A Note on the Interaction between Glycolipids and Wheat Flour Macromolecules[1]

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Recent studies, based on differences in solubility of gluten proteins, have shown that free polar wheat flour lipids (principally glycolipids) are bound to glutenin protein by hydrophobic, and to gliadin protein by hydrophilic, bonds (1). The results indicated that, in unfractionated gluten, the lipid apparently is bound to both protein groups at the same time.

Use of infrared spectroscopy in analyzing structures of proteins in dried membranes was introduced by Maddy and Malcolm (2). It is one of the few

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[1]Co-operative investigations between Department of Grain Science and Industry, Kansas State University, and Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture. Contribution No. 679, Kansas Agricultural Experiment Station, Manhattan.

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techniques which yield information on protein conformation and which are applicable to studies of intact membranes, albeit in the form of a dry film. Although several investigators (3,4,5) used the procedure of Maddy and Malcolm (2), deductions regarding viable biological membranes from studies of dried membrane films are open to criticism (6). Chapman et al. (5) investigated additional features of membrane infrared spectra, including those pertaining to the organization of the lipid chain. Chapman et al. (7) were the first to report use of nuclear magnetic resonance (NMR) spectroscopy in studies of membranes.

The purpose of this study was to investigate interactions between glycolipids and wheat flour starch and proteins by the recently published techniques.

METHODS

Wheat starch, gliadin, and glutenin were prepared according to the procedures described by Hoseney et al. (8,9,10).

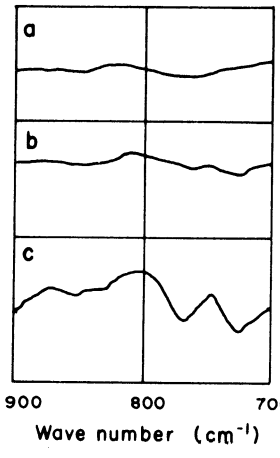
Wheat flour glycolipids were prepared by the method of Daftary et al. (11), and synthetic galactosyldioctanoylglycerol, as described by Wehrli and Pomeranz (12).

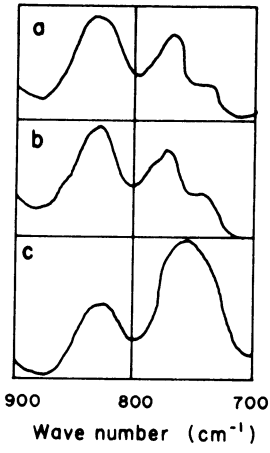
For infrared spectroscopy, protein-glycolipid complexes were prepared by thoroughly mixing, in a small test tube with a glass rod for 5 min., 25 mg. gliadin or glutenin, 5 mg. wheat-flour glycolipids, and 2 drops of water. Then, 1 ml. of water and 5 mg. of lactic acid were added. The suspension was stirred for 5 min., pipetted to an Irtran-2 disc (1 × 2 cm.), and dried overnight in a vacuum desiccator. Irtran, used as film carrier, is impermeable to infrared below 700 $cm.^{-1}$, but the band at 720 to 750 $cm.^{-1}$ is not impeded (Fig. 1). The resulting film was examined by infrared spectroscopy with a Perkin-Elmer 457 Grating Infrared spectrophotometer in a room maintained at 21°C. The smallest possible slit width was used; positions of peaks were calibrated against a standard polystyrene film. To extract "free lipids," the disc with the film was carefully shaken for 1 hr. in a beaker with petroleum ether and dried in vacuo. "Bound lipids" were extracted similarly with water-saturated butanol, followed by water. To detect a significant peak for C-C-C-C rocking at 720 to 750 $cm.^{-1}$, at least 0.3 mg. galactolipid was required (Fig. 1).

Raw starch was opaque to infrared. A complex of gelatinized wheat starch and glycolipid was prepared as the protein-glycolipid complexes, without addition of lactic acid. Drying for several days at 2 mm. Hg did not remove all water from the complex. Traces of water in the gelatinized starch did not interfere with interpretation of infrared spectra.

NMR spectra were measured at 30°C. in a 60 MC (Model A-60, Varian Associates, California) spectrometer in 99% D_2O. Deuterium oxide does not impair gluten proteins, although it increases elasticity and strength of gluten (13). Forty milligrams starch and 10 mg. synthetic galactosyldioctanoylglycerol were suspended in 1 ml. 99% D_2O, heated to boiling for 2 min., and examined in the NMR spectrometer. Then, the sample was diluted to 2 ml. and another spectrum was made. After addition of another 40 mg. of starch and heating for 2 min., a third spectrum—identical with the second—was obtained.

