Functional (Breadmaking) and Biochemical Properties of Wheat Flour Components. VI. Gliadin-Lipid-Glutenin Interaction in Wheat Gluten[1]

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ABSTRACT

Free polar lipids (principally glycolipids) are bound to the gliadin protein by hydrophilic bonds and to the glutenin protein by hydrophobic bonds. In unfractionated gluten, the lipid apparently is bound to both protein groups at the same time. The simultaneous binding of polar lipids to gliadin and glutenin may contribute structurally to gas-retaining complexes in gluten in a manner not previously proposed.

Wheat flour contains about 0.8% free lipids. The free lipids are extractable in petroleum ether and include unbound lipids as well as those held by hydrophobic bonds. The 0.8% of free lipids consist of 0.6% of nonpolar (mainly triglycerides) and 0.2% of polar (glycolipids and phospholipids) components. In addition, wheat flour contains 0.6% bound lipids (nonextractable in petroleum ether but extractable in water-saturated butanol). The bound lipids are essentially polar and soluble in petroleum ether after being removed from the protein by water-saturated butanol. When flour is wetted and mixed into dough, the 0.2% of free polar and about half of the 0.6% of nonpolar lipids become bound (no longer extractable in petroleum ether) and are presumed to form lipoprotein complexes with the glutenin fraction of gluten (1). Grosskreutz (2), on the basis of X-ray diffraction patterns, proposed that gluten structure consisted of protein platelets and bimolecular leaflets of phospholipids combined as lipoproteins. Ponte et al. (3) reported that lipids were bound to the gliadin fraction, but attributed the binding to a translocation of the lipid by the 70% ethanol used to fractionate the gluten.

The unique suitability of wheat flour for yeast-leavened products is attributable to the ability of gluten proteins to retain the carbon dioxide produced during fermentation. That ability is impaired when the free lipids are removed, but is restored completely when the polar fraction (especially the glycolipids) is returned to the flour (4).

Our results, reported here, show that the free polar lipids (principally monogalactosyl diglyceride and digalactosyl diglyceride) can be bound to the gliadin fraction of gluten by hydrophilic bonds, and to the glutenin proteins by hydrophobic bonds. In unfractionated gluten, those lipids apparently are bound to both protein groups at the same time. The gas-retaining complex can be pictured as gliadin- and glutenin-protein units that are bound together by polar lipids. That model probably is similar to the one recently proposed by McClare for the cell membrane in *Halobacterium halobium* (5).

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MATERIALS AND METHODS

The flour used was a composite (RBS-67) that contained 13.1% protein and 1.40% total extractable lipids. Analytical data are reported on 14% moisture basis. Gluten was washed from the flour as described previously (6). Gluten was solubilized by cutting it in small pieces and stirring in 0.005N lactic acid for 5 hr. The suspension was centrifuged at 1,000 X g for 20 min. to remove trapped starch. Approximately 8% of the total protein is removed by that centrifugation.

Gliadin and glutenin fractions (approximately equal quantities) were separated from the acid-soluble gluten by making the solution 70% ethanol, and then neutralizing with 0.1N Na_2CO_3. After the solution had stood at 4°C. overnight, the glutenin precipitate was separated by centrifugation. Ethanol was removed from the gliadins by vacuum distillation at 25°C. The two fractions were frozen and lyophilized.

A second glutenin fraction (100-5C) was separated by centrifuging the acid-soluble gluten at 100,000 X g for 5 hr. in a preparatory ultracentrifuge. The centrifugate (100-5C glutenin protein) was neutralized with 0.1N Na_2CO_3, frozen, and lyophilized.

Petroleum ether-extractable lipids were determined by exhaustive extraction in a Goldfisch extractor with petroleum ether (b.p. 35° to 60°C.). Petroleum ether in the flour was allowed to evaporate at room temperature, and the flour was re-extracted with water-saturated butanol as described previously (7). The butanol extract (bound lipids) was filtered, evaporated under reduced pressure, and redissolved in petroleum ether.

