Thermal Coagulation of Oat Globulin[1]

C.-Y. MA[2] and V. R. HARWALKAR[2]

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ABSTRACT

The thermal coagulating behavior of oat globulin was studied at low protein concentrations (<1%). The rate of formation of insoluble aggregates was affected by temperature, buffer salt composition, and protein-modifying reagents including dithiothreitol and sodium dodecyl sulfate, and was related to the degree of protein denaturation. Gel filtration chromatography of the soluble fraction of heated globulin revealed that a small amount of the globulin hexamer was associated into soluble aggregates, whereas the major part was dissociated into subunits. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate showed no significant dissociation of oat globulin into acidic and basic polypeptides, and there was no redistribution of these two polypeptide chains in the soluble and insoluble fractions of heated globulin. *N*-Ethylmaleimide inhibited formation of soluble aggregates but did not prevent protein precipitation. The data revealed a significant difference in heat coagulation of oat globulin and soy glycinin, although the two proteins have similar molecular structures.

Thermal coagulation is a widely observed phenomenon in proteins. As defined by Hermansson (1979), coagulation is the random interaction of protein molecules, leading to formation of aggregates that could be either soluble or insoluble (precipitates). For monomeric proteins, such as ovalbumin and bovine serum albumin, coagulation is normally preceded by denaturation, following the scheme $N \rightleftharpoons D \rightarrow A$, where N denotes native protein, D denatured molecule, and A the aggregate (Ferry 1948). For oligomeric proteins with complex quaternary structures such as soy glycinin (11S globulin), heat may cause association/dissociation of the oligomer, and disruption of the quaternary structure itself may result in aggregation (German et al 1982).

Oat globulin, the major storage protein fraction, is an oligomeric protein with a quaternary structure very similar to that of legumins. Legumin is made of six subunits each of which consist of an acidic and a basic polypeptide linked by a single disulfide bond. The six subunits are linked through noncovalent forces to form the hexamer (Millerd 1975, Derbyshire et al 1976, Nielsen 1985). Whereas heat coagulation of some legumins, particularly soy glycinin, has been extensively studied (Wolf and Tamura 1969; Catsimpoolas et al 1969, 1970; Aoki 1970; Yamagishi et al 1980; Mori et al 1982), little is known about thermal denaturation and aggregation of oat globulin. The present work investigated association/dissociation of oat globulin upon heat treatment, and the mechanism of thermal coagulation. Similarities and differences in heat aggregation of oat globulin and soy glycinin are discussed.

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[2]Food Research Centre, Agriculture Canada, Ottawa, Canada K1A 0C6.

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MATERIALS AND METHODS

Materials

Oats (variety Sentinel) were grown at the Central Experimental Farm, Ottawa, Canada. Dehulled groats were ground in a pin mill (model 160 Z) and defatted by Soxhlet extraction with hexane.

Preparation of Oat Globulin

Globulin was solubilized from defatted ground oat groats by sequential extraction (Osborne and Mendel 1914). Groats were mixed with 1.0*M* NaCl at a solvent-to-solid ratio of 10:1 and stirred at room temperature for 30 min. The slurry was centrifuged at $20,000 \times g$ for 30 min, and the residue was reextracted twice with 1.0*M* NaCl. The combined supernatant was dialyzed exhaustively against distilled water at 4°C, and precipitated globulin was separated by centrifugation and freeze-dried.

Heat Treatment

To study thermal coagulation of oat globulin, solutions of 1% (w/v) protein were prepared in 0.01*M* phosphate buffer, pH 7.4, containing 1.0*M* NaCl. More than 85% of the protein was soluble; insoluble materials were removed by centrifugation at $10,000 \times g$. Aliquots (1–2 ml) of globulin solution were pipetted into glass tubes and heated at 100 and 110°C, as oat globulin denatures around 110°C (Ma and Harwalkar 1984). For experiments at 100°C, each tube was stoppered loosely with a glass marble to minimize evaporation, and heated in a boiling water bath. For tests at 110°C, resealable 5-ml tubes and a hydrolysis heating block (Pierce Chemical Co., Rockford, IL) were used. After heating, tubes were cooled by immersing in an ice bath, and solutions were centrifuged to separate insoluble aggregated protein. Protein contents of supernatant and unheated globulin solutions were

determined by the method of Lowry et al (1951).

