THE RHEOLOGY OF CONCENTRATED GLIADIN SOLUTIONS

JOHN E. BERNARDIN, Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, CA 94710

ABSTRACT

Solutions of the purified wheat protein α-gliadin in its aggregated, fibrillar form spontaneously separate into a liquid crystalline phase and a more dilute, yet birefringent solution. Shearing such a solution produces a stable gel. The shear stress *vs.* shear rate diagram of the gelation is similar to a dough development curve. Since similar fibrillar forms have been demonstrated for other wheat proteins, the response of the solution to shear, the reversibility of the stress upon varying shear rates, the minimum shear rate required for a change in shear stress as well as the temperature dependence, all suggest that similar processes may be occurring in dough development.

Quantitative rheological studies of the wheat proteins in concentrated solutions have been limited to either the entire dough complex or to a whole gluten mixture. Because dough is a time-dependent and shear-dependent system, these measurements are usually 1) limited to the period where there is little change in shear stress with continued shearing, and 2) reflect the prior treatment the sample has received.

In this study, the entire rheological history of a purified wheat protein solution subjected to shear has been recorded. Simplifying the system to a single protein has allowed the definition of the size and shape of the interacting molecules. Because almost no attention has been previously given to the conformation or state of aggregation of the proteins in a dough, there has been little understanding of the proteins in the gluten complex that are responsible for the rheological properties as well as little knowledge of the chemical and physical interactions that occur during mixing. As is demonstrated in this study, the aggregation of a protein defines a shape which, in concert with the interaction potential of the subunits in the aggregate, produces larger structures. These larger structures have rheological properties of their own and largely define the properties of the macroscopic solution. By restricting the measurements to one protein where the size of the molecule is known, the change in rheological properties of the solution with shear can be translated to a molecular scale.

Rationale for Using a Purified Gliadin as a Model for the Gliadin Complex

General Properties of the Gliadin Proteins

The gliadin proteins have been described as that fraction of gluten soluble in 70% ethanol (1). However, since the wheat gluten proteins constitute a strongly interacting mixture of similar proteins (2,3), preparative schemes based on solubility criteria do not separate gluten into distinct protein classes (4,5). Studies on isolated gliadin protein have shown that gliadin can be generally characterized as those wheat storage proteins with molecular weights ranging from 30,000 to 37,000 (6) and which migrate in the low mobility region of an aluminum lactate buffered gel upon electrophoresis (7,8).

The different gliadin proteins separated by electrophoresis represent molecules with small differences in amino acid composition and amino acid sequence. Peptide mapping (9), peptide chromatography (10), and amino acid

sequences (11), suggest several gliadin proteins may have largely identical sequences. However, the few amino acid substitutions observed for the gliadin proteins are sufficient to alter the pH and ionic strength dependence of the protein aggregation as shown by the specific aggregation of α-gliadin in contrast to the other gliadin proteins (12). Therefore, in order to define as closely as possible the protein aggregates, it is desirable to make rheological measurements on a more homogeneous protein preparation than the whole gliadin mixture.

As a group of proteins the gliadins contain approximately 20% α-helix and little or no β-structure (13). The solubility of the proteins increases with increasing temperature (14) and the unfolding of the tertiary structure of the protein in concentrated urea solutions is reversible (15). The cysteine residues in the protein are all disulfide bonded since tests for free SH are negative (15,16). Whereas viscosity studies indicate a degree of assymetry (17), there is a further increase in viscosity upon complete disruption of the tertiary structure (15). The gliadin proteins thus appear to be compactly folded globular protein molecules (18).