Ten milligrams galactosyldioctanoylglycerol was suspended in 1 ml. hot D_2O. After cooling, 40 mg. of wheat starch was added, the suspension was shaken for 10 min., and a spectrum was made. Another 40 mg. of starch was added for a second spectrum. Since the starch rapidly settles to the bottom of the tube, the relevant





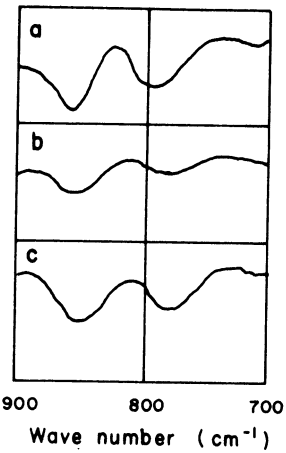


Fig. 1 (left). Infrared spectra in the 900- to 700-cm.$^{-1}$ wave-number range: a, Irtran disc alone; b, with 0.3 mg. wheat flour glycolipids; c, with 3.0 mg. wheat flour glycolipids.

Fig. 2 (center). Infrared spectra in the 900- to 700-cm.$^{-1}$ wave-number range: a, gelatinized starch and wheat flour glycolipids; b, as in a after extraction with petroleum ether; c, as in b after extraction with water-saturated butanol.

Fig. 3 (right). Infrared spectra in the 900- to 700-cm.$^{-1}$ wave-number range: a, glutenin and wheat flour glycolipids; b, as in a after extraction with petroleum ether; c, as in b after extraction with water-saturated butanol.

part of the spectrum (8 to 9 τ) must be measured within a minute after shaking. The two spectra were identical.

Thirty milligrams glutenin and 10 mg. galactosyldioctanoylglycerol were mixed to a dough with 2 drops of D_2O. The dough was suspended in 1 ml. D_2O containing 5 mg. acetic acid. After a first spectrum was obtained, the suspension was evaporated in vacuo, another 30 mg. of glutenin was added, and a second spectrum was made as before. The area under the peak at 8.7 τ in the latter spectrum was only half as high as in the former one.

Spectra of gliadin-glycolipid mixtures were made as described for glutenin-glycolipid complexes. The two spectra were identical.

For graphical presentation and calculation of peak areas, the average absorption, after subtraction of background noise, was drawn.

RESULTS

Infrared spectra of wheat flour glycolipids complexed with gelatinized starch, gliadin, and glutenin are shown in Figs. 2 to 4. Although the authors recognize the shortcomings of the infrared technique in investigations of viable biological systems, the results are deemed valid in studying complexes of lipids with wheat flour components, provided the limitations of the method are recognized.

Infrared spectroscopy reveals the presence of beta-protein in the film from the position of the amide-I and amide-II bands. In the beta-state, those bands are observed at about 1,630 and 1,520 cm.$^{-1}$, as contrasted to 1,650 and 1,540 cm.$^{-1}$ given by alpha-helix or random coil (14). The absorption band that occurs at 720

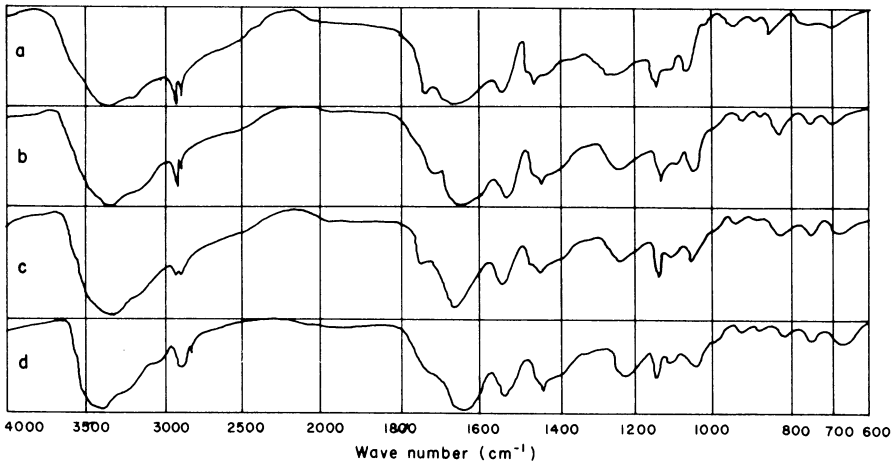


Fig. 4. Infrared spectra in the 600- to 4,000-cm.$^{-1}$ wave-number range: a, gliadin and wheat flour glycolipids; b, as in a after extraction with petroleum ether; c, as in b after extraction with water-saturated butanol; d, gliadin.

