Studies on a Simplified Dough System Composed of Gliadin, Glutenin, and Starch

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ABSTRACT

A synthetic dough made from wheat starch, glutenin, and gliadin gave a strong curve on the farinograph. This dough system was weakened by the addition of N-ethylmaleimide (NEMI) or dithiothreitol. When gliadin was treated separately with these reagents prior to combination with the other two components of the dough system, much weaker curves were obtained. Addition of water-soluble proteins of flour did not weaken the synthetic dough system. When the water-soluble proteins were pretreated with NEMI or dithiothreitol, no alteration in the mixing curve was observed.

The role of wheat flour proteins in the formation of a dough has received much attention from investigators. Much of this work so far has been on natural flour-dough systems, often with emphasis on the role of the SH groups of flour proteins in rheological mechanisms. One hypothesis is that interchange reactions between SH groups and SS bonds of flour proteins serve to relieve the stresses occurring in dough as it is mixed (1,2). An agent that blocks SH groups could upset this mechanism by preventing interchange between SH and SS groups (3).

The sensitive reaction of dough to SH agents was shown by early work of Sullivan and co-workers (4,5), who demonstrated that glutathione was responsible

for the dough-weakening effect of wheat germ. Molecular thiols of various types, such as dithiothreitol and reduced thioctic acid, have been observed to weaken a dough mixed in the farinograph (6). Recent work by Mauritzen (7) and Kuninori and Sullivan (8) provided evidence for the incorporation of thiols into flour proteins, presumably during the sulfhydryl-disulfide interchange. Since a natural flour dough is a very complex system of many components, the authors decided to study a simpler system of fewer components which, experimentally, could be manipulated and would give results that could be interpreted more clearly. A mixture of glutenin, gliadin, and wheat starch was found to give a mixing curve on the Brabender farinograph which was suitable for experimental study.

MATERIALS AND METHODS

Gliadin

Gliadin was prepared from commercial vital wheat gluten (hard wheat flour) by extraction with 35% isopropyl alcohol according to the method of McDonald (9). The preparation was not decolorized with activated carbon. The gliadin had a protein content of 92.2% (N X 5.7, Kjeldahl method).

Glutenin

After extraction of gliadin, the gluten residue was extracted four times with 35% isopropyl alcohol to remove any residual gliadin, and the extracts were discarded. The insoluble material, consisting mostly of glutenin, was washed several times with distilled water and freeze-dried (protein, N X 5.7, 71.3%).

Wheat starch was obtained from General Mills, Inc., Minneapolis, Minn. Reduced glutathione (A grade) and dithiothreitol (A grade) were purchased from Calbiochem, Los Angeles, Calif. NEMI was purchased from Sigma Chemical Co., St. Louis, Mo., and potassium iodate (analytical grade) from Mallinckrodt Chemical Works.

Water-Soluble Proteins of Flour

Two hundred grams of unbromated and unbleached patent wheat flour (hard spring-hard winter blend) was extracted with 800 ml. distilled water for 1 hr. The extract was freeze-dried.

Two 5-g. quantities of the water-soluble material were dissolved in 50 ml. distilled water and treated with 25 μmol. NEMI and 25 μmol. dithiothreitol, respectively. The amount of NEMI added was in excess of the amount required to alkylate the SH groups of the proteins in 5 g. of the material. After reaction for 30 min., the solutions were dialyzed exhaustively against distilled water to remove excess reagents and then freeze-dried. The dialyzed and freeze-dried materials had a protein content of 45.0% (N X 5.7).

Chemical Treatment of Gliadin

Four 10-g. quantities of gliadin were dissolved in 150 ml. distilled water and treated with 50 μmol. NEMI, 100 μmol. dithiothreitol, 100 μmol. reduced glutathione (GSH), and 50 μmol. potassium iodate, respectively. After standing for 30 min. at room temperature, the solutions were dialyzed exhaustively against distilled water to remove the excess reagents and freeze-dried. As a control, 10 g. gliadin was dissolved in 150 ml. distilled water, dialyzed, and freeze-dried. The protein content of the control and treated gliadin samples was 92.7% (N X 5.7).