The Hydration Phenomena

Wetting a flour particle or a section of a wheat kernel has been shown to produce a two-phase system of gluten protein fibrils in a highly concentrated form (19). Since gluten has been shown to contain as much as 75% gliadin protein

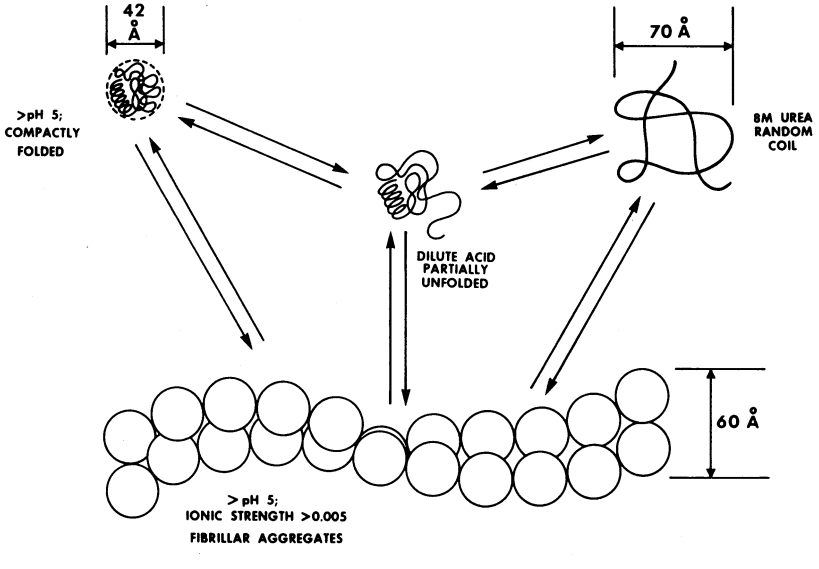

Fig. 1. A schematic representation of the size and shape of A-gliadin under varying conditions. The size of the molecule was calculated from the partial specific volume (12) and molecular weight assuming a spherical shape. The increase in volume associated with the conversion to the random coil form in 8*M* urea was calculated assuming a threefold increase in volume. The fibrillar schematic is only a possible arrangement of the A-gliadin subunits. However, the diameter of the fibrils is known from X-ray diffraction measurements (J. E. Bernardin, unpublished results).

(5), the properties of these concentrated protein fibrils must reflect largely the properties of the gliadin proteins. The protein fibrils form under solution conditions that are generally considered unsuitable for gluten or gliadin solubility (*e.g.*, 0.1 *M* NaCl) yet all of the protein in an endosperm cell is either in this hydrated form or soluble in a salt solution. The answer to this apparent contradiction lies in the solution properties of the gliadin proteins at high-protein concentration. Gliadin protein wet with aqueous solvents first forms a hydrated mass of protein which then dissolves to varying degrees depending on the ability of the solvent to dissociate the aggregate (20). The structure of the protein making up the aggregate has been shown to be protein microfibrils lying parallel to one another (21). This hydrated, liquid mass, therefore, represents a liquid crystalline form of the protein where the aggregate exposes a minimal surface to the surrounding solvent. The degree of dissolution is a reflection of the equilibrium concentrations in the two phases.

In only one case has sufficient characterized protein been isolated to allow rheological measurements. While there are at least four components (22) in the α-gliadin preparation described by Bernardin, *et al.* (12), these components all aggregate specifically to form linear fibrils under the same conditions of pH and ionic strength (23). They also exhibit the same fibrillar form and size as demonstrated for the liquid crystalline form of the gluten protein from wheat endosperm (19).

α-Gliadin (A-gliadin[1]) is isolated from a wheat flour extract as a globular protein aggregated into a fibrillar form with a particle weight of several million held together by secondary forces (12,23). This specific, reversible aggregation is utilized to purify the protein. The solution conditions which define the shape and size of the molecule are shown in Fig. 1. Solutions of A-gliadin in its fibrillar form spontaneously separate into a liquid crystalline phase and an isotropic phase at protein concentrations greater than 5% (Fig. 2). Microscopic examination of the birefringent portion of the solution showed that the lower phase was not homogeneous but was itself a biphasic solution of tactoids (a liquid crystalline mesophase) (Fig. 3) and a more dilute, yet birefringent solution. It was this two-phase solution that was used in this study.

