Aggregation of A-Gliadin: Gel Permeation Chromatography[1]

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ABSTRACT

Cereal Chem. 60(4):306–310

The reversible association of the storage protein fraction A-gliadin (aggregable α-gliadin) from the wheat cultivar Scout 66 into microfibrillar aggregates was studied at several different pHs, ionic strengths, and temperatures by gel permeation chromatography on Sephacryl S-300. At pH 3 the A-gliadin eluted from the column in a peak corresponding to the monomeric form of the protein (molecular weight near 30×10^3), whereas at pH 4 a peak corresponding to aggregated A-gliadin appeared, and at pH 5 the protein eluted mainly in the aggregated form at the excluded volume of the column, which indicated apparent molecular weights of 10^6 or greater for the aggregates. At pH 4 and pH 5, the percentage of the protein eluting in the aggregated form increased as ionic strength was increased from 0.005 to 0.01 or 0.015. Increase in temperature at pH 4 or pH 5 caused dissociation of the protein, and the aggregates showed greater stability with an increase in ionic strength at either pH. The ionic strength and temperature dependence of the aggregation indicated a major role for hydrogen bonding in stabilization of the microfibrillar aggregates; ionic interactions and hydrophobic interactions may be important, but apparently are of less importance. The pH dependence of the aggregation indicated that ionization of carboxylic side chains (and the C-terminal carboxyl group) is important for stabilization of the aggregated form, perhaps by permitting the formation of increased secondary and tertiary conformational structure in A-gliadin as the net positive charge on the protein is decreased.

A-gliadin is an aggregable α-gliadin fraction found only in some wheat varieties (Bernardin et al 1967, Platt et al 1974, Tobler et al 1982). Although A-gliadin is essentially monomeric at pH 3 with a molecular weight (mol wt) near 30×10^3 (abbreviated as 30k), it forms microfibrillar aggregates with apparent mol wts of several millions at pH 5 in the presence of 0.005*M* NaCl (Bernardin et al 1967, Kasarda 1980, Kasarda et al 1967). The aggregation is specific in nature, as indicated by the well-defined structure of the microfibrillar aggregates. It is also reversible; for example, by lowering the pH to 3, so the aggregation must involve only secondary bonding (noncovalent) forces such as hydrogen bonding, ionic interactions, or hydrophobic interactions. Aggregation of A-gliadin occurs under conditions in which the protein molecule assumes (or at least approaches) a compact conformation, similar to that of globular proteins, that has a significant amount of ordered secondary structure. Kasarda et al (1968) estimated that about one third of the polypeptide chain is in the α-helical conformation. The microfibrils of aggregated A-gliadin have some characteristics similar to those of wheat flour doughs, and Bernardin (1975, 1978) has used A-gliadin as a model for the more complex mixture of proteins that interact to form the cohesive matrix of wheat flour doughs and contribute the unique viscoelastic properties of these doughs.

The aggregation of A-gliadin has been studied mainly at pH 3 and at pH 5 with water or 0.005*M* NaCl as solvent. In the present study, we have used gel permeation chromatography (GPC) on Sephacryl S-300 to provide additional information about the aggregation of A-gliadin as a function of pH, ionic strength, and temperature. Our intentions were to provide a better understanding of the mechanism of A-gliadin aggregation and of the secondary forces that stabilize the aggregates, and eventually, to develop a molecular theory for the viscoelastic properties of doughs.

MATERIALS AND METHODS

Wheat Samples and Other Materials

The wheat flour was from the hard red winter wheat cultivar Scout 66 and was milled on a Brabender Quadrumat Sr. mill. Only the endosperm fraction was used to prepare A-gliadin. All solutions were prepared with deionized, deaerated water. The Sephacryl S-300 was from Pharmacia. The protein standards used to calibrate the S-300 column obtained from Sigma and Worthington Biochemical Companies were: thyroglobulin (669k), ferritin (420k), transferrin (81k), bovine serum albumin (68k), ovalbumin (43k), chymotrypsinogen A (25k), ribonuclease (13.7k), and horse heart cytochrome C (12.5k) (Fig. 1). All chemicals were of reagent grade.

