THE PHYSICAL STRUCTURE OF WHEAT PROTEIN

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SUMMARY

The physical structure of wheat proteins has been investigated using both wide- and small-angle X-ray scattering and electron microscopy. Dry wheat gluten, gliadin and the water-soluble proteins all give similar wide-angle diffraction patterns characteristic of folded polypeptide chains in the α_1-helix configuration. Small-angle scattering curves from stretched, dried glutens show marked orientation and can be interpreted in terms of parallel sheets of gluten platelets whose thickness does not exceed $\sim$70 Å. Electron micrographs taken of surface replicas of the stretched glutens confirm the sheet structure. Moist, relaxed glutens, while not exhibiting the orientation into sheets, still give the X-ray scattering characteristic of platelets.

Several small-angle diffraction peaks observable in both dry and moist glutens can be traced to the bound lipids which are extractable with water-saturated *n*-butanol. These peaks correspond to d-spacings of 62 Å and 48 Å. The extracted lipid exhibits the same structure and suggests that gluten originally consists of a lipoprotein complex. Removal of the bound lipid deprives the gluten complex of its elastic properties and it is not possible to obtain oriented small-angle X-ray patterns from stretched samples, although the platelet-scattering persists isotropically. Electron micrographs confirm the absence of sheet structure in lipid-free glutens. Gliadin films, prepared by evaporation of the ethanol extract exhibit a strong 42 Å diffraction which can be removed by extracting the bound lipid. The extracted lipid, however, exhibits its strongest diffraction at 48 Å, indicating that the lipo–gliadin complex is slightly skewed or compressed.

INTRODUCTION

The investigation of the microphysical structure of wheat protein has been largely confined to the work of HESS[1], and TRAUB, HUTCHINSON AND DANIELS[2], who studied the structure by means of X-ray diffraction and electron microscopy.

HESS showed that the native protein in the wheat grain could be physically separated into wedge (between starch grains) protein and bound (to the starch grains) protein. Two X-ray diffractions were obtained from these fractions, one representing a spacing d $\approx$ 45–47 Å and the other d $\approx$ 80-90 Å. TRAUB later showed that this latter spacing was due to starch. All spacings are observed to increase slightly with hydration of the flour. Dry glutens washed from flour doughs were also observed to give the $\sim$47 Å line. TRAUB succeeded in showing that this line was in reality

due to the phospholipid bound in wheat protein. Because of the disruptive effect on the elasticity of wheat doughs occasioned by the removal of the bound lipids[3], both HESS and TRAUB concluded that the lipid was intimately connected with the protein structure. HESS pictured the lipids forming layers between the protein fibrils and entering strongly into the hydration reaction; TRAUB suggests that the protein fibers are held together by layers of phospholipids in the form of bimolecular leaflets.

The present work seeks to elucidate further the wheat protein structure and the role of lipids in that structure by a combined X-ray and electron microscope approach. For the most part the earlier work of HESS and TRAUB is confirmed and some new results are reported.

EXPERIMENTAL

A. *Preparation of samples*

All protein samples were prepared from unbleached, untreated flours. Two were pure strains. Table I gives the data pertinent to the flours*.

TABLE I

Flour No.	*Description*	*% Protein*
434	Montana Hard Spring Wheat	13.8
3237	Pawnee (pure strain, short dough development time)	12.8
3238	C11Z871 (pure strain, long dough development time)	12.8

Glutens were washed from flour-water doughs in the usual way with distilled water and lyophilized to obtain dry samples. Lipid extraction was performed following the method of MECHAM AND MOHAMMAD[3], using water-saturated *n*-butanol. Gliadins were extracted from the dry glutens using 70 % ethanol. Water-soluble protein was obtained using the procedure of MATTERN AND SANDSTEDT[4].

Birefringence studies on moist, stretched gluten[5] have shown that relaxation is 90 % complete in $\sim$1 ½ h. Since most X-ray exposures and the preparation of electron microscope samples require at least this length of time, observation of the microstructure cannot be made unless relaxation is prevented. This has been achieved by first stretching the gluten unidirectionally as a thin sheet over a smooth metal button held at $\sim -70°$. The gluten freezes within a few seconds and is then transferred quickly to a small vacuum enclosure where it is dried by lyophilization. The dried sample, white in color, retains the size and shape of the original stretched specimen even to the oriented strain markings on the surface.