Effects of three protein structure modifying agents—dithiothreitol (DTT), *N*-ethylmaleimide (NEM), and sodium dodecyl sulfate (SDS)—on heat coagulation of oat globulin were studied. Globulin solutions were prepared in phosphate buffer containing 1.0*M* NaCl, and reagents were added as dry solids. Addition of SDS to globulin preparations in 1.0*M* NaCl, however, insolubilized protein. Hence, phosphate buffer containing 0.4*M* NaCl was used, and a separate control (no SDS) was also prepared in 0.4*M* NaCl buffer, with a protein concentration lower than that prepared in 1.0*M* NaCl. DTT and NEM were added at 10 m*M*, whereas SDS concentrations ranged from 10 to 50 m*M*.

High-Performance Liquid Chromatography (HPLC)

Gel filtration HPLC was performed on an LKB Ultrapac TSK 4000SW column with a fractionation range of 5,000 to 1,000,000 (LKB-Produkter AB, Bromma, Sweden). A Perkin-Elmer model 400 solvent delivery system and LC-75 UV-Vis detector were used. Aliquots (50 μl) of globulin samples containing 100–500 μg of protein were loaded onto the column. Isocratic elution at 1.0 ml/min (at an operating pressure of around 600 psi) was performed with 0.01*M* phosphate buffer, pH 7.4, containing 1.0*M* NaCl; the eluant was monitored at 280 nm using 0.2 absorbance units full-scale (AUFS). Chromatographic data were collected and analyzed using the Perkin-Elmer chromatographic 2 data handling system. The column was calibrated with standard proteins with known molecular weights (apoferritin, 440,000; human gamma globulin, 160,000; bovine serum albumin, 67,000; ovalbumin, 45,000; trypsin inhibitor, 21,000; and cytochrome c, 13,500). All standard proteins were purchased from Sigma Chemical Co. (St. Louis, MO).

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) was performed on 7.5% (w/w) gels (Ma 1983). Amount of protein loaded was 10–20 μg in 10 μl. For heated samples of low protein content, a Centricon 10 micro-concentrator (Amicon Corp., Danvers, MA) was used to concentrate samples. The sample buffer contained 6*M* urea with or without 5% (v/v) 2-mercaptoethanol (2-ME). Densitometric scanning of the stained gels was performed using an LKB 2002 Ultroscan laser densitometer (LKB-Produkter AB, Bromma, Sweden) equipped with a Hewlett-Packard 3390A reporting integrator.

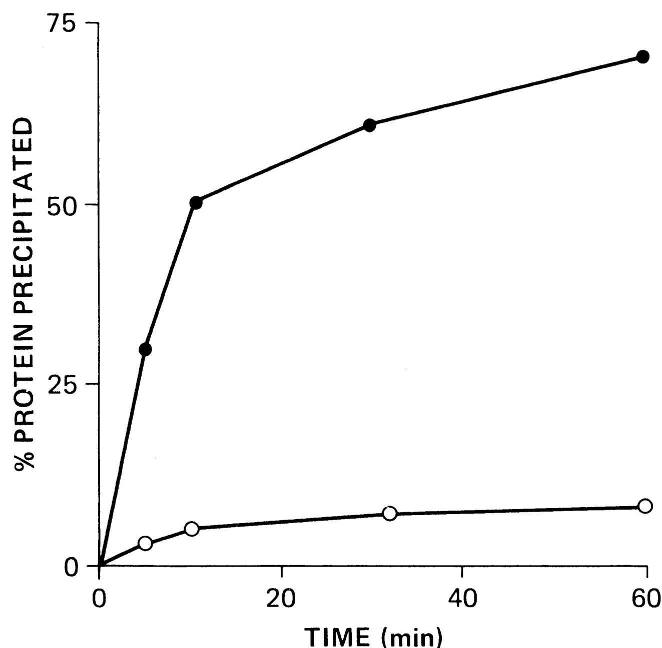

Fig. 1. Effect of temperature on the rate of heat coagulation of oat globulin. o, 100°C; ●, 110°C.

Differential Scanning Calorimetry (DSC)

Thermal characteristics of native and heated oat globulin were examined by DSC using a Dupont 1090 thermal analyzer equipped with a 910 DSC cell base and a high-pressure cell. Solid samples (approximately 1 mg) were weighed directly onto the pan, and 10 μl of buffer was added (protein:buffer about 1:10). A sealed empty pan was used as a reference. To study the effect of heat on thermal transition characteristics, oat globulin was first heated in a sealed pan on the reference or sample platform, preset to the desired temperature. After heating, the pan was cooled rapidly in an ice bath and reequilibrated to room temperature. The pan containing the heat-treated sample was again heated at a rate of 10°C/min from 30 to 140°C. Denaturation temperature (Td) and heat of transition (or enthalpy ΔH) were computed from the thermograms by the 1090 analyzer. Triplicate samples were evaluated by DSC; coefficients of variation ranged from 0.3 to 0.6% for Td and from 4 to 15% for ΔH.