A-gliadin was prepared according to the method of Bernardin et al (1967) and given a second GPC purification by dissolving 50-mg samples in 4.0 ml of 0.01*M* acetic acid, then chromatographing the samples on a 90 × 1.2-cm column (Kontes Glass Co.) of Sephacryl S-300 in 0.01*M* acetic acid as eluting solvent at room temperature (about 20°C). The protein eluted in two peaks: a small peak at the excluded volume of the column, and a second resolved peak. The first peak, which apparently consisted of irreversibly aggregated A-gliadin, was discarded. A center cut of the second resolved peak,

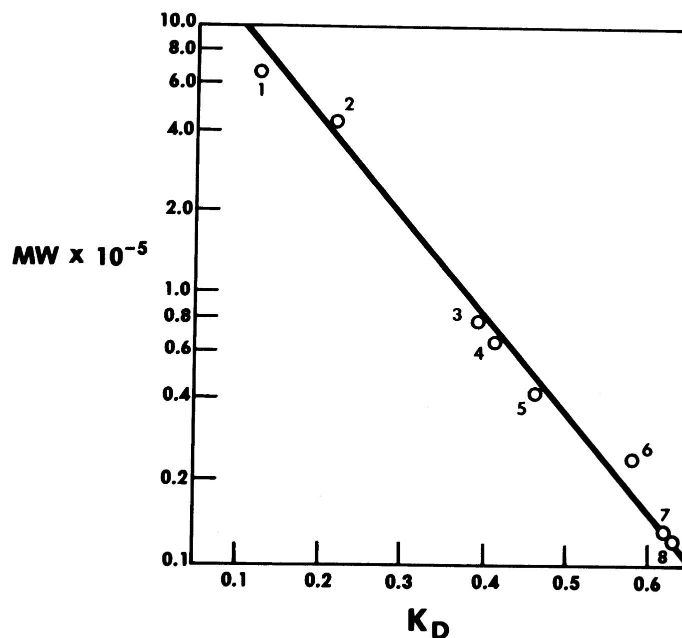

Fig. 1. Calibration curve for Sephacryl S-300 column with reference proteins in 0.2*M* sodium chloride + 0.01*M* ammonium acetate, pH 8.0, (pH adjusted to 8.0 with ammonium hydroxide). On curve, 1 = thyroglobulin, 2 = ferritin, 3 = transferrin, 4 = bovine serum albumin, 5 = ovalbumin, 6 = chymotrypsinogen, 7 = ribonuclease, 8 = horse heart cytochrome C.

[1]Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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which eluted at a position corresponding to a mol wt of about 30k, was freeze-dried directly; the resulting protein (about 35–40 mg recovered from each 50-mg sample) was used for our studies. Rechromatography of this fraction under the same conditions showed only the resolved peak. Two-dimensional (2-pH) electrophoretic analysis (Mecham et al 1978) of this A-gliadin preparation gave a pattern identical to that shown in Fig. 2 of Kasarda (1980).

Gel Permeation Chromatography

A water-jacketed Kontes column (90 × 1.2 cm) containing Sephacryl S-300 was used for all experiments. The column was packed at a downward flow rate of 30 ml/hr regulated by a peristaltic pump (Buchler Monostaltic pump). Column temperature was controlled by means of external heating (Haake model KT-62) or cooling (Brinkman model KT-63) circulators. Temperature of the buffer in the column was checked at the beginning and end of an experiment, and the temperature of the circulator reservoir was monitored during a run; temperature varied less than ±0.5°C in any experiment. All experiments were performed at a flow rate of 23 ml/hr by means of a peristaltic pump. Effluent absorbance was monitored at 280 nm by an Altex model 153 detector, and recorded with a Linear model 155MM recorder with an event marker that indicated fraction changes by a Gilson model VL fraction collector.

Total bed volume (V_t) of the column was determined by filling the column with water. The column was standardized after being packed with S-300 by running the set of protein standards in 0.2*M* NaCl that was 0.01*M* in ammonium acetate, pH 8.0. Blue Dextran 2000 (5 mg/ml) was used to determine the excluded volume (V_o). The internal volume (V_i) was obtained by loading 1 ml of 2*M* NaCl on the column and noting the elution volume at which solution conductivity sharply increased, as measured with a Radiometer model CDM 2d conductivity meter.