B. *X-ray apparatus*

Conventional wide-angle X-ray diffraction patterns were taken with a Laue camera using Cu-Kα radiation. Small-angle patterns were taken using either (1) the Laue camera with a film-specimen distance of 180 mm, or (2) a specially constructed small-angle camera using proportional counter detection of the X-rays.

The special small-angle camera was of the 4-slit type[6] and has been described

* Flour 434 supplied by Pillsbury Mills. Flours 3237 and 3238 supplied by Kansas State University.

elsewhere[7]. The collimating system is evacuated to forepump pressure ($\sim$1.0 μ) to reduce air scattering; however, the sample is at atmospheric pressure. "Mylar" (0.00025 in.) windows[8] are used at the entrances and exits of the vacuum system.

Monochromatization of the X-rays is achieved electronically using the characteristics of the proportional counter in conjunction with a single-channel pulse-height analyzer (Atomic Model 510). Copper Kβ is suppressed with a Ni filter.

C. *Electron microscope techniques*

An RCA EMU-2 electron microscope modified with a compensable pole-piece and Canalco astigmator was used in these studies. Resolution of the order of 40 Å could be obtained when the microscope column was clean and well aligned.

Wheat proteins do not lend themselves easily to direct observation under an electron beam; however, a solution of the difficulties is the use of surface replicas. Freeze-dried, stretched samples, prepared in the manner described above, were first shadowed in vacuum with Pt-Pd alloy (80–20 %) at a glancing angle of 14 degrees. Carbon was then evaporated normally onto the surface. The sample was then removed from the evaporator and pressed, under heat ($\sim$100°), replica down, into a piece of polystyrene sheet. This "sandwich" was washed alternately in 70 % ethanol and 0.2 % KOH to remove the gluten. Following washing, the sandwich (open-face) was submerged in ethylene dichloride which dissolved away the polystyrene leaving the shadowed carbon replica floating freely. The main advantage of this procedure is that the replica is made prior to any of the heat treatment or washing procedures and hence records faithfully the original state of the gluten surface.

RESULTS AND DISCUSSION

A. *X-ray*

1. *Wide-angle diffraction.* The proteins extracted from wheat are not crystalline in nature and should therefore not be expected to give a sharp wide-angle diffraction pattern. Fig. 1(a) is a typical diffraction pattern taken of dry, powdered wheat gluten. The patterns from dry gliadin, glutenin and the water-soluble wheat proteins are all essentially the same.

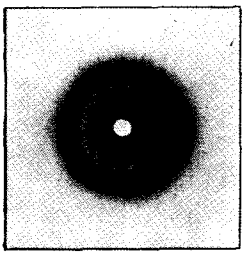
ARNDT AND RILEY[9] have shown that the patterns obtained from a wide range of animal proteins fall into three distinct classes which they term α, β, γ. The first group contains the largest number and consists of such corpuscular proteins as egg albumin, ribonuclease, and hemoglobin. The patterns for wheat proteins most nearly agree with the α-group proposed by RILEY AND ARNDT, which, according to these authors, consists of folded polypeptide chains in the α_1-helix configuration of PAULING AND CORY[10].

2. *Small-angle scattering.* Because of possible confusion introduced by extraneous components of the wheat (*i.e.*, starch, lipids, etc.), it is desirable first to isolate the contribution of these components to the small-angle scattering.

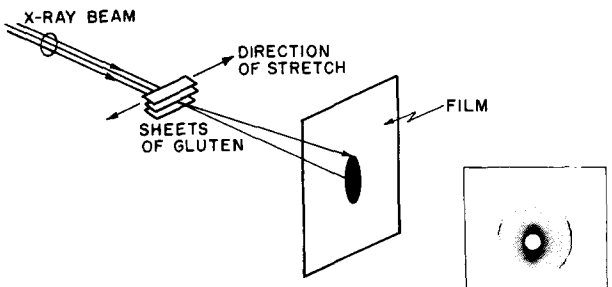
(a) *Doughs.* The structure of wheat protein is of interest here because it is related to the behavior of flour doughs used in bread making. One would therefore wish to observe this structure directly as it exists in a developed dough.

