

The Microstructure of Wheat Protein Fibrils

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ABSTRACT

Wheat endosperm protein is shown to be present in sheet form when wetted. It is suggested that these sheets result from a laminar deposition of the storage protein in the protein bodies of the developing wheat kernel. The sheets of endosperm protein rupture under stress forming fibrillar webs of protein which are composed of fibrils ranging in diameter from fifty to several thousand Angstroms. The larger fibrils are shown to be aggregates of the smaller fibrils interacting laterally. Two mechanisms are proposed for the elasticity of the gluten protein which could result from the interactions of these protein fibrils.

Microscopic fibrils of hydrated protein have been observed when wheat endosperm tissue is covered with a drop of water (1). These fibrils are elastic, interact with starch granules, exhibit viscous flow, and, in general, reflect the physical properties characteristic of gluten proteins. Similar fibrillar forms have been demonstrated in gluten (2) and dough (3). This paper reports an investigation of the fibrillar structure.

Since the order of a structure is frequently a reflection of the symmetry of the subunits that make up the structure, and since we had previously observed a microfibrillar form for one wheat protein, α -gliadin (4), we reasoned that these larger fibrils observed in the light microscope might be composed of microfibrils. This idea was further strengthened by our knowledge of the rheological properties of the α -gliadin fibrils (5). These microfibrils, along with more complex structures formed from them, may be the physical structures that give wheat dough its unique rheological properties.

For lack of a better descriptive term, in this paper fibril refers to all structures which have a large ratio of length to width. No lower limit is imposed upon a fibril diameter. This may result in some confusion in discussions where fibrils are composed of smaller fibrils. Qualifying phrases, such as "smaller, light microscope fibrils" and " α -gliadin fibrils" have been used in an attempt to clarify some of the discussions. α -Gliadin fibrils have a diameter of 80 Å. (4) and are not composed of smaller fibrils. Fibrils observed in the light microscope are larger than 0.5 μ (5,000 Å.). It is the substructure of these larger fibrils that is investigated in this paper.

MATERIALS AND METHODS

Flours used in this study included those from the varieties Justin (HRS), Comanche (HRW), and Scout (HRW). The varieties are identified for each micrograph even though no differences were found among the flours.

Specimens for microscopy were prepared by placing a few flour particles on a microscope cover glass or an electron microscope grid, wetting the flour with a drop or two of distilled water, and rapidly freezing the sample.

Transmission Electron Microscope Specimens

The electron microscope grids were first coated with a collodion membrane and carbon-surfaced. These grids, with the drop of water containing the flour particles, were frozen by placing them directly on a block of dry ice. The grids were then

transferred to freeze-dry flasks and lyophilized. When dry, the grids were shadowed with uranium for contrast in the electron microscope. Magnifications were obtained from a previous calibration of the microscope with polystyrene beads of known dimensions.

Scanning Electron Microscope (SEM) Specimens

Specimens for the SEM were frozen on cover glasses by immersion of the glass with water drop into 2-methylbutane containing 8% methylcyclohexane at -165° to -175°C . The samples were then warmed to -65°C . and held at that temperature during lyophilization. When dry they were coated with a 200- to 300-Å. layer of carbon and gold. Magnifications were obtained from a previous calibration of the instrument.

Light Microscopy

Light micrographs were photographed with light reflected from the gold coating on SEM specimens.