Solutions of A-gliadin for aggregation experiments were prepared by dissolving 20 mg of freeze-dried protein in 4 ml of 0.01*M* acetic acid (pH 3.2) with gentle stirring at room temperature. These solutions were applied to the column and allowed to equilibrate with the column buffer on the column. Samples were not dissolved directly in the column buffers because they dissolve quickly in acetic acid but are slower to dissolve in the column buffer. A few preliminary experiments indicated that this approach did not produce different results from experiments with samples of A-gliadin dissolved directly in the column buffer.

The partition coefficients (K_D) of protein standards and A-gliadin were calculated from the relationship:

$$K_D = (V_e - V_o)/(V_t - V_o)$$

where V_e is the elution volume of the protein, as indicated by peak maximum.

The following solutions were used for column equilibration and elution of A-gliadin in aggregation experiments: (pH 3.1), 0.001*M* HCl + 0.01*M* NaCl; (pH 4), 0.001*M* acetic acid + 0.005*M* NaCl adjusted to pH 4 with ammonium hydroxide; (pH 4), 0.001*M* acetic acid + 0.01*M* NaCl adjusted to pH 4 with ammonium hydroxide; (pH 4), 0.01*M* acetic acid + 0.01*M* NaCl adjusted to pH 4 with ammonium hydroxide; (pH 4), 0.01*M* acetic acid + 0.015*M* NaCl adjusted to pH 4 with ammonium hydroxide; (pH 5), 0.001*M* acetic acid + 0.005*M* NaCl adjusted to pH 5 with ammonium hydroxide; and (pH 5), 0.001*M* acetic acid + 0.01*M* NaCl adjusted to pH 5 with ammonium hydroxide. When measurements were made as a function of temperature at pH 4 and pH 5, the pH of the buffer was checked at the temperature of the experiment. No variations in pH greater than 0.15 pH units were noted at the temperatures (10–48°C) used.

Analysis of Chromatograms

Elution patterns were divided into areas corresponding to monomer and aggregates. A planimeter was used to measure areas, and amounts of monomer and aggregate were expressed as percentages of the total area.

RESULTS AND DISCUSSION

Column Calibration

The elution volumes of standards, along with constants, were used to calculate K_D, which was plotted against log mol wt of the standards (Fig. 1). A reasonably straight line was obtained, indicating that globular proteins with mol wts ranging from about 10^4 to 10^6 can be resolved on the S-300 column. Proteins were calibrated at pH 8 in 0.2*M* NaCl because some globular proteins behave anomalously in the pH range 3–5, probably because of conformational changes. The column did not change bed volume when equilibrated with the pH 3–5 solutions used to study A-gliadin aggregation, and we assumed that the column calibration determined at pH 8 was valid as long as no interactions occurred between the proteins being studied and the column matrix.

Effect of pH on Aggregation of A-Gliadin

Figure 2 shows the elution patterns obtained at pH 5, pH 4, and pH 3.1 for A-gliadin in 0.01*M* NaCl. All data (elution patterns and areas) represent the average of at least duplicate experiments. Specific conductivities (micromhos per centimeter) were 1,150, 1,148, and 1,480. We attempted to maintain equivalent conductivities at the different pHs, but 0.001*M* HCl was used at pH 3.1 instead of 0.001*M* acetic acid (which was titrated with ammonium hydroxide to give pHs 4 and 5), and the strong acid resulted in a slightly higher conductivity. All experiments were done at 25°C.

At pH 3.1, the A-gliadin eluted mainly in a peak corresponding to an apparent mol wt of about 25k on the basis of our column calibration at pH 8. A-gliadin has a mol wt of about 31,000 (Platt and Kasarda 1971). Our lower apparent mol wt may indicate slight retardation of the A-gliadin through interaction with the column, or it may simply represent inherent error in the column calibration, possibly because of altered pH. A small peak corresponding in elution volume to V_i (105 ml) was noted at pH 3.1 and slightly beyond V_i at other pHs. Examination of the protein eluted in this peak by two-dimensional PAGE (Mecham et al 1978) demonstrated that it was A-gliadin, evidently retarded through interaction with the column matrix. We ignored these small peaks