Fig. 2 shows the small-angle scattering from a straight flour–water dough and from the wet starch extracted from the dough. It is clear that starch, which comprises

~85 % of the dough, is responsible for the major shape of the scattering from dough, and that the protein must be extracted and studied separately if much is to be learned of its structure. The peak at ~12 milliradians seen in Fig. 2 corresponds to a Bragg d-spacing of ~125 Å. HESS[1] first ascribed this interference to the protein but TRAUB[2] has since shown it to arise from starch as is demonstrated in the present work.



(a)



(b)

Fig. 1(a). Wide-angle X-ray diffraction from amorphous gluten. Film-specimen separation, 4 cm.
Fig. 1(b). Small-angle X-ray scattering from stretched, dry glutens showing orientation effects. Film-specimen separation, 18 cm.

Whether the maximum is a true Bragg reflection or is due to interparticle interferences within the starch is not clear from any of the work done to date. It does not, however, appear pertinent to the structure of wheat protein, either isolated or within a parent dough.

(b) *Glutens.* The small-angle scattering from dry powdered gluten is shown as the uppermost curve in Fig. 3. The undulations observed in the angular range 20–40 mr. are reproducible and due to the lipid component (~7 % by weight). The scattering from the extracted lipid and the gluten residue are shown displaced beneath the parent gluten curve. It is usually not possible to remove completely the lipid lines from the gluten residue.

The interferences from the lipid are thought to be true Bragg diffractions with d-spacings 62 Å and 48 Å. HESS[1] assigned these lines to the protein, although he did report their disappearance from glutens washed from defatted flours. It is significant that (1) the removal of lipids does not seriously alter the general shape of

the gluten scattering curve, and (2) the positions of the diffraction lines from the lipid do not change from the bound to the isolated state, although their sharpness in the isolated state is strongly dependent on the degree of solidification of the sample. These observations would indicate that the lipid, while bound into a lipoprotein

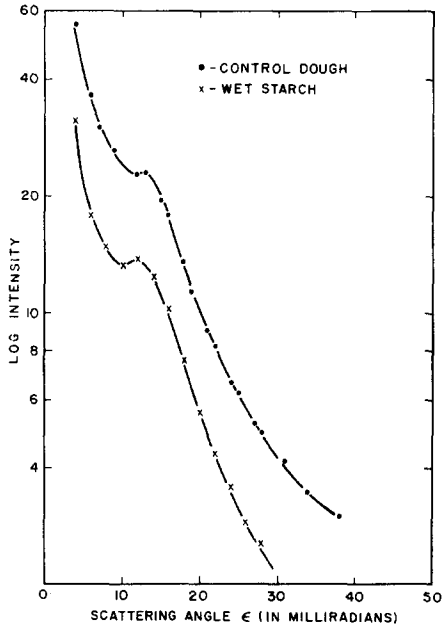


Fig. 2. Small-angle X-ray scattering from wheat flour dough and wheat starch.

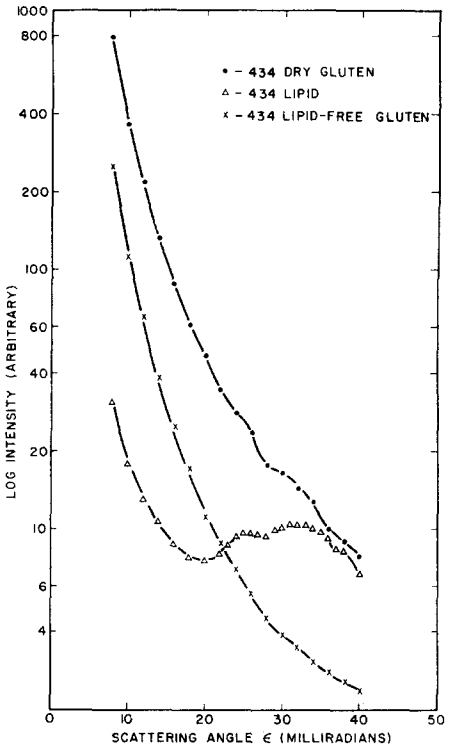


Fig. 3. Small-angle X-ray scattering from dry gluten, extracted lipid, and dry gluten residue.

complex in the gluten, separates essentially as a unit under the solvent action of wet *n*-butanol.