RESULTS

Initial scanning electron micrographs indicated that nearly all of the endosperm

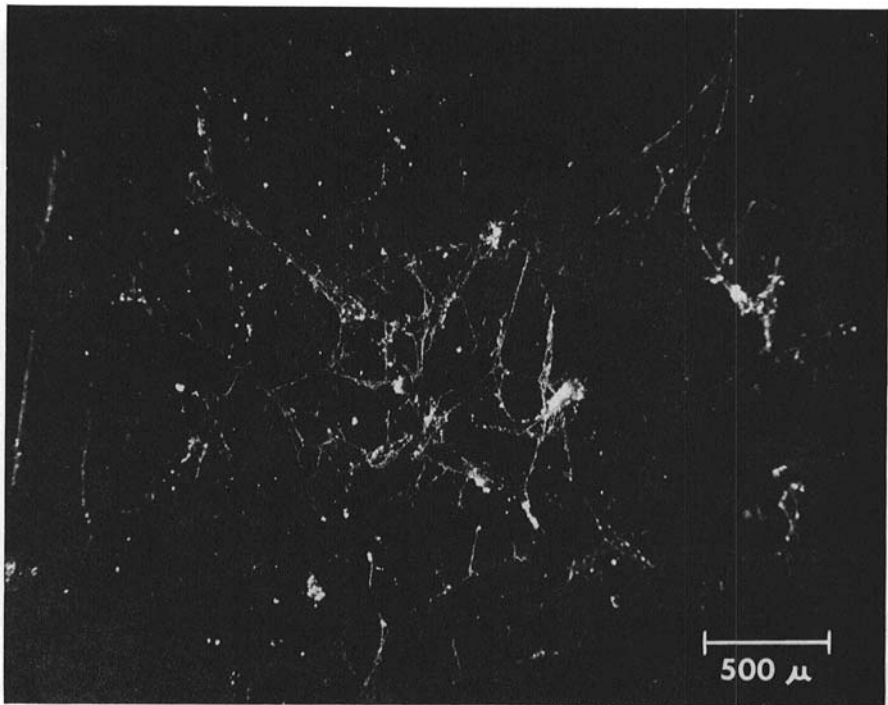


Fig. 1. A light micrograph of a SEM specimen photographed using light reflected from the gold coating on the protein fibrils and starch granules. The field covers fibrils from three flour particles. Scout flour.

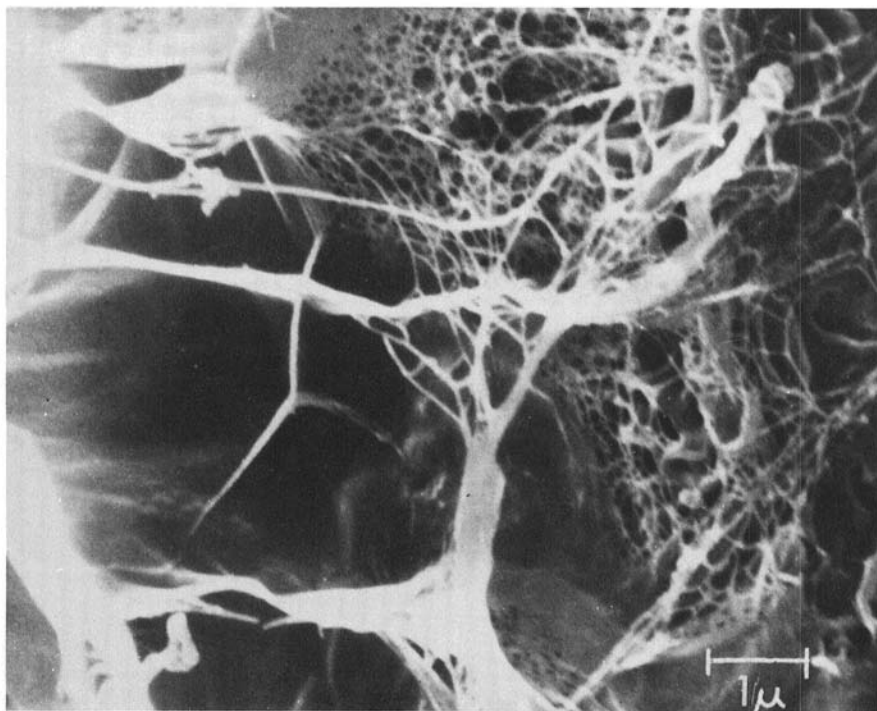


Fig. 2. Three-dimensional fibrillar structures produced by wetting particles of flour. Sheets of protein initially develop holes which become so numerous that eventually all that remains of the sheet is a fibrillar network. Justin flour.