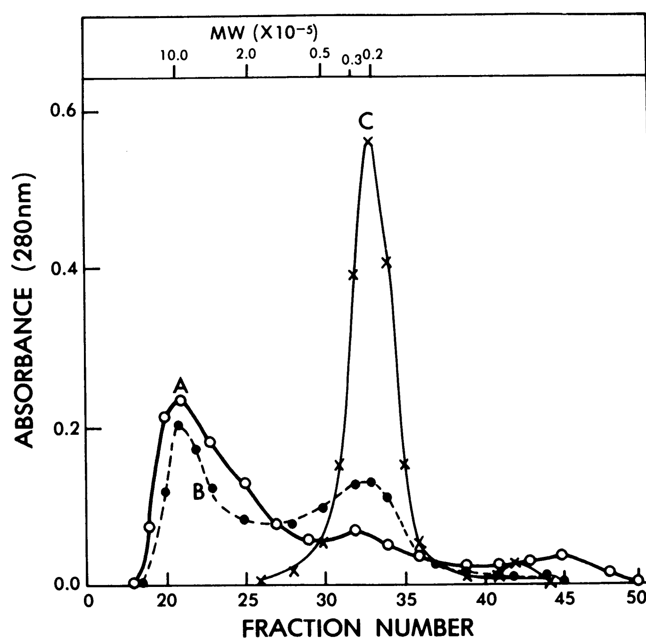

Fig. 2. Effect of pH on elution profile of A-gliadin aggregates separated on Sephacryl S-300. Profiles for A-gliadin eluted in: **A,** 0.001*M* acetic acid + 0.01*M* sodium chloride (pH 5.0); **B,** 0.001*M* acetic acid + 0.01*M* sodium chloride (pH 4, adjusted to 4.0 and 5.0 with ammonium hydroxide); and **C,** 0.001*M* hydrochloric acid + 0.01*M* sodium chloride (pH 3.1). Specific conductivities (micromhos per centimeter) = (A) 1,150, (B) 1,148, and (C) 1,480.

in analyzing the aggregation experiments insofar as the results were otherwise consistent with normal GPC behavior for A-gliadin. We speculate that a few strong binding sites on S-300 may readily become saturated with A-gliadin, whereas the bulk of the protein did not interact with the S-300. On rare occasions, we encountered severe retardation of A-gliadin at the lowest salt concentration studied (0.005*M* NaCl).

At pH 4, a peak appeared at or just beyond V_o tailed into the monomer peak. This peak must correspond to aggregated A-gliadin with mol wts of about 10^6 predominating, but ranging down in size to trimers and dimers. No turbidity was observed in the fractions excluded from the column.

To analyze patterns indicating both monomeric and aggregated protein, we divided the pattern at the minimum between the two peaks. Although a slight overestimation of the area corresponding to monomer results, we believe that a more elaborate approach is not warranted. We did, however, subtract an estimated Gaussian curve corresponding to the monomer peak from the total area in those few experiments in which no distinct minimum occurred (such as for curve C of Fig. 2). In this way, we estimated that 50% of A-gliadin is aggregated at pH 4.0 (0.01*M* NaCl), whereas about 70% is aggregated at pH 5.0 at the same salt concentration (Fig. 2). We found that A-gliadin is essentially monomeric at pH 3 at this ionic strength (Fig. 2), and becomes increasingly aggregated at higher pH into forms having apparent mol wts of 10^6 or greater at pH 5. We did not attempt measurements at pH higher than 5, but we expect that protein aggregation would continue to increase until precipitation occurred.

When A-gliadin aggregated at pH 5 was chromatographed on a column equilibrated at pH 3.1, a chromatogram equivalent to curve C of Fig. 2 was obtained, indicating complete reversibility of aggregation. Freeze-drying, however, apparently produces a small amount of irreversibly aggregated A-gliadin.

A-gliadin has about eight free carboxyl groups per mole (Kasarda et al 1976). These glutamic acid and aspartic acid side chains (plus the C-terminal carboxyl group) are largely protonated (neutral) at pH 3, becoming deprotonated and negatively charged as the pH increases. Ionization of carboxyl side chains in most proteins should be largely complete at pH 5 (Cohn and Edsall 1943), which should result in considerably reduced net positive charge on the A-gliadin molecule. A-gliadin has about 13 positive charges per mole at pH 3.1 on the basis of its amino acid composition (Platt and Kasarda 1971). A-gliadin also undergoes a conformational change in this pH range, leading to a more compact structure at pH 5 than at pH 3 (Kasarda et al 1968). We speculate that the decreased net positive charge of A-gliadin, and accompanying conformational change of A-gliadin as the pH is increased from 3 to 5, results in its specific aggregation to the microfibrillar form, although these concurrent changes in conformation and state of aggregation cannot, in actuality, be separated from one another in the analysis of our experiments (see Ohga et al 1981).