It is usual to analyze small-angle data in terms of the various theoretical expressions for scattering from various shaped particles[11]. This comparison is much easier if the effects of interparticle interference can be neglected, a situation which is satisfied for dilute solutions. In order to estimate the amount of interparticle interference in the scattering from concentrated glutens, curves were obtained from dilute dispersions of gluten in acetic acid (pH = 3.2) and compared with moist gluten as obtained by the usual washing procedures. It is clear from Fig. 4 that the effects of interparticle interference are small for moist gluten, resulting mainly in a slight depression of the curve at the smaller angles. The effect can be neglected in the analysis of the tail of the curves considered in the next paragraph. (Lipid lines are visible in the 20–40 mr. range for moist gluten.)

SCHMIDT[12] has calculated the scattering from polydisperse solutions of ellipsoids of revolution. For thin platelets, the intensity is proportional to ε^{-2} at moderately large angles, while at larger angles, the dependence changes to ε^{-4}; for long rods,

the scattering varies as ε^{-1} at moderately large angles, changing to ε^{-4} at larger angles. Spherical particles exhibit only the ε^{-4} dependence.

For purposes of experimental comparison, the functions ε^{-2} and ε^{-1} were integrated over the areas of the collimating and defining slits of the small-angle camera.

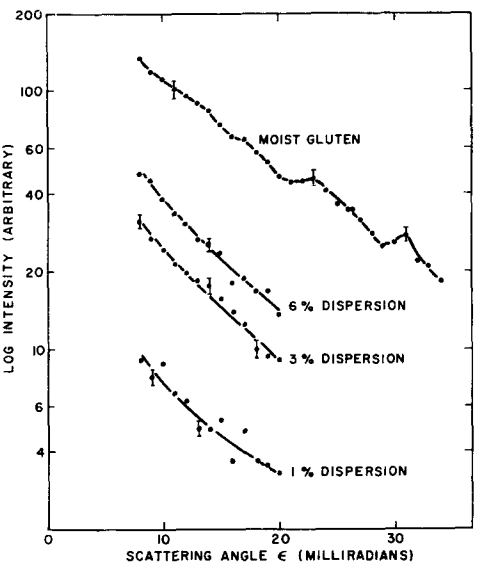


Fig. 4. Small-angle X-ray scattering from various gluten concentrations. Dispersions made in acetic acid (pH 3.2).

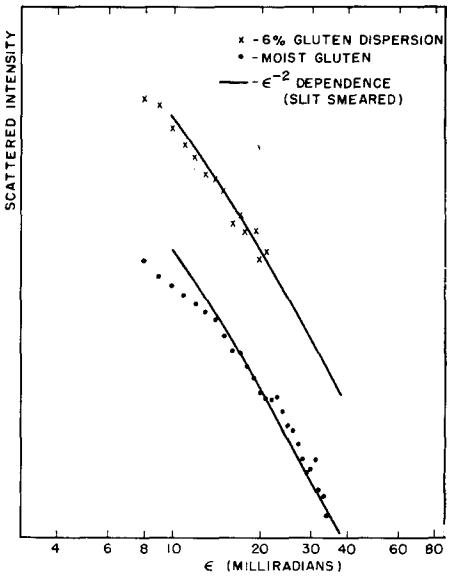


Fig. 5. Comparison of small-angle X-ray scattering from gluten with theoretical platelet, ε^{-2}, dependence.

Agreement with the ε^{-2} (platelet) dependence is shown in Fig. 5. An estimate of the platelet thickness can be determined by observing the angle at which the ε^{-2} dependence begins to change to ε^{-4}. Data for moist glutens were taken out to 100 mr. At about 50 mr. the curve began to flatten without over showing any ε^{-4} dependence. The reason for this flattening is thought to be due either to thermal diffuse scattering or to the beginnings of the leading edge of the diffuse interhelix peak whose maximum falls at $\sim$140 mr. Nevertheless, knowing that ε^{-2} scattering persists to at least 50 mr., the statement can be made that the platelet thickness is no greater than $\sim$70 Å.