MATERIALS AND METHODS

A-Gliadin was prepared according to Bernardin, *et al.* (12). The protein was dissolved in 0.001 *M* HCl to an initial concentration of approximately 10% protein. The solution was then filtered through a 5-μ millipore filter and transferred to dialysis tubing. Dialysis of the solution (2 to 4 ml) against 8 liters of 0.009 *M* KCl, pH 5.0 brought the protein to aggregating conditions. These operations diluted the solution and gave a final protein concentration of 5 to 6%. After dialysis the sample was viewed between crossed polarizers (see Fig. 2) and the lower phase was isolated for measurements. Protein concentrations were made by weighing a small sample of the solution into a volumetric flask and diluting with 0.001 *M* HCl. The pH change on dilution gave a homogeneous solution. The protein concentration in this diluted solution was determined

[1]Since the α-gliadin terminology is derived from the mobility of the protein upon electrophoresis, we have used A-gliadin to specify those α-gliadins which aggregate at pH 5.0, 0.005 *M* KCl and are derived from wheats having a distinctive pattern of α-gliadin (24).

spectroscopically from $E_{1\text{ cm}}^{0.1\%} = 0.58$ (22). Appropriate factors for dilution then gave the protein concentration in the concentrated solution. These concentrations are given in the figure legend.

Shear stress measurements were made on a Ferranti-Shirley cone-plate viscometer with a nominal 200 g cm spring in place of the 1200 g cm spring