protein formed the fibrillar webs of protein. A representative sampling is shown in Fig. 1. In every preparation there were areas where the fibrils were seen as an extension of sheets of protein; the fibrils apparently resulted from the breakdown of these sheets of endosperm protein (Fig. 2). The first stages in the rupture of a protein sheet are seen in Fig. 3 which is a higher magnification of a portion of the field of Fig. 3 in literature reference 1. Many small holes are visible in the sheet in the area where the sheet appears to be under stress. A similar sheet undergoing rupture is shown in Fig. 4, a transmission electron micrograph where, rather than surface features, variations in the sheet thickness are prominent. From this micrograph it is clear that the sheet rolls back upon itself following rupture.

In regions where the sheet has rolled back, the cross-sectional area is greater than through the extended sheet, producing thicker areas. Further rupture occurs in the extended sheet until it is entirely transformed into fibrils. Such a fibrillar web is shown in Fig. 5 and appears identical to the ruptured gluten sheet reported by Grosskreutz (2). While no detailed substructure of the fibrils is discernible, considerable variation in the diameter of the fibrils is apparent. Several fibrils appear to be 100 Å. in diameter, and many fibrils reach 600 Å. The larger fibrils seem to be composed of smaller fibrils.

In all of the micrographs no detailed substructure was clearly visible as long as

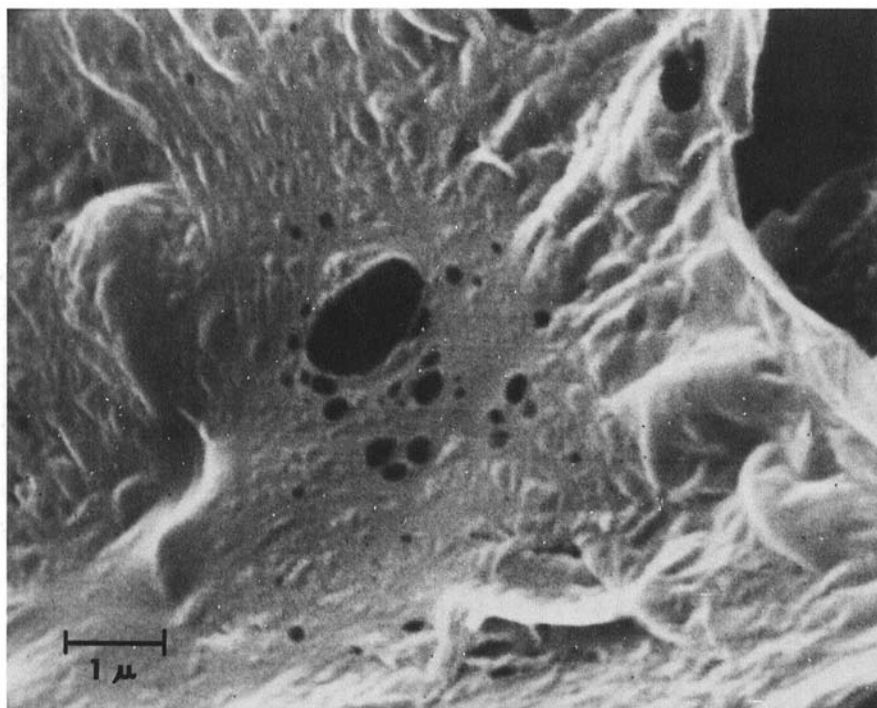


Fig. 3. A SEM of a stretched sheet of endosperm protein showing the initial stages in the transformation to a fibrillar structure. Scout flour.

the protein was suspended above the support film on the electron microscope grid. In areas where the fibrils had been drawn out to a very small diameter and most of the linkages characteristic of the webbed structure had been broken, fibrils deposited on the support film exhibited regions where parallel fibrils with diameters of approximately 100 Å. would separate and rejoin further along their length (Fig. 6).