cm.$^{-1}$ (13.9 μ) has been assigned to the main methylene (CH_2) rocking mode. In the presence of starch, the band is shifted to 745 cm.$^{-1}$ The band occurs in spectra of many long-chain molecules and is present in both liquid and crystalline phases. Appearance of the absorption band requires at least four uninterrupted methylene groups organized in an all-trans planar conformation (15,16). It should be noted that in dough, hydrogen bonds and hydrophobic bonds may occur. In the absence of free water, as in a dried film, hydrophobic bonds are excluded, by definition, and Van der Waals association of lipid hydrocarbon chains with nonpolar amino acid residues is postulated. Such attraction is generally considered not significant in the presence of stronger bonds (17).

Extraction of the gelatinized starch-glycolipid complex with petroleum ether did not reduce the C–H band at 2,920 cm.$^{-1}$ or the ester band at 1,720 cm.$^{-1}$ Part of the methylene groups were in the all-trans conformation as indicated by the presence of the 720 cm.$^{-1}$ band. Extraction of the starch-glycolipid complex with water-saturated butanol was accompanied by reduced intensity of the bands at 2,920 and 1,720 cm.$^{-1}$ and disappearance of the peak at 745 cm.$^{-1}$ The findings point to a gelatinized starch-glycolipid complex in which the polar sugar moiety of the lipid is bound to the starch by hydrogen bonds (indicated by lipid not extracted with petroleum ether), whereas the nonpolar lipid side chains form a layer in which the chains exist largely in the extended trans-conformation (indicated by the presence of the 745 cm.$^{-1}$ band).

Extraction of the glutenin-glycolipid complex with petroleum ether had no effect on the spectrum at 1,720 and 2,920 cm.$^{-1}$, indicating that the lipids were not bound by Van der Waals bonds alone. Even water-saturated butanol did not extract all the lipids. There was still a distinct band at 1,720 cm.$^{-1}$ from fatty acid ester bonds. The absence of any band at 720 cm.$^{-1}$ suggests a nonpolar interaction between glutenin and glycolipid. Consequently, it appears that under the conditions of the experiment the glycolipids are bound to glutenin by both Van der Waals and hydrogen bonds.

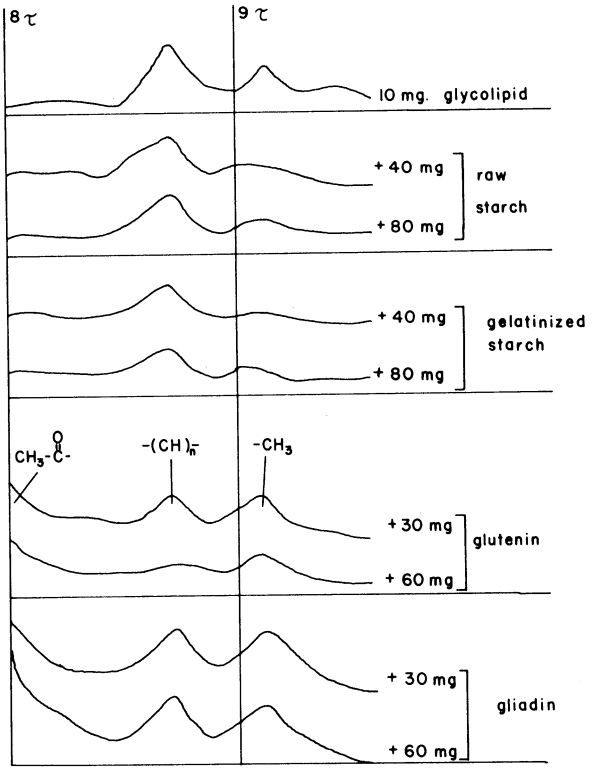


Fig. 5. NMR spectra of 10 mg. synthetic galactosyldioctanoylglycerol complexed with various amounts of starch, glutenin, or gliadin in 1 ml. D_2O.