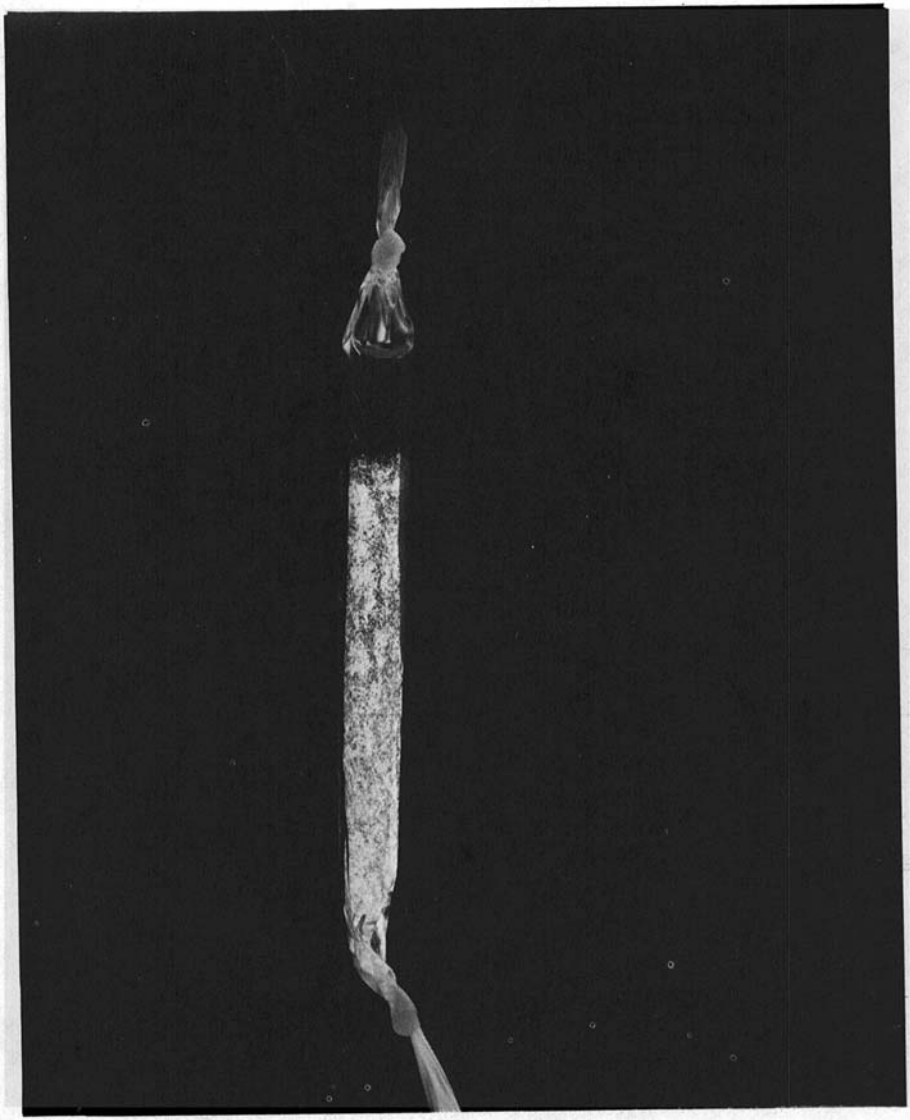

Fig. 2. A 5.7% solution of A-gliadin brought to aggregating conditions by dialysis against 0.009*M* KCl, pH 5.0, and examined between crossed polarizers. The lower phase is a birefringent solution of A-gliadin in tactoid form in equilibrium with an isotropic phase.

supplied with the instrument. The spring was calibrated as described in the instruction manual for the instrument and was determined to give a linear response to stress over the range 5.1 g cm to 173.4 g cm. Instrumental constants were as follows:

Cone angle = 0.00616939 radians
Cone diameter = 2.0 cm
Torque spring constant = 300.2 dyn/cm^2/division
Shear stress constant = 17.92 dyn/cm^2/division
Shear rate constant = 16.96 sec^{-1}/rpm
Rotary compliance of torque spring = 3.72×10^{-5} radians/dyn cm

Sufficient sample to just fill the gap between cone and plate was transferred to the plate by means of a 1-ml syringe with a large bore (2-mm) teflon needle at a sufficiently slow rate to preclude shear modification of the sample. A solvent trough with a hood lined with filter paper and saturated with water surrounded the plate. The temperature was controlled by circulating ethanol at 20° C through the cooling coil in the plate except for the one series of experiments designed to determine the effect of temperature.

RESULTS AND DISCUSSION

The effect of continuous shear at 20° C and constant shear rates of 85 and 170 sec^{-1} are shown in Fig. 4. The initial shear stress was less than the minimum

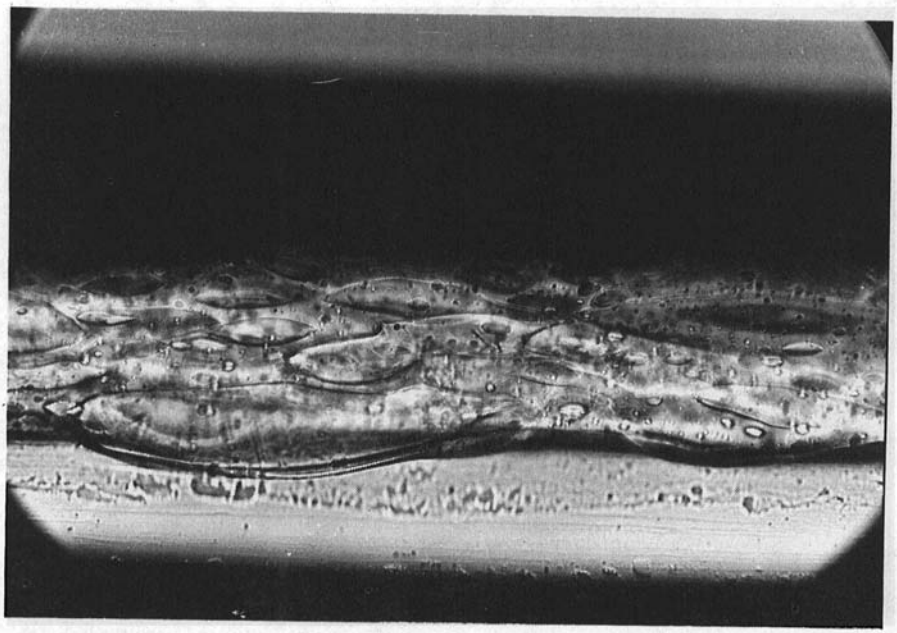

Fig. 3. The lower phase of the A-gliadin solution shown in Fig. 2 at a magnification of 288×. Tactoids and a less dense, although birefringent, solution in equilibrium.

sensitivity of the instrument and, therefore, less than 10 dyn/cm^2. However, the stress increased measurably after 7 min at both shear rates and continued with a nearly linear increase in shear stress as a function of time for several minutes. The maximum shear stress increased with increasing shear rate but only exhibited a constant period for shear rates less than 170 sec^{-1}. For greater shear rates there was a maximum in shear stress immediately followed by decreasing shear stress. The period of constant maximum shear stress decreased with increasing shear rate as shown for two different samples in Table I.