RESULTS

Effect of Heating Temperature and Time on Coagulation

When an oat globulin solution (<1%) in 0.01*M* phosphate buffer (pH 7.4) plus 1.0*M* NaCl was heated at 100°C, the amount of insoluble protein increased progressively with time, leveling off after 30 min (Fig. 1). Less than 10% globulin precipitated even after extended heating at 100°C. At 110°C, the quantity of insoluble protein was much higher, reaching over 70% after 60 min.

Oat globulin precipitation was also influenced by initial protein concentration. At concentrations above 4% and at alkaline pH where oat globulin had higher solubility, a gel was formed, and it became difficult to separate insoluble from soluble fractions. At protein concentrations less than 0.1%, heating at 100°C led to only slight turbidity development.

Effect of Anions on Heat Coagulation

When the anion of sodium salt was changed from Cl^- to Br^-, I^-, or SCN^-, the rate of protein precipitation at 100°C increased progressively at the same salt concentration (1.0*M*) and pH (Fig.

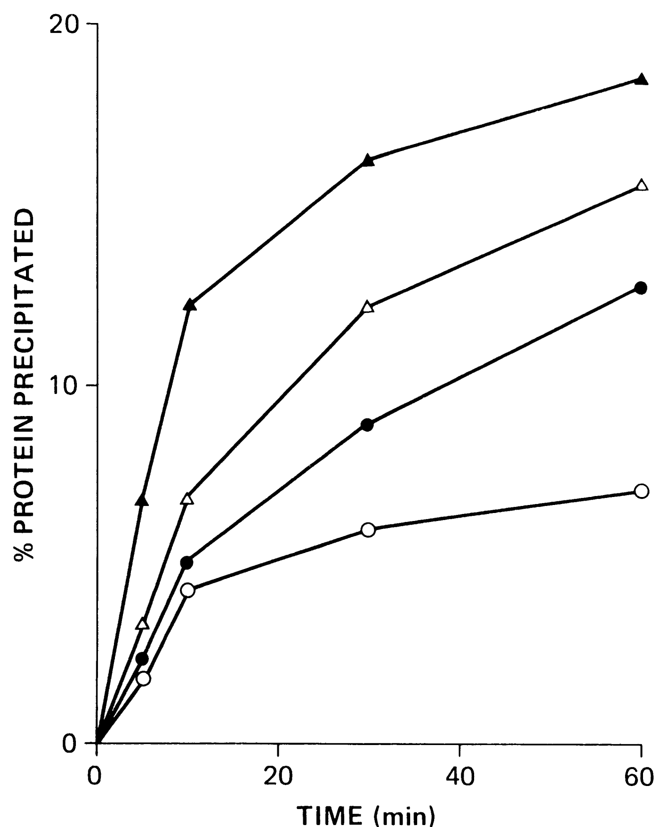

Fig. 2. Effect of anions on rate of heat coagulation of oat globulin. Heating temperature was 100°C. o, Cl^-; ●, Br^-; Δ, I^-; ▲, SCN^-.

2). Starting protein concentrations for this study varied slightly (0.9–1.0%) because oat globulin was more soluble in sodium thiocyanate and iodide than in bromide or chloride.

Effect of Protein Modifying Reagents on Heat Coagulation

Three chemical reagents known to modify protein conformation were added individually to globulin solutions to assess their effects on heat coagulation. At 100° C in the presence of 10 m*M* DTT (a disulfide reducing agent), the rate of protein precipitation markedly increased (Fig. 3). Addition of 10 m*M* NEM (a sulfhydryl-blocking agent) caused no significant change in amount of protein coagulated. Addition of 20 m*M* SDS (an anionic detergent) significantly reduced the rate of precipitation (Fig. 3). All heating curves followed a similar trend, leveling off after 30 min. Similar responses to these additives were observed at 110° C (data not shown).

