, since a complete dissociation of subunits can be achieved in the presence of SDS and 2-mercaptoethanol at boiling temperature. The same treatment at ambient temperatures, however, gave a partial dissociation (Figure 3), suggesting that peptide aggregation may hinder the reduction of some disulfide bonds.

It is generally accepted that the heating process deteriorates protein functionality. Native F-1-p is expected to be more conformationally flexible than the heated one because of its lack of disulfide bonds and a weaker hydrophobic association. Thus, any of these structural modifications in the preheated protein or during heating could be partially responsible for changes of functional properties. Flexible protein molecules are susceptible to denaturation at air–water and oil–water interfaces. When protein undergoes surface denaturation, the surface hydrophobicity increases and results in good foaming and emulsifying properties. Less molecular flexibility in the heated F-1-p could lower foaming capacity and stability (Table II). Similar explanations may be applied to the

decrease in emulsion viscosity, at least in the case of the preheated pH 8.5 preparation. The degree of lowering in fat-binding capacity of the preheated protein and the unheated pH 3.0 sample could be related to the magnitude of increased hydrophobic association after denaturation. Such structural changes appear to be independent of water and oil absorption, which probably represents entrapment as the inherent characteristics of the analytical methods (Table II).

Barbeau and Kinsella (1986) reported that functional properties of F-1-p from spinach leaves were related to physical behavior and affected by pH and salt. For tobacco F-1-p, the electrostatic forces between adjacent peptides at pH 8.5 could be decreased by salt; and as a result, the conformation of native protein became unstable. When the salt-added protein solution was heated, the prompt denaturation could cause random formation of disulfide bonds that led to gelation as a heat-setting phenomenon. The irreversibility of the pH 8.5 gel may be due to extensive disulfide linkages among the subunits. Disulfide bonds in accompaniment with the exposure of hydrophobic groups upon heat denaturation of F-1-p at pH 8.5 may also explain the low solubility index of the dehydrated powder of preheated protein. In contrast, the pH 3.0 condition caused a limited unfolding of peptides to facilitate the formation of electrostatic and hydrogen bondings between adjacent peptides. These interactions favor additional hydrophobic association but without disulfide cross-links to stabilize the partially denatured protein. The fine network of the pH 3.0 F-1-p film (Figure 1C,F) and the integrity of the oligomer (Figure 2B) substantiate these conclusions. The strong hydrophobic interactions could limit disulfide bond formation upon heating of the pH 3.0 protein in solution. It also permits ready formation of electrostatic and hydrogen bonds among the unfolding peptides, which may underlie the molecular mechanism for thermally reversible gelation and heat-setting. This is consistent with the inhibition of gelation by NaCl but not by 2-mercaptoethanol. Likewise, the predominance of the above molecular interactions other than disulfide bonding could render the dehydrated protein powder resoluble upon heating to boil (Table I). The present study showed the possible manipulation of molecular interactions among the subunits of tobacco F-1-p by temperature in combination with pH and salt for altered functionality. How F-1-p interacts with other proteins and ingredients in food systems on food formulation needs further investigation. Such studies are being undertaken.

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