During the increase in shear stress there was a pronounced change in the sample. The solution was free flowing although viscous when applied to the plate. In a series of experiments the sample was shown to be in various stages of gelation with increasing time of shearing by stopping the experiment and examining the sample. After 16 min the sample had gelled sufficiently so that it would not flow under gravity. Development of the solution to the maximum shear stress produced a gel which was stable for at least 24 hr as indicated by its

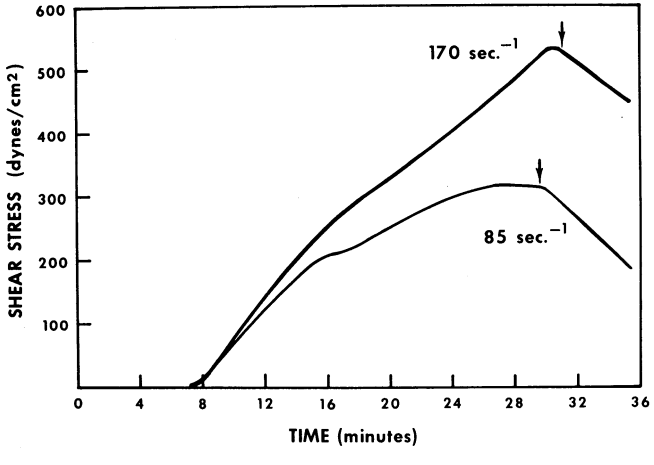

Fig. 4. The change in shear stress with time of shearing for shear rates of 85 sec^{-1} and 170 sec^{-1} at 20°C for a 5.7% solution of A-gliadin. The 85-sec^{-1} shear rate experiment exhibited constant shear stress with continued shearing for the period from 26 to 30 min whereas the 170-sec^{-1} shear rate experiment exhibited constant shear stress for less than 1 min. The arrows indicate the point of gel breakdown and the beginning of decreasing shear stress with continued shearing.

TABLE I

Shear Rate	Stability of Maximum Stress (min) Sample No. 1	No. 2
170 sec^{-1}	<1	<1
85 sec^{-1}	4	4
51 sec^{-1}	7	8

response to strain; shearing at 85 sec^{-1} was stopped after 28 min and resumed 24 hr later whereupon an identical shear stress was measured.

Shearing, if continued for a sufficiently long time, eventually resulted in a decrease in shear stress in every case where there had been a measurable increase in shear stress. Examination of the sample after a decrease in shear stress had been observed showed the gel had broken down, the protein precipitated, and free solvent had been expressed from the gel. While the solution and gel were both birefringent, neither the expressed solvent nor the precipitated protein was birefringent.

Microscopic examination of the sample demonstrated that during shear the biphasic nature of the solution was retained but the tactoids became elongated and greatly increased their surface area. Regions were observed where the tactoids had fused and had begun to develop a three-dimensional network even after short periods of shearing. The eventual effect of shear is the gelation of the solution resulting from the entrapment of solution by the network of fibrils formed from elongation of the tactoids. This is shown schematically in Fig. 5. The temperature dependence of the gelation was determined by measurements at 20°C, 35°C, and 40°C (Fig. 6). In contrast to the data shown in Fig. 4, this sample at 20°C had a measurable resistance to shear at 84.8 sec^{-1} due to a greater protein concentration. However, it required the same period of shearing at this rate to produce an appreciable increase in shear stress, *i.e.*, 7 min. Whereas increasing the temperature to 35° or 40°C decreased the initial resistance to shear, the sample required less time for gelation. A decrease in the viscosity within a tactoid but no significant alteration in the equilibrium between the tactoid and the surrounding, more dilute solution, *i.e.*, retention of the biphasic nature of the solution, would be consistent with these observations. The decrease in the internal viscosity of the tactoid would require less shearing for the elongation of the tactoids. Retention of the two-phase equilibrium would result in gelation upon collision of the elongated tactoids with subsequent entrapment of the more dilute solution. And the resistance of the gel to shear would be less than that observed at higher temperatures because of the decreased viscosity of the tactoids. This temperature dependence is the same as that observed for the resistance to shear shown by dough (25) but additional measurements are necessary to determine if the energy of the interactions are similar to those measured in a dough (26).