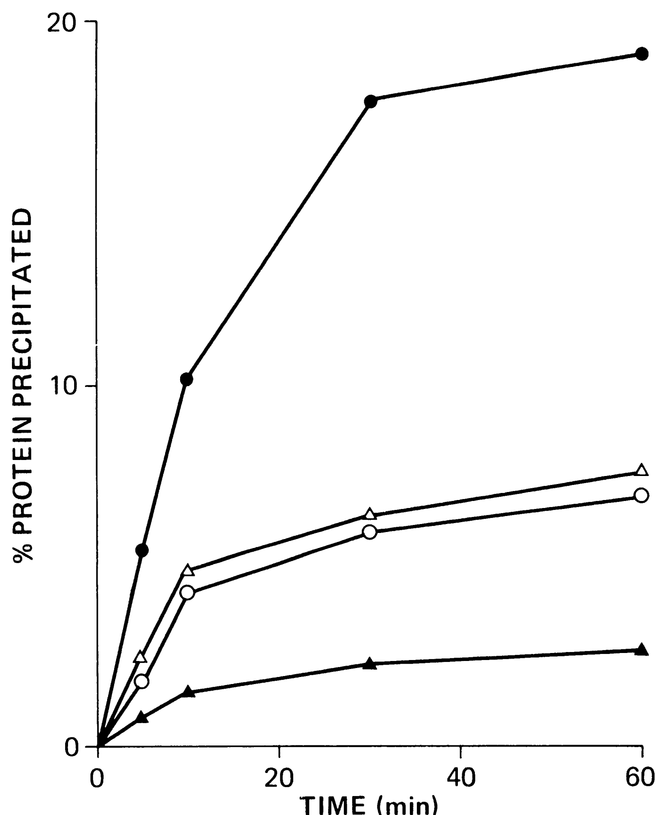

Fig. 3. Effect of protein modifying reagents on rate of heat coagulation (100° C) of oat globulin. All samples (except that in the presence of sodium dodecyl sulfate [SDS]) were heated with 1.0*M* NaCl (or 0.4*M* NaCl for SDS) in 0.01*M* phosphate buffer, pH 7.4. Protein concentrations were about 0.5% (0.4*M* NaCl) and 0.9% (1.0*M* NaCl). o, Control (no additive); ●, +10 m*M* dithiothreitol; Δ, +10 m*M* (*N*-ethylmaleimide); ▲, +20 m*M* SDS.

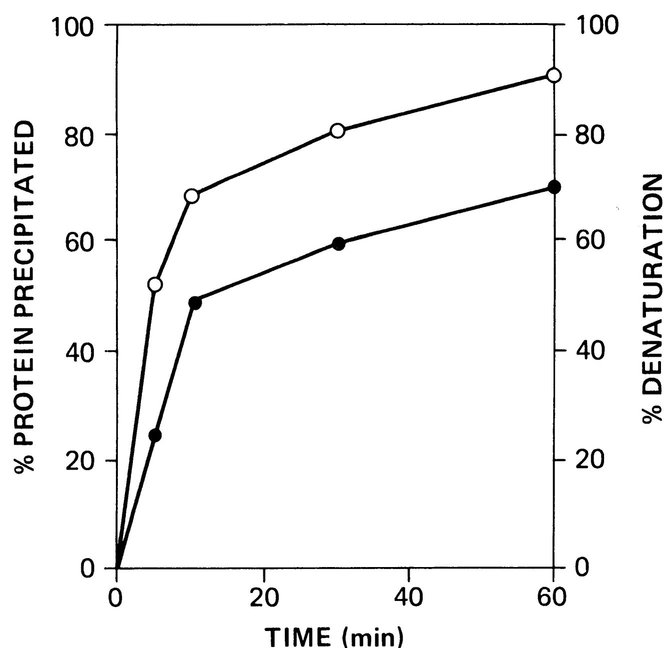

Fig. 4. Relation between heat coagulation at 110° C and denaturation of oat globulin. ●, % Protein precipitated; o, % denaturation.

Thermal Transition Properties

Figure 4 shows the relation between coagulation of oat globulin at 110° C and degree of denaturation, measured by DSC from enthalpy values. At 110° C, a progressive decrease in ΔH indicates gradual protein denaturation, as native protein would require more heat energy to denature than partially unfolded protein. There was a concurrent increase in the amount of protein precipitated, but percent denaturation was higher than percent precipitation at any time.

Table I shows effects of various anions on thermal transition properties of oat globulin. At 1.0*M*, Td progressively decreased as the anion changed, in the order Cl^-, Br^-, I^-, and SCN^-. Enthalpy also decreased in the above order, although not as dramatically as Td. The Td of oat globulin in water was about 110° C, considerably lower than in 1.0*M* NaCl. Data indicate that thermal stability of oat globulin changed markedly with ionic environment.

Table II shows the effect of SDS on DSC characteristics of oat globulin. Both Td and ΔH were progressively reduced with increased concentration of detergent, but the decrease in ΔH was much more pronounced than the decline in Td, indicating a slight decrease in thermal stability and a marked denaturation of oat globulin by SDS. The amount of protein precipitated at 110°C decreased significantly with increased SDS concentration (Table II).

HPLC

Changes in molecular weight distribution of oat globulin upon heating were studied by gel filtration HPLC. Soluble heated globulins (separated by centrifugation) were analyzed. Unheated globulin was resolved into five peaks (Fig. 5Aa). The first peak was eluted at void volume and constituted less than 1% of total protein.