Gliadin is highly susceptible to chemical (18) and functional (10) modifications during extraction, so that interpretation of infrared spectra of gliadin complexes must be made with reservation. Lipid binding of gliadin was lower than binding of gelatinized starch or glutenin, as extraction with petroleum ether reduced bands at 1,720 and 2,920 $cm.^{-1}$ (Fig. 4, b). Generally, the spectra of gliadin-glycolipid complexes resembled those of the corresponding glutenin complexes. Prior to butanol extraction, part of the protein was in the beta-conformation. After the extraction, the shoulder at 1,630 $cm.^{-1}$ had disappeared and there was a sharp amide band at 1,650 $cm.^{-1}$ The tertiary structure of the protein was, therefore, changed as a result of the absence of glycolipids or of butanol extraction. Since gliadin without added lipid exists also largely in the beta-conformation (Fig. 4, d), it can be concluded tentatively that glycolipid is not essential to stabilize that conformation, and that the change during butanol extraction is due to the solvent effect rather than to absence of lipid. However, as the samples were prepared with lactic acid, the possibility that the infrared absorption around 1,630 $cm.^{-1}$ might be due, in part, to lactate salts of the protein cannot be excluded. No peak is detectable at 720 $cm.^{-1}$ in any spectrum of the gliadin-glycolipid complex. Thus it appears that under the conditions of the experiment glycolipid is bound to gliadin

by both Van der Waals (indicated by absence of the 720 cm.$^{-1}$ band) and hydrogen bonds (indicated by the fact that the glycolipid was not extractable with petroleum ether).

A dispersion of synthetic galactosylglyceride in D_2O produces a well-defined peak at 8.7 τ (Fig. 5). No ultrasonic dispersion was required, because galactosyldioctanoylglycerol is much more hydrophilic than the phospholipids used by Chapman et al. (19). The inhibition of the (CH_2) signal at 8.7 τ in dispersions of erythrocyte membranes indicates a high local viscosity of the hydrocarbon lipid chains in the membrane (19). It can be explained by hydrophobic lipid-protein interaction. It is difficult to imagine that a mere sandwich of a phospholipid bilayer between protein monolayers, according to the Danielli membrane layer model (as postulated by Grosskreutz (20) to exist in wheat gluten), could itself cause inhibition of the chain signal.

The spectra of complexes between starch or gelatinized starch and galactosylglyceride are characterized by a slight broadening of the methylene peak and large broadening of the methyl peak. The area under the peak is not significantly reduced. Thus in the presence of starch the glycolipid cannot move freely, indicating some binding between starch and glycolipid. Theoretically, the binding may involve the galactosyl group as well as the methyl groups of fatty acids (whose environment must have a rather high local viscosity), but not the whole fatty acid chains, because the methylene peak is only slightly modified by starch. There is no significant difference between complexes with raw and gelatinized starch.

Glutenin binds galactosylglyceride strongly (Fig. 5). It is remarkable, however, that whereas the methylene peak (8.7 τ) is substantially reduced, the methyl peak (9.2 τ) remains almost unchanged. Glutenin alone produced no methyl peak. It is difficult to visualize how only the middle part of the lipid molecule (namely the methylene groups) is bound to a protein. Since one end, namely the methyl groups, is apparently free, it is feasible that the polar end of the lipid is also involved in the complex formation. The evidence for such a model is rather indirect.

Gliadin has little influence on the spectrum of galactosylglyceride in D_2O (Fig. 5). The relative height of the methylene peak is somewhat reduced, indicating that hydrophobic binding might occur to a small extent only. Thus, if glycolipid is actually bound to gliadin, the complex must be stabilized by hydrogen bonds between polar amino acid residues and the galactosyl moiety. Hoseney et al. (1) reported that such hydrogen bonds are broken by dilute acetic acid but not by lactic acid. Since the methyl group of lactic acid produces a peak at about 8.7 τ, it could not be used to disperse gliadin, and acetic acid was used instead.

The results of this study indicate that, in dough, glycolipids can be bound by hydrogen bonds to starch and to gliadin and glutenin. In the presence of water, binding to glutenin is mainly by hydrophobic bonds.

SUMMARY

Interactions between glycolipids and raw starch, gelatinized starch, gliadin, and glutenin were investigated by infrared and NMR spectroscopy. Infrared spectroscopy indicated hydrogen bonds between glycolipids, gelatinized starch, and gluten components; and Van der Waals bonds between glycolipids and gluten components. By definition, hydrophobic interactions cannot be ascertained by

infrared spectroscopy in the absence of water. NMR spectra point to hydrogen bonds with starch and hydrophobic (and limited hydrogen) bonds with glutenin.

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[Received January 28, 1969. Accepted October 6, 1969]