It is well known that the small-angle scattering from particles of any shape will follow the approximate experimental law of Guinier (ref. [11], p. 26) at or near $\varepsilon = 0$. Because of the demonstrated platelet structure of gluten, one would not expect close agreement with the Guinier expression except at very small angles. Nevertheless, log I (ε^2) plots show some degree of linearity for the gluten dispersions and moist gluten (Fig. 6). The slope of the straight-line portion can be interpreted in terms of a radius of gyration whose value is 31.5 Å for the dilute dispersion. Interparticle interference effects depress the slope somewhat for moist gluten. The relation of this number to the platelet thickness is not obvious in view of the lack of information on the details of the platelet shape. Nevertheless, an approximation is to state that the thickness $\sim 2R_0$, or 62 Å. This is consistent with the other data yielding $t < 70$ Å.

Although dry glutens do not show, as do the moist glutens, the marked agreement

with the predicted platelet scattering, several conclusions can be drawn about their structure. Firstly, the tail of the dry scattering curve falls somewhat more rapidly than ε^{-2}. This would be true for oblate ellipsoids with axial ratios larger than those found in the moist state. At first glance this appears contradictory since one would

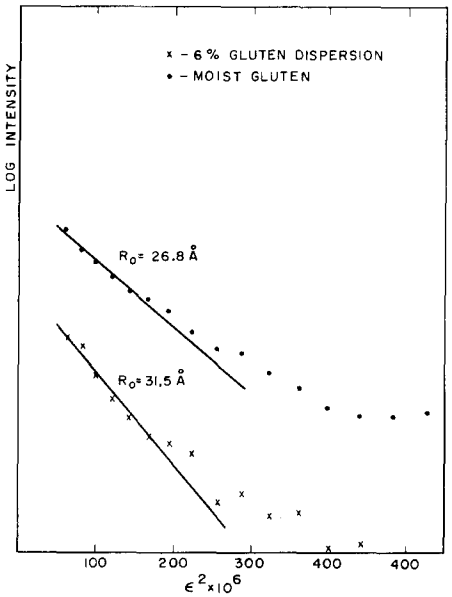


Fig. 6. Guinier plot of small-angle X-ray scattering from gluten. R_0 = radius of gyration.

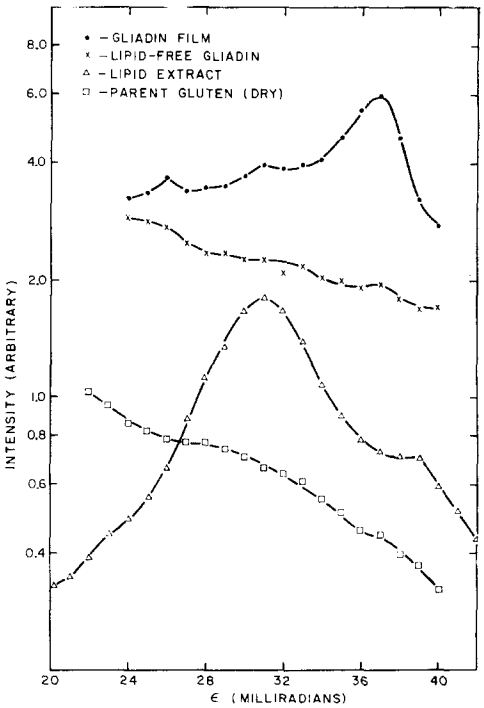


Fig. 7. Small-angle X-ray scattering from gliadin film, lipid extract and gliadin residue.

expect shrinkage rather than swelling to occur with loss of water. However, the agglomeration of several thin platelets into a single unit while drying could account for the observations. This is further supported by the behavior of the dry curves at the smaller angles. Unlike the moist glutens, there is a rapid increase in scattered intensity for $\varepsilon < 15$ mr. No good radius of gyration can be obtained from the data, but it is evident that larger values are contributing to the scattering. KRATKY AND POROD[13] have shown that scattering from lamellae in densely packed systems gives rise to a "minimum angle" scattering which increases rapidly as ε approaches zero.

Additional evidence for the platelet structure was obtained from photographs of the X-ray scattering at small angles from stretched, freeze-dried gluten. Fig. 1(b) shows the experimental arrangement and the oriented pattern that was obtained. Clearly the platelet must be oriented flat into the plane of stretch. (Photographs taken with the X-ray beam perpendicular to the plane of stretch show a diffuse unoriented scattering with a faint oriented pattern superimposed. This latter is thought to be the result of a few platelets which are rotated to some degree with respect to the plane of stretch.) The dependence of scattered intensity on scattering angle in the plane perpendicular to the stretch has been measured using a counter and found to be the same (except for the greatly increased intensity) as that from

powdered glutens. One then obtains the picture of stretched gluten existing in parallel sheets, made up of platelets which have bonded together under the action of hydration and mechanical mixing. The majority of these sheets are oriented parallel to the plane of stretch, although a few, through folding, are to be found at all angles with respect to the majority.