TABLE I
Effect of Anions on Thermal Transition Properties of Oat Globulin[a]

Anion	Td[b] (°C)	ΔH[c] (J/g)
Chloride	114.2[d]	26.5[d]
Bromide	110.8	21.8
Iodide	104.5	19.2
Thiocyanate	98.4	16.6

[a] Buffers were 0.01*M* phosphate, pH 7.4, containing 1.0*M* sodium salt of the respective anion.
[b] Denaturation temperature.
[c] Enthalpy.
[d] Averages of three determinations.

TABLE II
Effect of Sodium Dodecyl Sulfate (SDS) on Thermal Transition Properties and Heat Aggregation of Oat Globulin[a]

SDS Concentration (m*M*)	Td[b] (°C)	ΔH[c] (J/g)	% Protein Precipitated[d]
0	112.0[e]	26.7[e]	42.5[f]
10	111.6	23.6	30.2
20	111.4	16.3	13.0
35	109.8	9.2	5.8
50	109.2	7.3	5.2

[a] Oat globulin was solubilized in 0.01*M* phosphate buffer, pH 7.4, containing 0.4*M* NaCl; SDS was added as a dry solid.
[b] Denaturation temperature.
[c] Enthalpy.
[d] Samples were heated at 110° C for 30 min.
[e] Averages of three determinations.
[f] Averages of two determinations.

Fig. 5. High-performance liquid chromatography (HPLC) of oat globulin, **A,** heated at 100° C. Protein samples were heated in 0.01 *M* phosphate buffer (pH 7.4) containing 1.0 *M* NaCl. Soluble protein was eluted with the same buffer. Samples were (**a**) unheated, or heated for (**b**) 10 min, (**c**) 60 min, or (**d**) 180 min. **B,** HPLC of oat globulins heated at 110° C. Other conditions were as in **A**. Samples were (**a**) unheated, or heated for (**b**) 2 min, (**c**) 10 min, (**d**) 60 min, or (**e**) 180 min. **C,** HPLC of oat globulins heated at 100° C in the presence of *N*-ethylmaleimide (NEM). Proteins were heated in 0.01 *M* phosphate buffer (pH 7.4) containing 1.0 *M* NaCl and 10 m*M* NEM. Soluble protein was eluted with the same buffer without NEM. Samples were (**a**) unheated, or heated for (**b**) 2 min, (**c**) 10 min, or (**d**) 60 min.

The second peak (about 9%) was a shoulder of the third major (about 75%) fraction. Estimated molecular weights of proteins in the second and third peaks were 700,000 and 350,000, respectively. Peaks 4 and 5 corresponded to estimated molecular weights of 58,000 and 30,000, and constituted about 12 and 4%, respectively, of total protein.

When heated at 100° C for 10 (5Ab) and 60 (5Ac) min, there was a progressive increase in size of the void volume peak, from <1% up to a maximum of about 7% of total protein, with a corresponding decrease in peaks 2 and 3, from 84 to 76%, indicating formation of high molecular weight aggregates. After extended (180 min) heating (5Ad), peaks 2 and 3 were further decreased to about 70% of total protein, whereas peaks 4 and 5 were increased to about 17 and 6%, respectively.

When heated at 110° C (Fig. 5B), the void volume peak was increased to 3% of total protein after 2 min (5Bb) but was absent after 10 min of heating (5Bc). With further heating, peak 2 disappeared and peak 3 was decreased from 75% (5Ba) to 25% (5Be) of total proteins. Peaks 4 and 5 were increased by about 40% (in absolute quantity), and from 12 and 4%, respectively, of total protein (5Ba) to 60 and 15% after 120 min of heating (5Be).

Figure 5C shows molecular weight distributions of oat globulin heated at 110° C in the presence of 10 m*M* NEM. Because of the strong absorbance of NEM, eluting near V_T, globulin peak 5 was incompletely resolved. A progressive decrease in peaks 2 and 3 occurs with increased heating time; and peak 3 represents only about 25% of total protein after 60 min (5Cd), considerably lower than that (45%) without NEM (5Bd). The void volume peak was completely lost when heated for 2 min at 110° C (5Cb). The progressive increase in peak 1 at 100° C (Fig. 5A) was completely prohibited by NEM addition (data not shown), indicating that formation of soluble aggregate was hindered.