The effect of varying shear rate and the reversibility of the shear stress for the A-gliadin solution was determined as shown in Fig. 7. Three successive flow curves were measured on the same sample with no time between cycles. The instrument was programmed for a linear increase in shear rate to a maximum of 84.8 sec^{-1} over a 4-min period and then decreased to zero linearly in another 4-min period. The total time of the measurements shown in Fig. 7 is, therefore, 24 min or three cycles of acceleration-deceleration. As in the continuous shear experiments, there was almost no increase in shear stress for the first seven min at shear rates above 20 sec^{-1}. The first flow curve thus served to initiate a measurable increase in shear stress. While the linear increase in shear stress with shear rate shown for the second flow curve would ordinarily indicate a Newtonian fluid, the constant shear stress with decreasing shear rate clearly demonstrates the complex rheological behavior of the gliadin solution. More data are required for a detailed analysis of this behavior. However, the

requirement for a minimum shear rate to produce an increase in shear stress is evident for both the second and third flow curves. Almost no increase in shear stress is found for shear rates less than 20 sec^{-1} with increasing shear rate. Also, for decreasing shear rates, a decrease in shear stress was observed for shear rates less than 20 sec^{-1}. A similar requirement of a minimum shear rate for dough development has recently been reported (27). A change in mixing rate produced a partially reversible change in dough resistance similar to the data of Fig. 7.

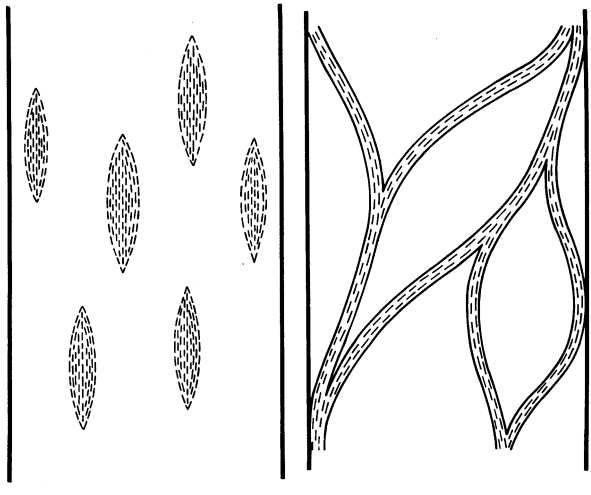

Fig. 5. A schematic representation of the change in shape of the tactoids with shearing and gel formation. Parallel lines within the tactoids represent the A-gliadin fibrils. The more dilute solution is trapped between large fibrils formed from the interacting A-gliadin fibrils.

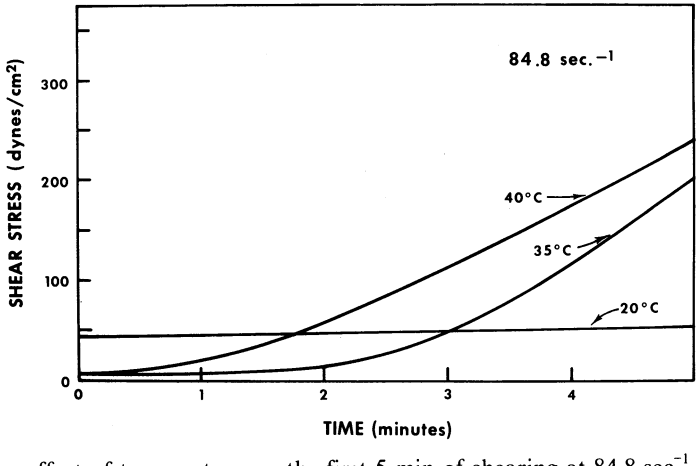

Fig. 6. The effect of temperature on the first 5 min of shearing at 84.8 sec^{-1}. Increased temperatures result in a more rapid formation of the gel. Protein concentration 6.4%.