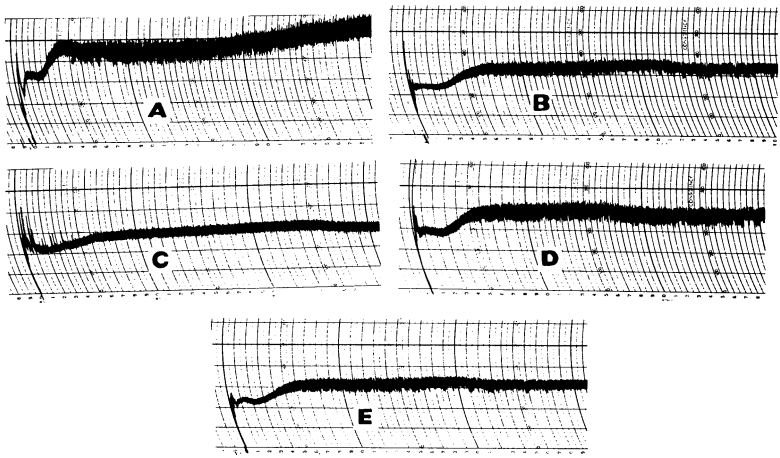

Fig. 1. Synthetic dough systems composed of glutenin, gliadin, and starch. A, control gliadin; B, NEMI-treated gliadin; C, diethiothreitol-treated gliadin; D, GSH-treated gliadin; E, potassium iodate-treated gliadin.

A synthetic dough system, consisting of 11.0 g. glutenin, 4.0 g. gliadin (control or chemically treated), 32.0 g. wheat starch, and 33.0 ml. water, was studied in the farinograph (50-g. bowl, 62 r.p.m.). The protein content of this synthetic flour, consisting of glutenin, gliadin, and starch, was 24.53% by calculation. All the ingredients were weighed separately into the bowl and mixed for 5 min. before the water was added. The farinograms are shown in Fig. 1.

The effects of adding NEMI and dithiothreitol to the synthetic flour system containing control gliadin are illustrated in Fig. 2. In the former, 33.0 ml. of a NEMI solution (100 μmol. in 100 ml. water) was added directly to the system and, in the latter, 28.0 ml. water and 5.0 ml. of a solution of dithiothreitol containing 40 μmol.

The effect of adding water-soluble material of flour to the synthetic flour was studied by mixing 11.0 g. glutenin, 4.0 g. gliadin (control), 31.0 g. starch, 1.0 g. water-soluble material (control or chemically treated), and 33.0 ml. water in the farinograph. The farinograms are shown in Fig. 3.

SH Content of Gliadin

Only the control gliadin and NEMI-treated gliadin were assayed for SH content by amperometric titration with ethylmercuric chloride (Aldrich Chemical); the method of Tsen and Anderson (10) was used, with suitable modifications. A Radiometer Polariter, Type PO4d, was used. Thirty milligrams of gliadin was stirred in 2 ml. absolute ethanol in the polarograph vessel until the large lumps were broken up. To this, 20 ml. buffer pH 9.20 was added (0.2M potassium chloride, 0.1M ammonium chloride, 0.1M ammonium hydroxide, 0.001M EDTA, and 8.0M urea). No foam-reducing agent was added. The vessel was flushed for at least 30 min. with nitrogen, and the gliadin solution was titrated with 2×10^{-3}M ethylmercuric chloride at –0.65 volts against the saturated calomel electrode. The ethylmercuric chloride solution was added from a 0.20-ml. microburet graduated in microliter units.

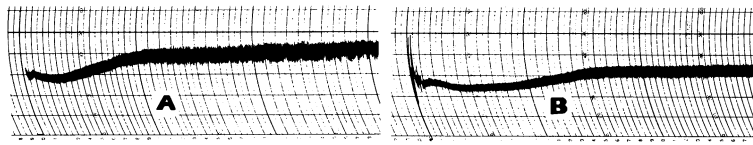

Fig. 2. Synthetic dough system mixed in the presence of A, 33 μmol. NEMI; B, 40 μmol. dithiothreitol. Control curve shown in Fig. 1, A.

The control gliadin and NEMI-treated gliadin had SH contents of 7.53 and 1.27 μmol. per g., respectively.