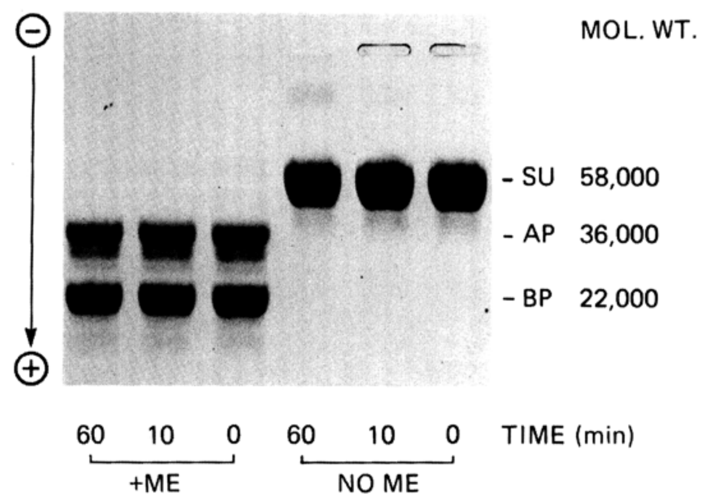

Fig. 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of oat globulin heated at 100° C. **SU,** Subunit; **AP,** acidic polypeptide; **BP,** basic polypeptide; **2-ME,** 2-mercaptoethanol.

SDS-PAGE

Figure 6 shows SDS-PAGE patterns of oat globulin heated at

100°C for 0, 10, and 60 min. When samples were analyzed in the absence of 2-ME, one major band was present (estimated mean molecular weight, 58,000). Heating at 100°C only slightly increased the intensity of a minor 90,000 mol wt band. When electrophoresis was performed in the presence of 2-ME, both the 58,000 mol wt and the slow migrating bands disappeared, and components with estimated mean molecular weight of 36,000 and 22,000 were observed. Heating did not markedly change the SDS-PAGE pattern (Fig. 6).

Figure 7 shows SDS-PAGE patterns of oat globulin after heating at 110°C for 0, 10, 60, and 120 min. Soluble and precipitate fractions were separated by centrifugation and analyzed in the presence of 2-ME. Soluble and insoluble fractions had similar patterns, except that some high molecular weight components in the precipitate did not enter the gel. Heating did not markedly change the ratio of 22,000 and 36,000 mol wt bands (shown by densitometry to range from 1.20 to 1.30). SDS-PAGE patterns of oat globulin heated at 110°C and run in the absence of reductant were similar to those at 100°C (data not shown).

DISCUSSION

Our data show that insoluble aggregates formed when a dilute (<1%) solution of oat globulin in 0.01 *M* phosphate buffer (pH 7.4) containing 1.0*M* NaCl was heated. The rate of precipitation was affected by temperature.

Oat globulin has fairly high heat stability. At 100°C, less than 10% of the total protein precipitated, even after extended heating; this could be attributed to an exceptionally high denaturation temperature of oat globulin (114°C in 1.0*M* NaCl). Most monomeric globulins that are heat coagulable, e.g., egg white proteins and bovine serum albumin, have Td's well below 100°C (Privalov and Khechinashvili 1974). Soy glycinin, an oligomeric protein, has a Td of 104°C (Wright and Boulter 1980), considerably lower than that of oat globulin. When dilute (0.5–1%) soy glycinin in 0.4–0.5*M* NaCl was heated at 100°C, more than 50% of soy protein precipitated after 10 min (Wolf and Tamura 1969, Yamagishi et al 1980, Mori et al 1982), much higher than that of oat globulin. Both oat globulin and soy glycinin can be heat coagulated below their Td. For oligomeric proteins, heat may disrupt the quaternary structure, resulting in aggregation without extensive denaturation of constituent monomers (German et al 1982).

More extensive precipitation occurred when oat globulin was heated at 110°C. DSC was used to monitor the extent of protein denaturation upon heating. Because of the sensitivity limit (10 μW)

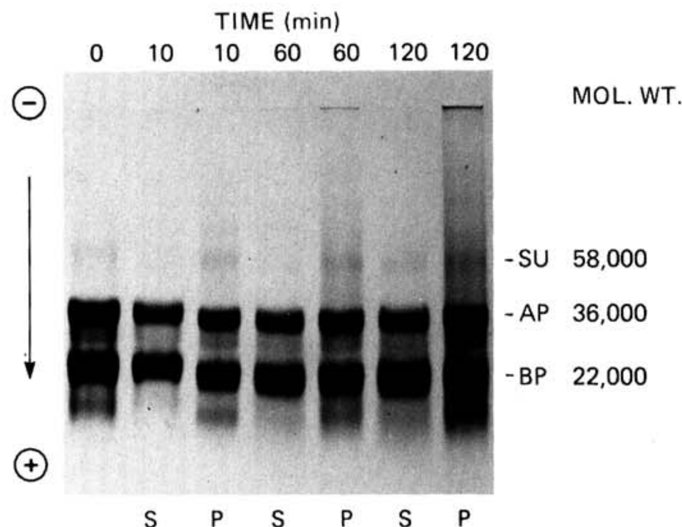

Fig. 7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of oat globulin heated at 110°C. Heated samples were separated by centrifugation into supernatant (**S**) and precipitate (**P**), and run in the presence of 2-mercaptoethanol. **SU**, Subunit; **AP**, acidic polypeptide; **BP**, basic polypeptide.