It was not possible to obtain an oriented small-angle pattern from glutens extracted with wet *n*-butanol. One therefore concludes that the sheet-like structure of ordinary stretched gluten owes its existence to the presence of lipid, most probably in the form of a lipo-protein complex. No significant difference was found in the X-ray scattering from the two pure wheat strains, 3237 and 3238.

(c) *Gliadins.* TRAUB *et al.*[2], reported a sharp diffraction line from gliadin film at 47 Å and attributed the interference to the lipid component which, in a film evaporated to dryness from the ethanol extract, remains with the gliadin. The uppermost curve in Fig. 7 shows the scattering obtained in this investigation from a dry, pulverized gliadin film. The main peak at 37 mr. corresponds to a spacing of 42 Å. Measurements made on gliadins extracted from six other flour types all showed the interference at 42 Å to within a few tenths of an angstrom.

The second and third curves in Fig. 8 show the scattering from lipid-free gliadin and from the lipid which was extracted with wet *n*-butanol. The main interference from the lipid falls at 31 mr., or 49.7 Å. This peak can be seen in the original gliadin curve as a small bump which disappears after lipid extraction. Extraction of the lipid also removes the strong 42 Å line from gliadin. There is a knee on the lipid curve at 42 Å, but with nothing of the intensity originally observed for gliadin. The curve from the parent (unextracted) gluten is also shown and the influence of the lipid interferences can be seen.

B. *Electron microscopy*

Electron micrographs were taken of the replicas of stretched, freeze-dried gluten surfaces. The method of preparing the replica was described in an earlier paragraph. Fig. 8(a) shows the surface of 3237 gluten at fairly low magnification (8000 ×). Notice

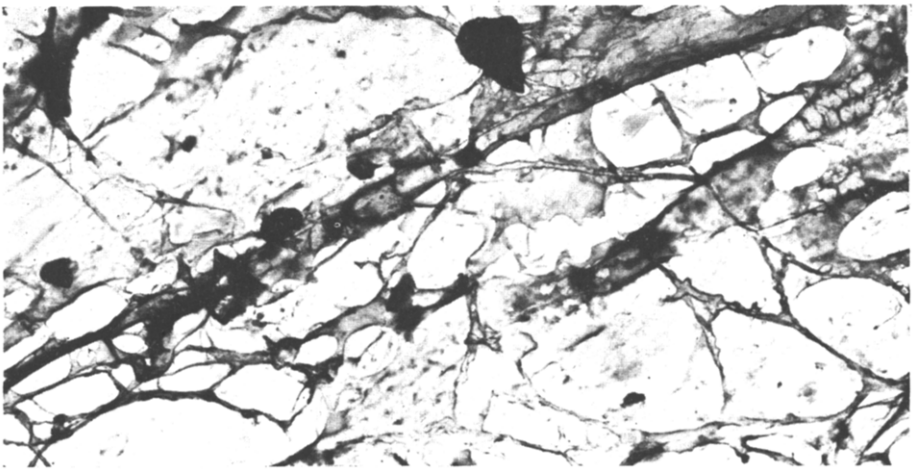


Fig. 8(a). Electron micrograph of surface of stretched, dry gluten (carbon replica 8,000 ×).