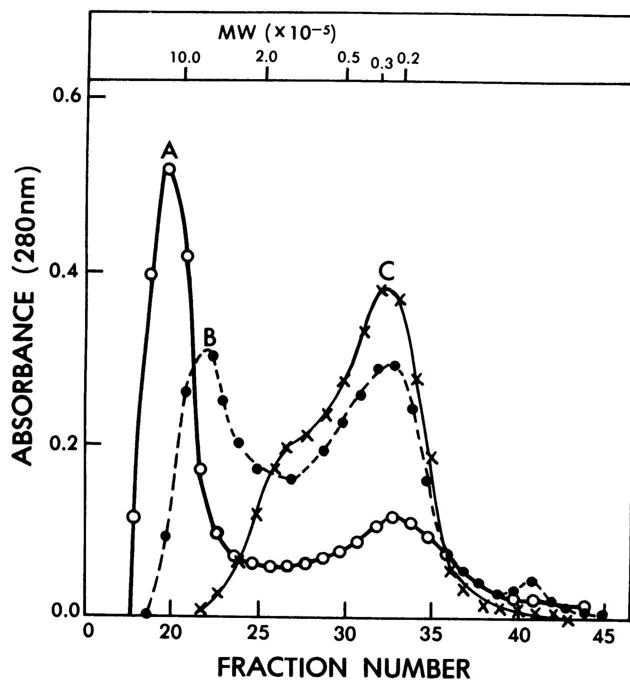

Fig. 3. Effect of salt concentration on elution profile of A-gliadin aggregates separated on Sephacryl S-300, pH 4.0, 25°C. Profiles for A-gliadin eluted in: **A,** 0.01*M* acetic acid + 0.015*M* sodium chloride, 2,000 μmho/cm; **B,** 0.001*M* acetic acid + 0.01*M* sodium chloride, 1,150 μmho/cm; **C,** 0.001*M* acetic acid + 0.005*M* sodium chloride, 630 μmho/cm (pH adjusted to 4.0 with ammonium hydroxide).

Effect of Ionic Strength on Aggregation of A-Gliadin

Figure 3 shows the effect of changed ionic strength (NaCl concentration) on A-gliadin aggregation at pH 4.0. In 0.005*M* NaCl (curve C), A-gliadin eluted largely in the monomeric form with only about 25% in the form of aggregates having mol wts near 10^5. When the NaCl concentration was increased to 0.01*M*, about 50% of the A-gliadin was aggregated and the predominant mol wt had increased to about 500,000. Increasing the salt concentration to 0.015*M* NaCl (curve A) caused about 70% of A-gliadin to be aggregated into a form eluting mainly at V_o and having mol wts greater than 10^6.

These results indicate that A-gliadin aggregation is a function of both ionic strength and pH. Apparently, positive charges that may prevent A-gliadin from assuming a compactly folded, aggregated form can be diminished through shielding of these charges by increasing the ionic strength of the solution. According to the Debye-Huckel theory (Edsall and Wyman 1958), at an ionic strength of 0.01 the influence of a positive charge on the surface of a protein would extend about 30×10^{-8} cm (30 Å) before being effectively neutralized by the ion atmosphere of surrounding salt ions. This theoretical approximation might err considerably in either direction compared with actuality. For example, our estimate ignores the effect of possible neighboring charges on the surface of the protein. Nonetheless, the estimate indicates that charges on the surface of an A-gliadin molecule expected, on the basis of its mol wt, to have a diameter of about 50 Å, might act independently to some extent, allowing specific interactions with other protein subunits despite significant net positive charge on the protein. The relatively low charge density of A-gliadin (Kasarda et al 1976), which has few amino acid side chains that can assume either positive or negative charge in comparison with most other proteins, might also tend to diminish net charge effects. We therefore believe that increased ionic strength could effect aggregation at pHs other than those studied (pH 4.0 and 5.0), for example, at pH 3.1.

It appears that ionic interactions between oppositely charged side chains (salt linkages) of A-gliadin molecules do not predominate in stabilizing aggregates because aggregation is enhanced by increased ionic strength, which should disrupt such salt linkages. It is conceivable, however, that in the pH range we used (3.1–5.0), positive charges on the proteins prevent protein or protein interactions until repulsive forces are diminished by increasing the ionic strength. Once protein subunits interact, however, ionic linkages might be buried and consequently protected from disruption by the surrounding salt solution. Additional experiments are required to test these speculations.