When the three-dimensional fibrillar structures were deposited on the support film and shadowed with uranium for transmission electron microscopy, smaller fibrils were clearly seen (Fig. 7). These fibrils, with diameters ranging from 50 to 100 Å., were usually aligned parallel to one another and frequently were organized in large aggregates 500 to 2,000 Å. across, with the small fibrils twisted about one another to form a large fibril. Large areas were found where the fibrils were parallel to one another for distances of several thousand Å. Linkages from one bundle of fibrils to another resulted from a crossing over of one or more fibrils (Fig. 8).

DISCUSSION

The general fibrillar nature of the endosperm protein is clearly seen in the micrographs. However, an obvious question concerns the source of the sheets of endosperm protein. Deposits of storage protein in endosperm are large, irregularly

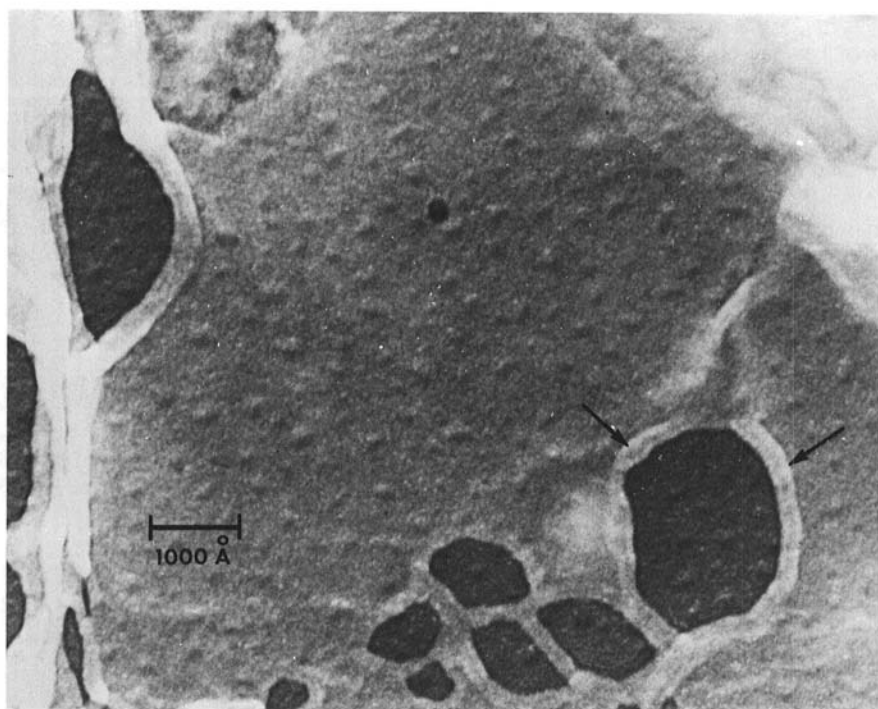


Fig. 4. A transmission electron micrograph of a protein sheet undergoing rupture similar to that shown in Fig. 3. Instead of the surface contours shown in the SEM, the thickness variations are prominent in the micrograph. Arrows indicate where the sheet has rolled back at the ruptured edges forming a thicker protein mass. Comanche flour.

shaped masses of protein (6) with occasional inclusions of endoplasmic reticulum. This protein contains small amounts of lipid (7) and may incorporate remnants of subcellular organelles present in the developing seed (8). In the developing endosperm this protein is deposited in protein bodies (8,9,10). As the seed grows, these bodies are distorted by the growth of starch granules and by the dehydration of the kernel after maturity. It seems reasonable to suggest that the protein is deposited in laminar form around the protein body during its development in much the same manner as the laminar structure of starch granules develops (11). When endosperm tissue is wet, such laminar structures might disperse in the form of sheets of protein. Wheat proteins have been shown to have areas which will bind hydrophobic molecules (12), and globular proteins are known to have charge-bearing amino acids located predominantly on the surface of the protein (13). This hydrophobic/hydrophilic character of the wheat proteins may stabilize the sheets of protein and their interactions. For example, a sheet may be formed with predominantly hydrophobic forces stabilizing the lateral fibrillar interactions, and polar forces stabilizing the interactions between sheets.