Thin-layer chromatography was performed on 100 γ of lipids. One half plate was developed with chloroform to determine nonpolar lipids; the other half was developed with chloroform-methanol-water (65:35:4) to determine polar lipids. Plates were sprayed with a saturated solution of $K_2Cr_2O_7$ in 70% (v./v.) of aqueous sulfuric acid, and charred at 150°C. for 30 min. The plates were photographed under ultraviolet light.

Starch-gel electrophoresis was performed in a vertical position with pH 3.2 aluminum lactate and lactic acid buffer containing 3.0M urea. Electrophoresis was performed at 30 ma. and approximately 325 v for 6 hr. at room temperature. The gel was sliced and stained with 0.1% amido black 10B. Details of the procedure have been given previously (8).

TABLE I. LIPID CONTENTS OF GLUTEN AND CERTAIN OF ITS FRACTIONS

Sample	Yield %	Pet. Ether-Extractable %	Bound Lipid %	Total Lipids %
Flour (RBS-67)		0.80	0.60	1.40
Gluten	100	0.56[a]	5.84	6.40
Gliadin	53	4.29	3.90[b]	8.19
Glutenin	47	1.40	2.97	4.37
100-5C	15	13.47	6.67	20.14
100-5C (Def)	15	0.81	3.95	4.76

[a]Nonpolar.

[b]Polar.

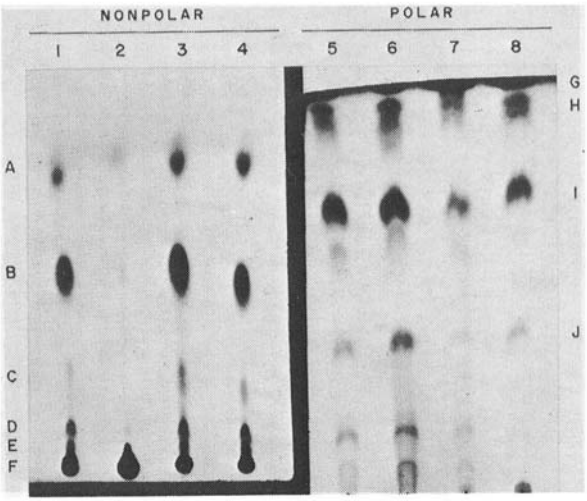

Fig. 1. Thin-layer chromatograms of lipids from gluten fractions. Patterns 1 and 5 represent bound lipids that were extracted from gluten. Patterns 2 and 6 represent bound lipids that were extracted from gliadin (soluble in 70% ethanol). Patterns 3 and 7 represent bound lipids that were extracted from glutenin (insoluble in 70% ethanol). Patterns 4 and 8 represent lipids that were extracted with petroleum ether from the 100-5C glutenins. Spots are tentatively identified as follows: A, hydrocarbons and steryl esters; B, triglycerides; C, free fatty acids; D, diglycerides; E, monoglycerides; F, unresolved polar lipids; G, unresolved nonpolar lipids; H, monogalactosyl diglycerides; I, digalactosyl diglycerides; and J, phosphatidyl choline.

RESULTS AND DISCUSSION

Gluten washed from flour contained 6.40% total extractable lipids, only 0.56% of which was extractable in petroleum ether (Table I). During gluten fractionation experiments (9), the glutenin proteins that sedimented at 100,000 × g (100-5C) contained 20.14% lipids. A glutenin fraction (100-5C defatted) that was obtained under comparable conditions from petroleum ether-defatted flour contained only 4.76% total lipids. Thus, the flour lipids that were originally free (extractable in petroleum ether) became bound to the glutenin proteins during gluten formation. These data confirm the conclusions of Alcott and Mecham (1). Since those lipids were sedimented with the glutenin proteins in an aqueous system, they were assumed to be held by the protein. Free lipids migrated upward and formed a layer at the top of the centrifuge tube. The 100-5C glutenins contained 13.47% lipids that were extractable with petroleum ether. Those lipids were identified to be both nonpolar and polar by thin-layer chromatography (Fig. 1). Since both the polar and nonpolar lipids were extractable with petroleum ether, nonpolar parts of both apparently were associated with the glutenin and, therefore, the bonds presumably were hydrophobic.