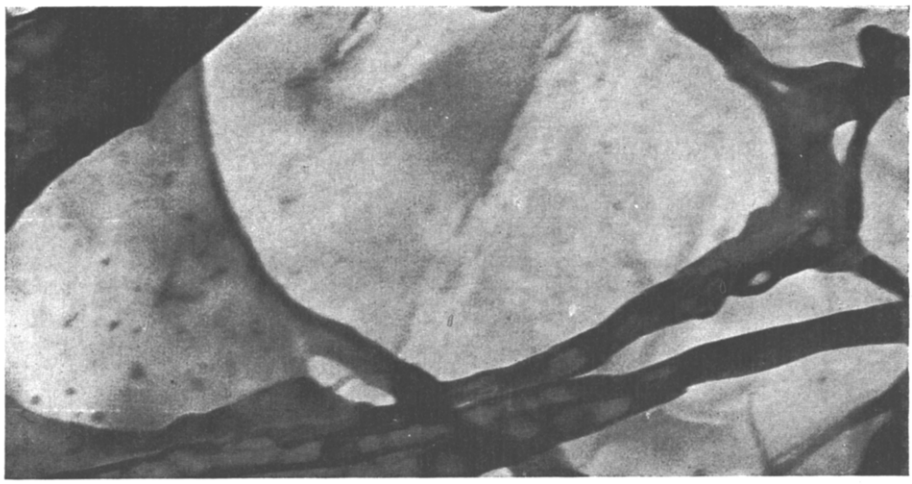


Fig. 8(b). Electron micrograph of surface of stretched, dry gluten (carbon replica 43,600 ×).

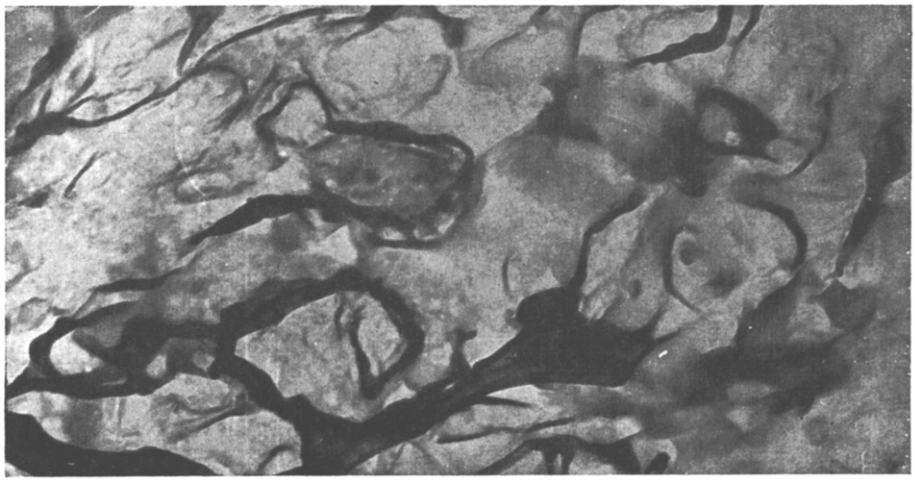


Fig. 8(c). Electron micrograph of surface of stretched, dry, lipid-free gluten (carbon replica 43,600 ×).

the filamentary structure (direction of stretch ~30° to the horizontal) which is not necessarily all aligned in one direction. Fig. 8(b) shows the typical sheet structure of stretched gluten which was also inferred from the X-ray photographs. This picture is a magnified portion of the upper right-hand part of Fig. 8(a). Three layers of gluten can be distinguished.

A careful examination of the replicas under high magnification has revealed many broad expanses of unruptured gluten sheet. In regions of high stress concentration, however, these sheets first develop holes which grow until the sheet is reduced to a network of filaments. Using present procedures it has not been possible to distinguish individual platelets in the gluten sheet. If their thickness is less than 70 Å, however, their presence would be betrayed only by undulations ~35 Å high in the surface of

the gluten. This is at the limit of resolution of the EMU-2 microscope presently in use.

Lipid removal results in the gluten structure shown in Fig. 8(c). Only fragmentary "sheets" can be seen.

CONCLUSIONS

The physical structure of wheat protein as revealed by X-ray and electron microscopy has been shown to be:

1. Folded polypeptide chains in the α_1-helix configuration of PAULING AND CORY.

2. Molecular platelets of a thickness not greater than about 70 Å. Under hydration these platelets bond together into sheets which can be oriented by an applied stress parallel to one another and in the plane of stretch.

3. Dependent on the presence of lipids (wet *n*-butanol extractable) to the extent that lipid removal, while not affecting the basic platelet, destroys the ability to bond into sheets.

Gliadin films, prepared by evaporation of the ethanol extract, were shown to have a structural periodicity of 42 Å. This is apparently not a true lipid interference, but removal of the lipid destroys the periodicity. Lipids themselves all show a strong diffraction maximum at ~48 Å, with subsidiary peaks at ~42 and 62 Å.

No significant differences, other than the well-known macro-elastic properties, have been found in the microstructure of different wheat protein strains.

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