We have not attempted to calculate an association constant for the aggregation of A-gliadin. The appearance of two or more peaks in our chromatograms suggests that the association is not rapidly reversible, which would instead tend to produce a single asymmetric peak; hence, the association cannot be treated by equations applicable to equilibrium systems (Cann 1970). Because rechromatography at pH 3 of A-gliadin aggregated at pH 5 indicated virtually complete dissociation of the aggregates to monomer, however, the A-gliadin system cannot be considered to have negligible re-equilibration during chromatography. A-gliadin seems likely to be undergoing slow but significant re-equilibration

during gel filtration chromatography. We are not aware of suitable theoretical treatments of such a system, but it seems likely that more information about the kinetics of the association-dissociation reactions would be required for theoretical treatment.

Effect of Temperature on Aggregation of A-Gliadin

The degree of aggregation of A-gliadin as a function of temperature at pH 4 in 0.01 *M* NaCl, is plotted in Fig. 4 (curve B). A similar plot (curve A) shows the aggregation characteristics of A-gliadin in pH 5 solutions at the same salt concentration. At pH 4 (curve B), the proportion of aggregated A-gliadin decreased as the temperature was increased from 5 to 40°C until only the monomeric form was evident at 45°C. At pH 5 (curve A), aggregated A-gliadin was more stable than at pH 4 in the lower temperature range (up to about 25°C) but rapidly dissociated at higher temperatures until dissociation was complete at 48°C.

Figure 5 shows A-gliadin aggregation at pH 5, as in Fig. 4, but at a lower NaCl concentration (0.005 *M*). The results were similar to those found at pH 5 with 0.01 *M* salt (Fig. 4) except for an apparent deviation from a smooth curve between 25 and 30°C. Results were less reproducible at this lower ionic strength. We have not drawn curves through the points of Fig. 5 because we are not certain of the significance of the irregularity just above room temperature. A light-scattering study[3] under conditions similar to those of Fig. 4 gave generally similar results without any obvious anomaly; the apparent mol wt of A-gliadin showed a slight decrease from 10°C to about 25°C (the apparent mol wt was about 2×10^{-6} in this range), and then decreased rapidly as temperature was increased above 25°C.

Kasarda et al (1968) found evidence for unfolding of A-gliadin above 30°C. Because we also observe dissociation of A-gliadin between 25 and 50°C, it seems possible that a loss of conformational structure destabilizes the aggregated form, although it is not possible to separate the two processes in our experiments.

[3]D. D. Kasarda and S. G. Platt, unpublished results.

The temperature dependence of A-gliadin aggregation apparently indicates that hydrophobic bonds (Kauzmann 1959) do not predominate in stabilization of A-gliadin aggregates. Thermodynamic considerations suggest that an increase in temperature should favor hydrophobic interactions, but we observe dissociation of aggregates under these conditions. The nature of hydrophobic interactions is imperfectly understood, however, and the conclusion that hydrophobic interactions do not predominate in A-gliadin aggregation should not be considered as established beyond question. Our results certainly do not exclude some contributions of hydrophobic bonding to the stabilization of A-gliadin aggregates.

Secondary Forces Contributing to Stabilization of Aggregates

Our results indicate that neither ionic nor hydrophobic interactions are the main forces stabilizing the microfibrillar aggregates of A-gliadin; rather hydrogen-bonding seems to be the predominant interaction. This is in accord with gliadin's large number of glutamine residues, which serve as both donor and acceptor in hydrogen bonding. Along with hydrophobic bonds, salt linkages may also contribute to the stabilization of aggregates, but they apparently are less important than hydrogen bonding.

Interaction of A-Gliadin with S-300

A number of solutes may be adsorbed to Sephacryl at pH 4; to prevent this, ionic strengths of at least 0.05 are recommended.[4] These recommendations seem to be somewhat arbitrary, however, and we needed to perform our studies in the pH range of 3–5 and at relatively low ionic strength because of the properties of A-gliadin.

[4]Manual from Pharmacia Fine Chemicals, "Gel Filtration: Theory and Practice."