Protein bodies in cross-section would appear layered if the protein were distinguishable as laminar sheets. In fact, a laminar structure at the periphery of

sections of protein bodies has been reported (9) and described as a lipoprotein membranous array on the basis of its staining properties. However, this staining pattern does not necessarily require lipoproteins; it might result from a variation in protein density due to the laminar deposition of the protein. A laminar form has also been observed in storage protein deposits in mature seeds (14). While these are referred to as laminar artifacts (14), they bear a striking resemblance to the fibrillar arrays shown in our micrographs. The presence of the fibrils (or a laminar form of protein) in the protein bodies remains unproved. Once wet, however, the fibrils are clearly present and must affect the rheological properties of dough.

The thinnest sheets observed in our micrographs were approximately 100 Å. thick. Some areas were found which were considerably thicker; these showed no tendency to roll back upon the main mass at the edges, as did the thinner sheets. Perhaps the sheets are stabilized once a minimum thickness is reached. These thicker sheets provide a structure which would allow expansion of a protein film without rupture. Layered sheets could expand by a sliding of one sheet over another to produce a thinner protein layer covering a larger area. Once the minimum thickness was reached, rupture would occur. The thinnest sheets observed corresponded well with the thickness of the gluten sheets measured by Grosskreutz

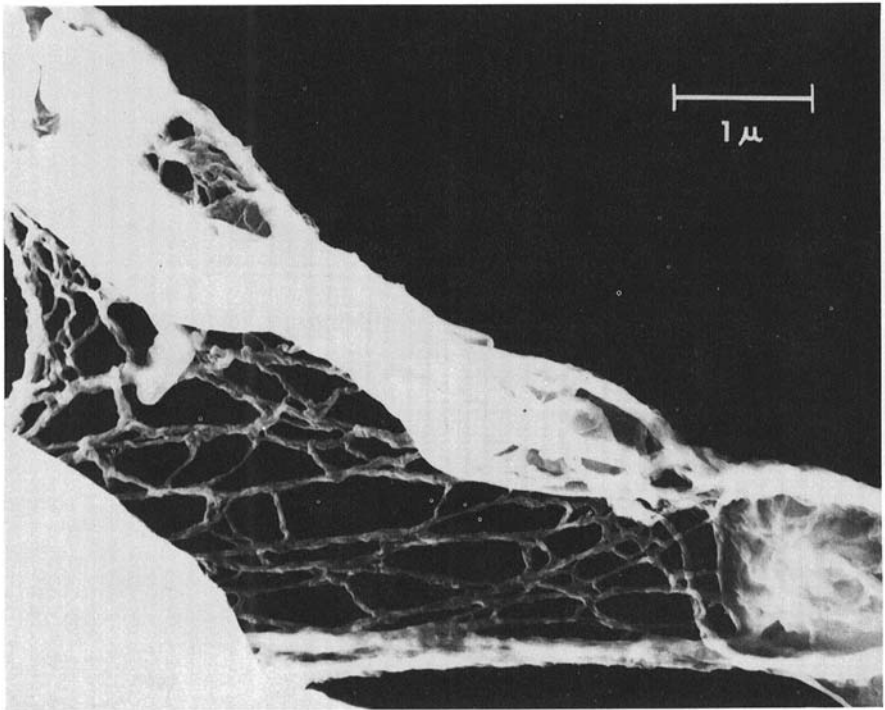


Fig. 5. The fibrillar network produced by a sheet of protein after wetting. Intact sections of the original sheet are visible as well as the rolling of the edge of the ruptured sheet. Comanche flour.