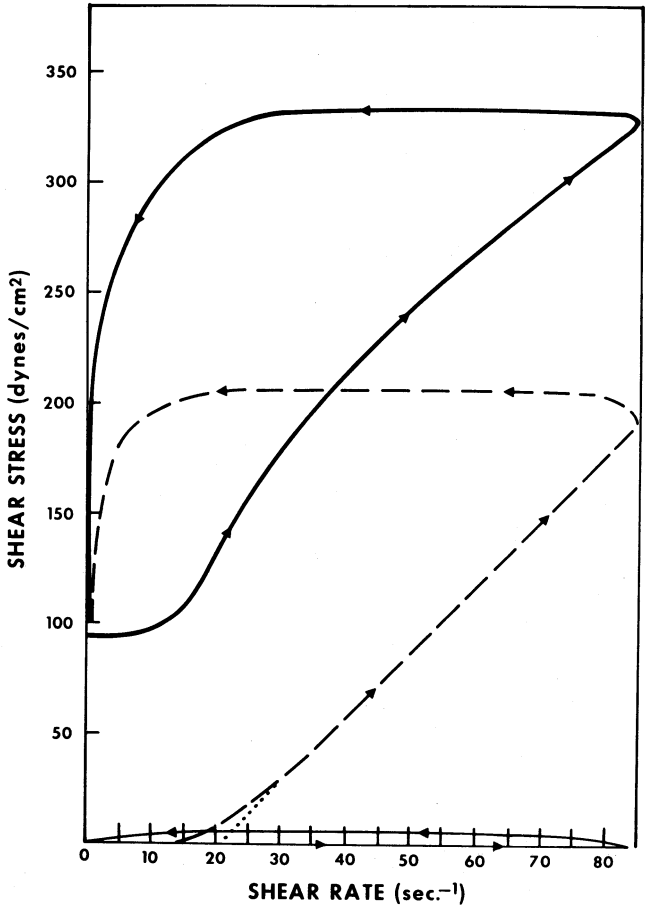

Fig. 7. Three successive flow curves for a single sample of A-gliadin. Shear rates of 20 sec^{-1} or greater are required for an increase in shear stress or to maintain the shear stress observed at the higher shearing rates. Protein concentration 5.7%.

In summary, the microfibrils, composed of globular protein molecules of A-gliadin and interacting through secondary forces develop a three-dimensional network. This network is stable if left undisturbed yet shows partial reversibility to varying shear rates. Similar microfibrils of *gluten* protein have been demonstrated in hydrated wheat endosperm (19). The microfibrils are aligned parallel to one another forming macrofibrils. The macrofibrils are visible in the light microscope (21) and appear identical to those demonstrated in dough (28). While this does not prove that dough development results from a similar buildup of a three-dimensional structure of fibrillar protein aggregates, the response to shear, the reversibility of the stress upon varying shear rates, the minimum shear rate required for a change in shear stress as well as the temperature dependence, all suggest that similar processes may be occurring in dough development.