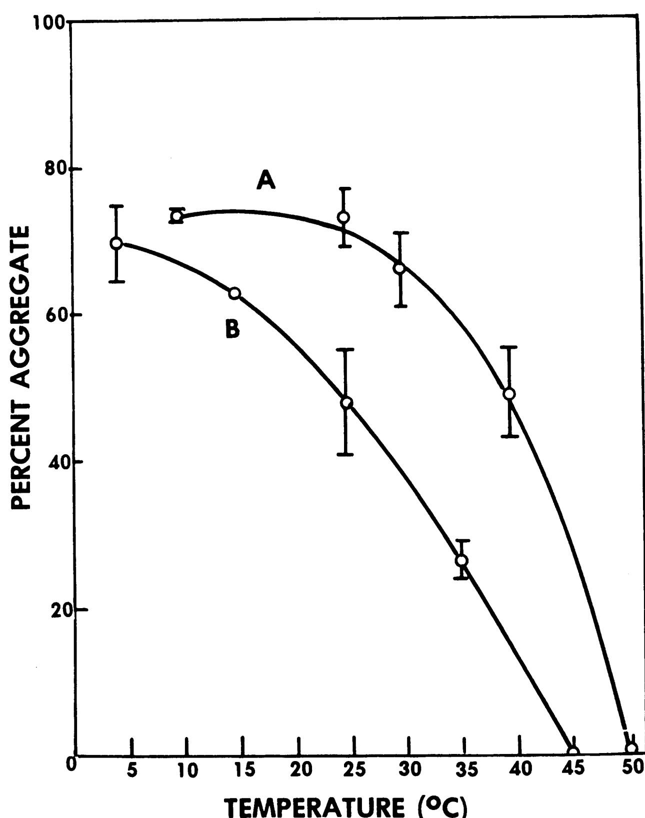

Fig. 4. Aggregation of A-gliadin in 0.01 *M* sodium chloride as a function of temperature: **A,** pH 5.0, 0.001 *M* acetic acid, 1,150 μmho/cm; **B,** pH 4.0, 0.01 *M* acetic acid, 1,600 μmho/cm (pH adjusted to 4.0 and 5.0 with ammonium hydroxide).

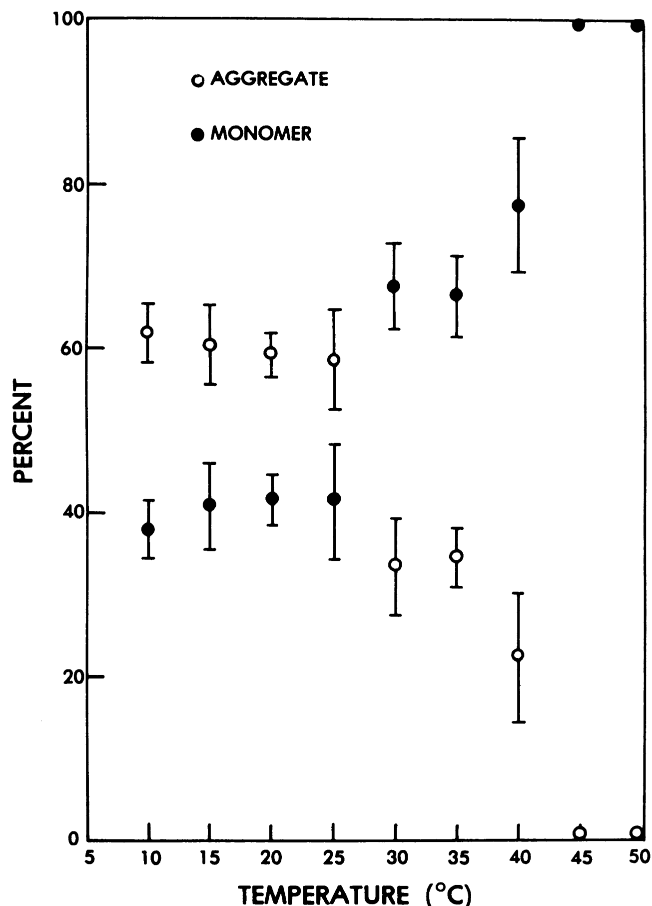

Fig. 5. Aggregation of A-gliadin in 0.001 *M* acetic acid + 0.005 *M* sodium chloride, pH 5.0, 630 μmho/cm, as function of temperature (pH was adjusted to 5.0 with ammonium hydroxide).