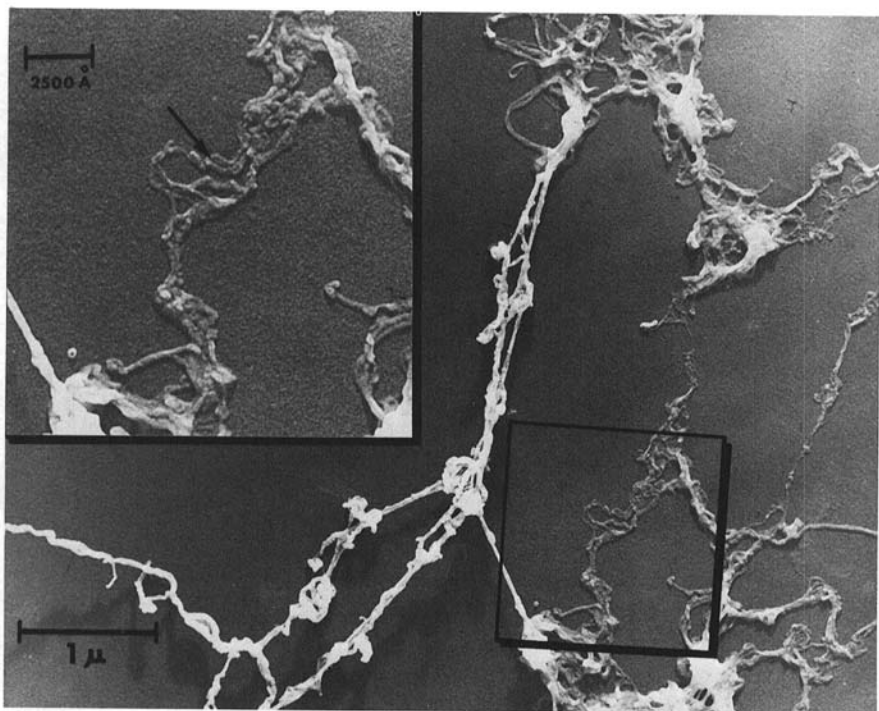


Fig. 6. Fibrils of endosperm protein suspended above the support grid as well as deposited on the membrane surface and shadowed. Fibrils with diameters of 50 to 100 Å are evident. Inset is a higher magnification of the outlined area. The arrows indicate three 80-Å. fibrils which formed a larger fibril and then separated on the membrane surface. Scout flour.

(2). His platelet model, however, does not incorporate a fibrillar form.

While we have not isolated a fibrillar form and shown that it is composed of gluten protein, except in the case of α -gliadin, the similarities of the other gluten proteins to α -gliadin suggest they may also form fibrillar aggregates. While some varieties of wheat do not have aggregatable α -gliadin of the type described by Bernardin et al. (15),¹ we have observed fibrils in these wheats as well as in those that have aggregatable α -gliadin. The small fibrils with diameters from 50 to 100 Å. are probably composed of specific aggregates of globular proteins, as are α -gliadin fibrils (4). The fibrils are also not a result of a lipid-protein interaction, since the fibrils are found to be present and to retain their elastic properties in defatted flours.

Even in the higher-magnification micrographs, where the fibrils are clearly seen, there is no indication of structure within the fibrils. This is consistent with the earlier micrographs of α -gliadin fibrils (4). If the subunits are assymmetric and have one dimension which is smaller than 20 Å., the resolution of the microscope would probably not be sufficient to resolve them. From the known molecular-weight

¹Platt, S. G., Qualset, C. O., and Kasarda, D. D., unpublished results.