of the DSC analyzer, protein concentrations equivalent to those used for heat aggregation experiments (<1%) could not be used. Furthermore, soluble proteins were used for heat coagulation tests, whereas DSC experiments were performed on slurries containing insoluble globulin. Despite these differences, DSC provides information on conformations of oat globulins upon various treatments. Results (Fig. 4) indicate that denaturation was closely followed by coagulation. Heating near Td could disrupt the oligomeric structure and denature monomers, initiating further rearrangement and/or aggregation. A close correlation between aggregation and denaturation has also been observed for ovalbumin (Hegg 1978) and conalbumin (Hegg et al 1978) under the influence of SDS.

Oat globulin precipitation rate was also affected by buffer composition, increasing as the counterion changed from Cl^- to Br^-, I^-, or SCN^- (Fig. 2) following the lyotropic series (Hatefi and Hanstein 1969). Salts profoundly influence protein conformation and function by affecting the physical state of water, breaking the hydrogen-bonded structure of water and weakening intramolecular hydrophobic interactions of protein (von Hippel and Schleich 1969, Kinsella 1982). Those anions higher in the lyotropic series have a greater tendency to denature protein by reducing the free energy required to transfer polar groups to water, thus decreasing thermal stability (von Hippel and Wong 1964). This is consistent with our data (Table I), which show a progressive decrease in both Td and ΔH by salts following the lyotropic anion series. The increase in heat-induced aggregate formation could therefore be attributed to lower thermal stability and partial denaturation of oat globulin under the influence of neutral salts.

The effects of some protein-modifying agents on rate of precipitation were also studied. DTT increased the rate of protein precipitation. A similar observation was reported for soy glycinin (Wolf and Tamura 1969). These results indicate that oligomeric structures are stabilized by disulfide bridges between acidic and basic polypeptides. Dissociation of the oligomer into monomers may promote formation of aggregates. NEM did not affect the rate of protein precipitation. This is in contrast to soy glycinin, where NEM inhibited formation of insoluble aggregates or gel (Wolf and Tamura 1969, Mori et al 1962). SDS decreased oat globulin aggregation at both 100°C (Fig. 3) and 110°C (Table II), and caused a decline in ΔH (Table II), indicating partial denaturation. SDS binds to proteins noncovalently and causes denaturation (Steinhardt 1975). Increased negative charge on proteins could hinder protein-protein interaction and prevent formation of aggregates. In conalbumin, heat coagulation was suppressed by SDS due to electrostatic repulsion (Hegg 1978).

HPLC reveals the molecular weight distribution of heated oat globulin that remained buffer soluble. Oat globulin is a hexamer composed of six pairs of acidic and basic polypeptides with a molecular weight of 320,000–370,000 (Peterson 1978, Brinegar and Peterson 1982). Acidic (AP) and basic (BP) polypeptides, with molecular weights of 22,000–24,000 and 32,000–37,000, respectively, are linked by disulfide bonds into a subunit (SU) with a molecular weight of around 60,000 (Brinegar and Peterson 1982). HPLC data suggest that the major soluble oat globulin species is the hexamer. The shoulder eluted before the major component could be the dimeric form also detected by ultracentrifugation (Peterson 1978). Peak 4 may correspond to SU, while peak 5 may represent AP and BP mixture.

The increase in the void volume peak when oat globulin was heated indicates formation of soluble aggregates. When fractionated on Sepharose CL-6B, having a higher fractionation range (data not shown), soluble aggregates also eluted at the void volume, corresponding to a molecular weight of over 4×10^6. The decrease in the void volume peak after further heating at 100°C and its rapid disappearance at 110°C suggest that soluble aggregates either precipitate or dissociate. Because a small amount (<7%) of soluble aggregates formed, it was difficult to determine the exact fate of associated globulin.

When heated at 110°C, the progressive decrease in oligomeric peaks 2 and 3 and increase in subunit and polypeptide peaks 4 and 5 suggest dissociation of oat globulin. As in legume 11S globulins,

the subunits are linked by noncovalent forces (Pernollet and Mosse 1983). Heating may disrupt these forces and dissociate the hexamers. Oat oligomers may also be converted to insoluble aggregates, leading to a marked decrease in the soluble fraction.