RESULTS AND DISCUSSION

Commercial vital wheat gluten was separated roughly into two major fractions, glutenin and gliadin. The protein soluble in 35% isopropyl alcohol was considered to consist largely of gliadin; the insoluble residue was termed glutenin. No attempt was made to determine the homogeneity of the gliadin fraction or to purify it further. The dough system, consisting of glutenin, gliadin, wheat starch, and water, produced a strong curve on the farinograph (Fig. 1, A). The curve was weakened greatly by addition of the SH-blocking agent, NEMI, or the SS-cleaving agent, dithiothreitol (Fig. 2). When gliadin was reacted separately with these reagents prior to its combination with the other ingredients of the system, much weaker curves were obtained (Fig. 1, B and C). Similarly, pretreatment with GSH or iodate gave weak curves (Fig. 1, D and E). These results indicate that gliadin is sensitive to reagents which block or oxidize SH groups or reduce SS bonds. Presumably, dithiothreitol and GSH reduced the intramolecular SS bonds of the gliadin to SH groups, thereby altering its tertiary structure. NEMI blocked 83% of the SH groups in the gliadin fraction, and this resulted in loss of its viability. This change in viability suggests that one or more of the SH groups of the gliadin fraction were involved in dough formation in this synthetic flour system. These SH groups appear to be oxidized by iodate.

The effect of adding water-soluble proteins of flour is seen in Fig. 3, A. There was no weakening of the curve; on the other hand, the consistency of the dough slightly increased. There was no change in the effect produced by the water-soluble proteins in this system when they were reacted separately with NEMI or

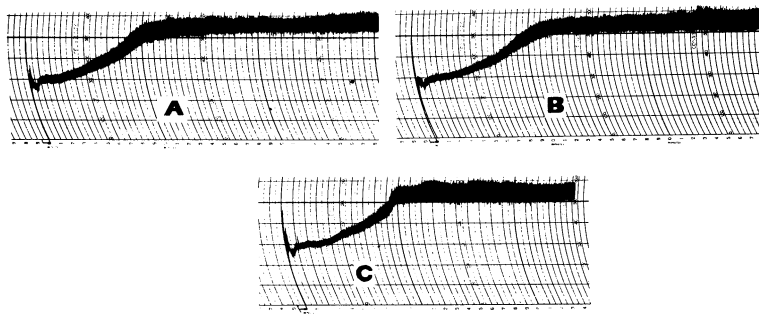

Fig. 3. Synthetic dough system composed of glutenin, gliadin, water-solubles of flour, and starch. A, control water solubles; B, NEMI-treated water solubles; C, dithiothreitol-treated water solubles.

dithiothreitol prior to their addition to the synthetic flour (Fig. 3, B and C). At the level employed, no alteration in mixing behavior was induced when they were reacted with the reagents which caused a change when reacted with gliadin.

The insoluble glutenin fraction was not treated with NEMI or dithiothreitol, since it was believed that any solvent capable of dispersing glutenin might cause appreciable denaturation, giving results which would be difficult to interpret.

One of the interesting findings of this study was the sensitivity of gliadin to attack by either a SH-blocking agent or a thiol. Gliadin in natural flour is likely to be vulnerable in a similar way to these and other reagents. However, one cannot rule out that the action of SH-blocking agents or thiols on proteins of natural flour other than gliadin produces changes in the rheological properties of dough.

A recent study by Nielsen et al. (11) showed that the SS bonds of gliadin are mostly intramolecular; cleavage of these bonds by mercaptoethanol resulted in physical changes which correspond to the unfolding of the molecule. Assuming that a similar unfolding occurs in gliadin on treatment with a thiol, it is evident that the physical conformation of gliadin is a critical factor of mixing stability. Its ability to associate or complex with other proteins (glutenin in this study) might be altered. One can only speculate on the actual mechanism of the rheological change induced by chemical modification of gliadin.

Smith and Mullen (12) reported that the albumin and globulin fractions of flour proteins, although containing 55% of the total sulfhydryl of flour, had only minor effects on the dough-mixing properties of flour. Recently Dahle and Pinke (13) demonstrated disulfide-reducing activity of yeast enzymes. In their study, salt-soluble proteins of flour were added to an intact flour system; no dough-weakening effects were observed unless molecular thiol also was present. It is possible that the steric requirements of protein thiol groups limit their reactivity in comparison to that of molecular thiols. Redman and Ewart (14) made some studies on the extent of the interchange between cystamine and cereal flour proteins in an effort to shed light on the extent of possible protein-protein disulfide interchange.

The study reported in this paper involved a comparatively simple dough system consisting of two major protein fractions of flour, glutenin and gliadin. It must be kept in mind that a natural flour dough system is more complex and contains many more variables. While it cannot be concluded from this study that the entire rheological effects produced by adding SH-blocking (or oxidizing) and SS-reducing agents to natural flour doughs are attributable to gliadin exclusively, the work does provide a method for studying independently the effects of SH reagents on various fractions of flour proteins in relation to changes in rheological properties.

Acknowledgment

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