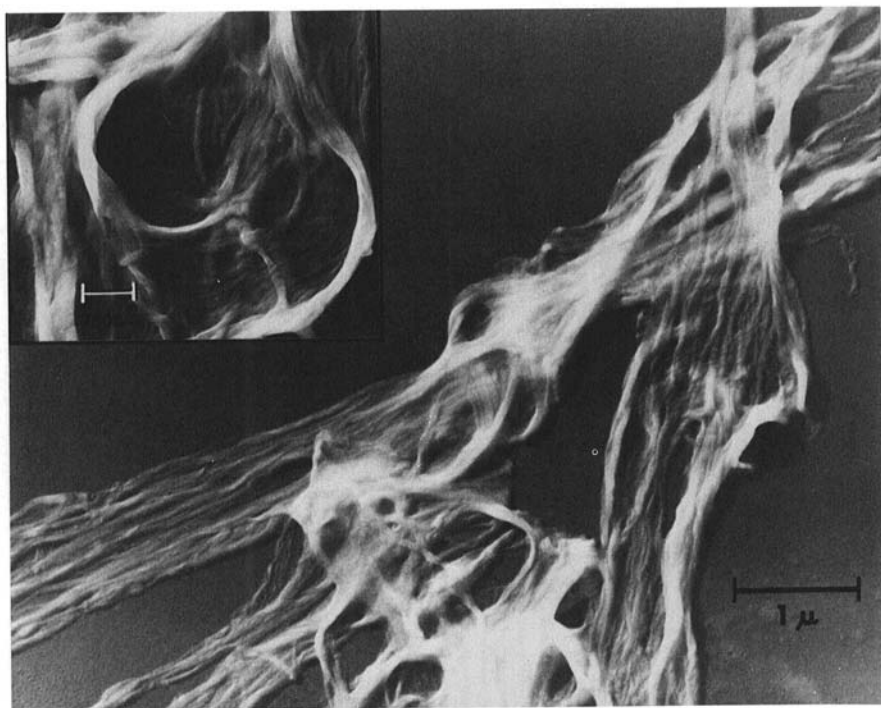


Fig. 7. The collapse and deposition of the three-dimensional fibrillar web of protein on the support membrane produce several layers of fibrils. Within one layer or bundle, the fibrils are aligned parallel to one another. The inset is a higher magnification of a similar area where the smaller fibrils that formed the larger bundles are clearly evident. Comanche flour.

range of the gliadin proteins this is possible. The observation of small subunits may also be limited by the lack of contrast development by the uranium shadow at the subunit level.

Most of the literature attempting to understand the rheological properties of dough has been based on the hypothesis of a disulfide cross-linked system. Our observations do not conflict with the earlier work demonstrating the effects of hydrogen bond-breaking reagents or disulfide/sulphydryl reagents on the dough system; these observations do not require a disulfide cross-linked system. We hypothesize that these reagents effect a conformational change that alters the interior structure as well as the surface of the protein subunits. This can best be understood by using the α -gliadin fibrils as a model for all wheat protein fibrils. Although intramolecular disulfide bonds stabilize the protein subunits that make up the fibrils (16), no intermolecular disulfide bonds join the subunits of the fibrils or the fibrils to one another (4). The breakage or interchange of an intramolecular disulfide bond, however, would most likely affect the conformation of the protein. This conformational change would be enhanced if the alteration occurred through a disulfide interchange reaction where a change was introduced into the interior of the globular protein. Glutathione or a similar sulphydryl bearing amino acid or