Literature Cited

1. OSBORNE, T. B. The Proteins of the wheat kernel, Carnegie Inst., Wash. Publ. No. 84 (1907).
2. HOLME, J., and BRIGGS, D. R. Studies on the physical nature of gliadin. Cereal Chem. 36: 321 (1959).
3. HUEBNER, F. R., and WALL, J. S. Wheat glutenin subunits. Cereal Chem. 51: 228 (1974).
4. ORTH, R. A., and BUSHUK, W. Studies of glutenin, I. Comparison of preparative methods. Cereal Chem. 50: 106 (1973).
5. BIETZ, J. A., and WALL, J. S. The effect of various extractants on the subunit composition and associations of wheat glutenin. Cereal Chem. 52: 145 (1975).
6. BIETZ, J. A., and WALL, J. S. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49: 416 (1972).
7. WOYCHIK, J. H., BOUNDY, J. A., and DIMLER, R. J. Starch gel electrophoresis of wheat gluten protein with concentrated urea. Arch. Biochem. Biophys. 94: 477 (1961).
8. LAWRENCE, J. M., HERRICK, H. E., and GRANT, D. R. Analysis of wheat flour proteins by polyacrylamide gel electrophoresis. Cereal Chem. 47: 98 (1970).
9. BIETZ, J. A., and ROTHFUS, J. A. Comparison of peptides from wheat gliadin and glutenin. Cereal Chem. 47: 381 (1970).
10. BIETZ, J. A., HUEBNER, F. R., and ROTHFUS, J. A. Chromatographic comparisons of peptic digests of individual gliadin proteins. Cereal Chem. 47: 393 (1970).
11. BIETZ, J. A., and ROTHFUS, J. A. Differences in amino acid sequences of gliadin and glutenin. Cereal Chem. 48: 677 (1971).
12. BERNARDIN, J. E., KASARDA, D. D., and MECHAM, D. K. Preparation and characterization of alpha-gliadin. J. Biol. Chem. 242: 455 (1967).
13. CLUSKEY, J. E., and WU, Y. V. Optical rotatory dispersion of wheat gluten, gliadin and glutenin in acetic acid and aluminum lactate systems. Cereal Chem. 43: 119 (1966).
14. MacRITCHIE, F. Effects of temperature on dissolution and precipitation of proteins and poly amino acids. J. Colloid Interface Sci. 45: 235 (1973).
15. BECKWITH, A. C., WALL, J. S., and JORDAN, R. W. Reversible reduction and reoxidation of the disulfide bonds in wheat gliadin. Arch. Biochem. Biophys. 112: 16 (1965).
16. MURTHY, P. R., and DAHLE, L. K. Studies on a simplified dough system composed of gliadin, glutenin, and starch. Cereal Chem. 46: 463 (1969).
17. NIELSEN, H. C., BECKWITH, A. C., and WALL, J. S. Effects of disulfide bond cleavage on wheat gliadin fractions obtained by gel filtration. Cereal Chem. 45: 37 (1968).
18. WU, Y. V., and DIMLER, R. J. Conformational studies of wheat gluten, glutenin, and gliadin in urea solutions at various pH's. Arch. Biochem. Biophys. 107: 435 (1964).
19. BERNARDIN, J. E., and KASARDA, D. D. Hydrated protein fibrils from wheat endosperm. Cereal Chem. 50: 529 (1973).
20. MEREDITH, P. On the solubility of gliadinlike proteins. II. Solubility in aqueous acid media. Cereal Chem. 42: 64 (1965).
21. BERNARDIN, J. E., and KASARDA, D. D. The microstructure of wheat protein fibrils. Cereal Chem. 50: 735 (1973).
22. PLATT, S. G., and KASARDA, D. D. Separation and characterization of alpha-gliadin fractions. Acta Biochim. Biophys. 243: 407 (1971).
23. KASARDA, D. D., BERNARDIN, J. E., and THOMAS, R. S. Reversible aggregation of alpha-gliadin to fibrils. Science 155: 203 (1967).
24. PLATT, S. G., KASARDA, D. D., and QUALSET, C. O. Varietal relationships of the alpha-gliadin proteins in wheat. J. Sci. Food Agr. 25: 1555 (1974).
25. BAYFIELD, E. G., and STONE, C. D. Effects of absorption and temperature upon flour-water farinograms. Cereal Chem. 37: 233 (1960).
26. BLOKSMA, A. H., and NIEMAN, W. The effect of temperature on some rheological properties of wheat flour doughs. J. Texture Studies (in press).
27. TIPPLES, K. H., and KILBORN, R. H. "Unmixing" – The disorientation of developed bread doughs by slow speed mixing. Cereal Sci. Today 19: 386 (Abstr. 48) (1974).
28. MOSS, R. A study of the microstructure of bread doughs, CSIRO Food Res. Quarterly 32: 50 (1972).