When SDS-PAGE was conducted in the absence of reducing agent, disulfide bonds linking AP and BP of oat globulin remained intact, and SU was the major band in the unheated oat globulin. Hexamers were dissociated by SDS. The appearance of a minor, high molecular weight component and its increase upon heating at 100°C (Fig. 6) indicates formation of soluble aggregates, as suggested by HPLC. However, SU was a major component even after extensive heating at 100 or 110°C, indicating that disulfide bonds were not disrupted by heat treatment. In contrast, SDS-PAGE of glycinin in the absence of 2-ME showed that heating caused the disappearance of the SU band and the formation of zones corresponding to AP and an oligomer of AP (Yamagishi et al 1980).

When SDS-PAGE was run in the presence of reductant, oat globulin subunits separated into AP and BP. Densitometry showed that heating did not cause pronounced changes in the BP/AP ratio of the supernatant and precipitate. Difference in affinity of BP and AP for the protein stain, Coomassie Blue, may account for the deviation of the BP/AP ratio from unity, as expected from the structure of oat globulin (Brinegar and Peterson 1982).

Our data reveal similarities and differences between heat-coagulating behavior of oat globulin and soy glycinin. Because oat globulin is structurally similar to glycinin, we attempted to compare the steps in heat coagulation of the two proteins.

Thermal changes in glycinin, as proposed by Mori et al (1982), include the following steps: 1) formation of soluble aggregates (average sedimentation coefficient, 80S; molecular weight, 8×10^6); 2) dissociation of the soluble aggregates to acidic and basic polypeptides; 3) association of basic polypeptides to insoluble aggregates, whereas acidic polypeptides remain soluble.

At early stages of heating, more than 60% of total soy protein was converted to soluble aggregates (Wolf and Tamura 1969, Yamagishi et al 1980, Mori et al 1982). Soluble aggregates formed to a relatively minor extent when oat globulin was heated and thus may not be key intermediates in its thermal coagulation.

Soluble aggregates of soy glycinin dissociate into AP and BP upon subsequent heating (Wolf and Tamura 1969, Yamagishi et al 1980, Mori et al 1982). Similar dissociation was also observed for oat globulin, particularly at higher temperatures, but the predominant product was SU, with little AP and BP. Free SH groups in soy and oat globulins (Draper and Catsimpoolas 1978, Brinegar and Peterson 1982) may participate in thiol-disulfide exchange, a reaction promoted by heat, leading to cleavage of disulfide bonds linking the polypeptide chains. Our data suggest that thiol-disulfide interchange is limited in oat globulin, and heat only disrupts noncovalent bonds linking the subunits into soluble aggregates.

The final step in thermal coagulation of glycinin is formation of insoluble aggregates from dissociated polypeptide chains, mainly BP. Because SU were the major dissociated molecules in oat globulin, no significant redistribution of AP and BP into the supernatant and precipitate was observed. The results suggest that insoluble aggregates form directly from dissociated SU.

A main feature in thermal coagulation of soy glycinin is that NEM prevents formation of insoluble aggregates by blocking free sulfhydryl groups and preventing thiol-disulfide interchange. Soluble aggregates that formed have a molecular weight (4×10^6) considerably lower than that (8×10^6) when heated in the absence of NEM (Mori et al 1982). It was proposed that a NEM-soluble aggregate was formed before the higher molecular weight soluble aggregate (Mori et al 1982). For oat globulin, NEM did not prevent protein precipitation, confirming that thiol-disulfide interchange is not involved in formation of insoluble aggregates. However, formation of soluble aggregates was hindered by NEM. Hence, thiol-disulfide interchange may be involved in formation of soluble aggregates.

Thus, despite the similar quaternary and subunit structures of oat globulin and soy glycinin, the two proteins have significantly different heat aggregation behavior. Based on our data, a scheme for thermal coagulation of oat globulin is outlined in Figure 8. According to this proposed scheme, a small portion of oligomeric oat globulin associates to soluble aggregates at early stages of heating (at 110°C). Upon further heating, these aggregates may dissociate to subunits or further aggregate and precipitate. Insoluble aggregates may also be formed from partially denatured subunits through protein-protein interaction or from oligomers via a disruption of the quaternary structure.

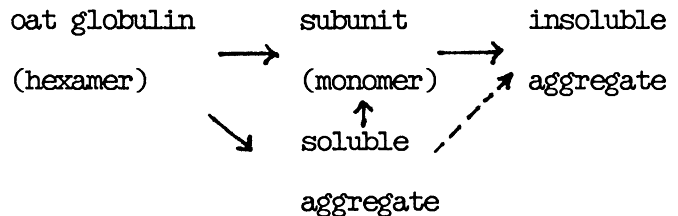

Fig. 8. Proposed scheme for thermal coagulation of oat globulin.

ACKNOWLEDGMENTS

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