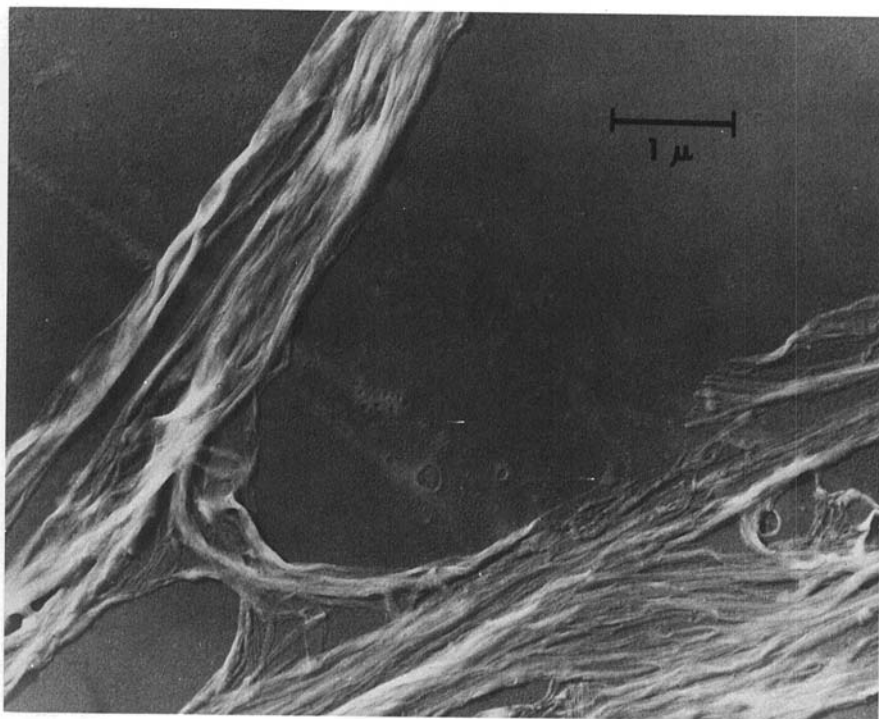


Fig. 8. Two bundles of fibrils which have layers onto the support membrane, showing that the link between the larger fibrils consists of a number of fibrils which change lateral interaction from one bundle to another. Comanche flour.

peptide would have charges at the N-terminal, C-terminal, and on the glutamic acid side chain. Aspartic acid, lysine, and arginine would also be charged at the pH of dough. Also, there would be an additional space requirement for the peptide. This is similar to the suggestion of Jones and Carnegie (17) for a disulfide exchange mechanism and the effect of charges on protein conformation. Their model places charges at the interior of globular protein molecules prior to disulfide interchange, however, and we do not feel this is consistent with the conditions in the developing wheat kernel or generally accepted concepts of protein structure.

Since the formation of linear fibrils requires a specific set of interactions, a conformational change in the subunits would probably prevent one or more of these interactions from occurring, which in turn could favor a dissociated form of the protein subunits. Disruption of the fibrils would produce dramatic effects upon dough structure and properties.

The large number of glutamine and asparagine residues in the gliadin proteins make hydrogen bonds certain to exist between subunits within a fibril and between fibrils. A hydrogen bond-breaking reagent, such as urea, would compete with the amide side chains for hydrogen-bonding sites and thereby weaken the fibrils and interactions between fibrils. The substitution of D_2O for H_2O would enhance the stability of the hydrogen bonds and strengthen the intra- and interfibrillar

interactions. Both of these effects are in agreement with the effect of these reagents on dough (18,19,20).

The cross-linked web of protein fibrils shown in Fig. 5 would provide a very efficient system for incorporating elasticity into the dough system. Only a small deformation of the bonds between any pair of fibrils would allow a large deformation of the protein web. Small deformations could recover by restoration of the bonds to their minimum energy position. Bonding interactions between fibrils which are ruptured as a result of large deformations could be recovered by means of a zipper-type mechanism whereby fibrils with a nucleus of lateral interaction could reform bonds sequentially along their length, provided that the nucleus of the interaction was not lost by too great a deformation. This restoration of bonds along the fibrils provides a mechanism for elastic recovery of the system. Neither bond-deformation nor lateral-bonding interactions between fibrils would prohibit viscous flow, since the fibrils would be free to slide along one another and form new bonding interactions once the force was removed. The same noncovalent bonding interactions could therefore allow both elastic recovery and viscous flow.

The size of the interacting subunits and the range of intermolecular interactions demonstrated in this study differ considerably from those previously proposed for the wheat protein and/or dough system. Instead of single polypeptide chains with diameters of approximately 10 Å. cross-linked by disulfide bonds between the chains, the interacting units are protein fibrils with diameters of 50 to 100 Å. These fibrils can exhibit lateral interactions that form larger fibrillar structures with diameters ranging up to 10,000 Å. The observation of the protein fibrils provides a new physical structure upon which a model for dough structure can be built. This model will not be restrained to the covalently cross-linked polypeptide chains previously proposed, but can conform to the interacting but nondisulfide cross-linked form consistent with rheological data (21).

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