Gliadin and glutenin fractions that were prepared from 70% ethanol are characterized electrophoretically in Fig. 2. The gliadin fraction contained 3.90% bound lipids (extractable in water-saturated butanol) and 4.29% lipids that were extractable in petroleum ether (Table I). The glutenin fraction contained 2.97%

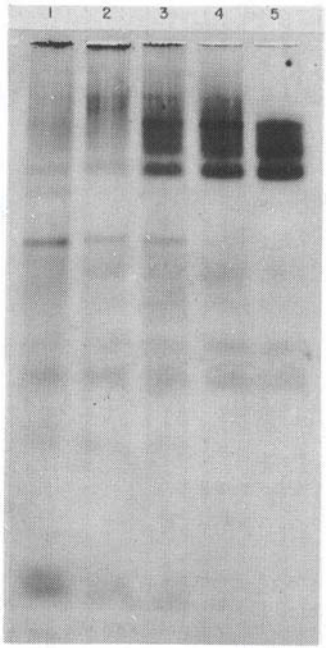

Fig. 2. Starch-gel electrophoretic patterns of gluten protein fractions. Pattern 1, 100-5C glutenins; 2, glutenins insoluble in 70% ethanol; 3, total gluten; 4, gliadin-rich fraction extracted from flour with 0.005N lactic acid; and 5, gliadins soluble in 70% ethanol.

bound lipids and 1.40% lipids that were extractable in petroleum ether. The lipids that were bound to glutenins were mainly nonpolar triglycerides (Fig. 1). The lipids that were bound to gliadins were practically all polar and mainly glycolipids (monogalactosyl and digalactosyl diglycerides). Polar lipids that were bound in the gliadin fraction were similar in composition to those that were extracted with petroleum ether from the 100-5C glutenin fraction.

Gliadins (soluble in 70% ethanol) were fractionated from gluten that was washed from petroleum ether-defatted flour. Those gliadins contained essentially no extractable lipids. When those gliadins were reconstituted with starch plus water-solubles and total free lipids (petroleum ether extract of flour) and mixed into a dough, nearly all the polar lipids became bound (no longer extractable in petroleum ether). Thus, gliadin proteins preferentially bound the polar lipids. Since that binding occurred in the absence of alcohol, the suggested translocation of lipid by 70% ethanol during gluten fractionation (3) does not appear to be relevant. Since nonpolar lipids did not participate in the binding to gliadin proteins (pattern 2, Fig. 1), the bonds presumably involved the hydrophilic parts of the polar lipids.

In unfractionated gluten all polar lipids were bound, because none were extracted with petroleum ether. Since the polar lipids were petroleum ether-extractable from the glutenin proteins, presumably they were bound to the gliadin proteins in unfractionated gluten. Also the polar lipids presumably were held to the glutenin proteins, since they sedimented with the glutenins under

ultracentrifugation. It is visualized that the bonds (probably hydrogen) between the polar lipids and gliadins were sufficiently weakened by the acid so that they were broken during ultracentrifugation.

The results suggest that the glycolipids were bound to both the gliadin and glutenin fractions of wheat gluten. Binding with the gliadin fraction involved the hydrophilic part of the glycolipid, and binding with the glutenin fraction involved the hydrophobic part of the glycolipid. The lipid apparently was simultaneously bound to both protein groups of unfractionated gluten.

The occurrence of a gliadin-glycolipid-glutenin complex would explain, at least in part, the importance of hydrophobic and hydrogen bonding in gluten structure. The proposed complex also would explain the relatively large effect of small amounts of glycolipids on the breadmaking performance of wheat flours (4). The proposed complex is of special significance in view of the high sensitivity to heat of hydrogen bonds (10) and increased stability of hydrophobic bonds at elevated temperatures (11). Since the nature of gliadin proteins governs loaf volume potential (9), the strength of their hydrogen bonding with glycolipids at oven temperatures would seem to control the stability of the lipid-protein complex, and to be of critical importance.

Numerous studies have shown the ubiquitous occurrence of glycolipids in animal, plant, and microbial cells, and have suggested that they have a fundamental role in the living cell. The binding of two types of proteins by polar lipids in such widely different systems as halophilic bacteria and wheat gluten suggests that the binding model may be of general occurrence.

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