Our results indicate that the behavior of A-gliadin was essentially normal, despite the low ionic strength used, perhaps because of the low charge density of A-gliadin compared to most other proteins.

We did, however, encounter strong retardation of A-gliadin on three occasions. On two of these, the column previously had been used successfully for many experiments; the problem was once encountered with a newly-packed column. The change was abrupt, and no explanation was apparent for the sudden change in column characteristics.

We tried several approaches to restore normal behavior to the column, including treatment with ethylenediaminetetraacetic acid, with 0.1*M* NaOH, and with concentrated urea solutions; none was effective. We are at loss to explain the retardation. It occurred only at 0.005*M* NaCl; at 0.01*M* NaCl, normal column behavior was observed. We have included the data obtained at 0.005*M* NaCl despite these occasional problems, since dozens of experiments performed at this ionic strength indicated no abnormal behavior, ie, protein eluted as a monomer at pH 3 and as both monomer and aggregates at pH 4 and pH 5 (Figs. 3 and 5).

On the basis of our experience, we conclude that GPC of α-gliadins on Sephacryl S-300 should be performed with 0.01*M* or 0.015*M* NaCl included in the elution solvent. When it is desirable to suppress protein aggregation, 0.01*M* acetic acid plus 0.01*M* NaCl seems a good combination because these conditions should not produce major loss of conformational structure. Preliminary experiments indicated, however, that ω-gliadins may be retarded in these solvents. If retention of structure is not a consideration, GPC can be carried out in strong dissociating agents such as 2*M* sodium thiocyanate (Preston 1982), which disrupt conformational structure extensively.

Relations to Dough Properties

Aggregated A-gliadin has a number of characteristics similar to those of wheat flour doughs. Application of shear produces gelation of concentrated A-gliadin solutions (Bernardin 1975, 1978), which may be analogous to dough development through mixing. Continued shear breaks down the A-gliadin gel (Bernardin 1975), which may be analogous to dough breakdown. Increased salt concentration enhanced the A-gliadin aggregation (Fig. 2); similarly, addition of salt to doughs increases rigidity (Bennett and Ewart 1965). Increasing temperature from 25 to 50°C (Fig. 4) dissociated A-gliadin aggregates. Doughs heated in this range also show a rapid decrease in apparent viscosity (Bloksma 1980).

Our study provides evidence that hydrogen bonding is the predominant secondary force stabilizing A-gliadin aggregates. Hydrogen bonding has also been suggested as playing a major role in the formation of dough structure (Jankiewicz and Pomeranz 1965) insofar as addition of urea to a dough results in a rapid decrease in dough consistency.

Because the effects of salt concentration, temperature, and dissociating agents indicate that hydrogen bonding is the major force in A-gliadin aggregation, and because effects of these variables on doughs and on A-gliadin aggregation are similar, we suggest that hydrogen bonding is the most important secondary force contributing to dough structure and properties. Ionic and hydrophobic bonds probably also contribute to dough structure, but these are less important.

Previous studies of A-gliadin (Bernardin 1975, Bernardin and Kasarda 1973, Kasarda et al 1976) suggested that aggregation of protein subunits to fibrillar structures plays an important role in dough formation and that this aggregation involves specific interactions determined by the conformational structure of the interacting endosperm proteins. The present study supports these concepts by providing additional information about A-gliadin aggregation.

In the future, we expect to explore aggregation of other wheat storage proteins to further evaluate the relation of conformationally-determined specific interactions to dough properties. The relative importance of specific and nonspecific interactions, and of disulfide bonds to protein polymerization and to dough properties also remains to be evaluated. Even if covalent intermolecular crosslinks in proteins are major contributors to dough properties—and some question about their importance remains (Batey 1980)—specific secondary interactions may still be important in determining interactions of proteins with each other and with nonprotein components in doughs (Ewart 1977).

ACKNOWLEDGMENTS

We thank William Van B. Robertson, Stanford Children's Hospital, Stanford, CA, (presently National Science Foundation, Washington, DC) for his generous gift of most of the A-gliadin used in this study; and V. A. Johnson, USDA, Lincoln, Nebraska for supplying wheat grain. J. V. Torres was visiting scientist while holding an IRI Research Institute, Inc. fellowship.

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[Received October 18, 1982. Accepted March 15, 1983]