HYPOTHESIS FOR THE STRUCTURE OF GLUTENIN IN RELATION TO RHEOLOGICAL PROPERTIES OF GLUTEN AND DOUGH

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ABSTRACT

The suggested molecular unit of glutenin is the concatenation. This consists of a variable number of polypeptide chains, not necessarily of the same type, which are joined together by disulfide bonds to form a super-molecule possessing a linear (*i.e.*, unbranched) configuration. Concatenations can adopt very complex conformations in water, and will often be entangled with one another. Regions of strong interaction and entanglement points (nodes) form the cross-links that are essential for rubber-like elasticity. Individual polypeptide chains in a concatenation may be unfolded by stress: the natural tendency of such chains to return to a compact state of lower free energy accounts for elasticity. Since nodes depend on secondary forces only, they are capable of being unraveled by stress. This process is merely a special case of molecular slip, and it is the latter that accounts for viscous flow in gluten and dough. The model explains the sensitivity of dough and gluten to disulfide-bond breaking agents, since the probability of entanglement decreases rapidly as the average length of a concatenation falls. The tensile strength expected for such a system is of the order observed.

Glutenin is the only constituent of wheat flour that exhibits significant viscoelasticity when isolated. It is reasonable, therefore, to assume that this protein is the major determinant of the viscoelasticity of dough. Glutenin comprises about 5% of the flour weight in a standard British bread flour having 11% protein on a 14% moisture basis.

Any hypothesis of glutenin structure must satisfactorily explain the remarkably rapid transition from flour, where the protein particles are unquestionably discrete, to dough, where the protein forms an apparently homogeneous structure in which the starch granules are embedded. This transition occurs in less than a minute when flour is mixed with water. In fact, if flour is merely wetted with water, viscoelastic strands can be pulled out almost at once. As there is no evidence for the presence in flour of appreciable quantities of high-energy compounds which could initiate or drive a rapid chemical reaction, and as the dough properties appear concomitantly with the diffusion of water into the stirred mass, it is postulated that the potential for viscoelasticity is already inherent in the glutenin molecules and is simply activated by wetting and mixing.

Elasticity

According to the kinetic theory of rubber elasticity, a three-dimensional molecular network is an essential requirement. The network should be cross-linked to provide continuity of structure, and must be capable of extension. This latter factor means that segments between cross-links will normally exist in a contracted conformation, and have adequate length, and furthermore, that intra- and inter-molecular forces will be weak in order not to hinder extension.

It has been assumed for many years in cereal chemistry that glutenin is cross-linked by disulfide (SS) bonds into a three-dimensional network. Such a hypothesis can reasonably account for elasticity, because glutenin chains are long and like most proteins they normally adopt contracted conformations,

stabilized in a large part by the cooperation of feeble hydrophobic forces (1). Water will weaken intermolecular forces that are due to hydrogen bonding or involve dipoles by competition and will screen ions, greatly lowering the strength of electrostatic bonds.

Electrostatic Forces in Glutenin

In view of the strength of the electrostatic bond, even in water, and the fact that ion pairs are common in concentrated salt solutions, electrostatic forces should cause glutenin chains to aggregate. A simple calculation will indicate the contrary, however. The amino acid analyses of glutenin show that in a mole of protein of molecular weight (mol wt), say 44,000, there will be, at the pH of dough, 28 positive and 25 negative groups. If two of these glutenin chains were in close proximity in dough, and even if all charged groups were sterically capable of interacting, on probability grounds about half the groups of one kind of charge would find themselves closest to positive groups and half to negative groups on the opposite chain. The resultant would be a virtual cancelling out of attractions by repulsions over the whole system. In fact, there would probably be a slight repulsion owing to the net positive charge, and this may be one of the factors responsible for the swelling of glutenin in water.

Viscous Flow

Glutenin, therefore, satisfies the conditions necessary for rubber-like elasticity. However, glutenin does not show true rubber-like elasticity except at low extensions. At large extensions, or even at low stress for an extended period, it exhibits viscous flow. Flow, however, is not observed in elastic substances for if extended beyond the limit these materials rupture.

Glutenin Network not Covalently Linked

The inference appears inescapable that, during dough mixing, the discrete flour protein particles do not join to form a three-dimensional network by disulfide bonds, nor indeed covalent bonds of any kind. In further corroboration of this fact, there is plenty of evidence in the literature that viscoelastic doughs can be formed when powerful SH-blocking reagents are present right from the start: these reagents block SH-groups which would catalyze the SS-interchange reaction that is the only practicable means of forming a network interlinked by SS-bonds. SH-groups occur naturally in flour, to the extent of about 1 μmol per g. Oxidation of these to SS-bonds to form a significant protein network is ruled out both by the immediately preceding argument and by probability considerations: the chance of two SH-groups being in juxtaposition is remote (2), and in any case most of them are attached to small molecules and not proteins.

Glutenin Properties Depend on SS-Bonds

Conversely, it is well known that agents such as thiols, sulfite, or powerful oxidizing agents which break SS-bonds, rapidly destroy the viscoelastic properties of dough. Indeed, it was this fact that for so long sustained the belief in a three-dimensional, SS-cross-linked system in dough. Even if such a system existed in the flour particles before they were wetted in the dough mixer, earlier reasoning denies the possibility that they could link up through SS-bonds. Nor could they form adequate secondary cross-links by molecular entanglement

because there would only be short lengths of polypeptide chains free on the surfaces of the protein particles.

Hypothetical Structure for Glutenin

A way of reconciling the sensitivity of dough viscoelasticity to SS-bond-breaking chemicals with the objections put forward to an SS-network can be found if a novel structure is proposed for glutenin.

Glutenin is agreed by most workers in the field to be a mixture of high mol wt proteins, built up from an assortment of polypeptide chains ranging in mol wt from about 10,000 to 130,000: the links between chains are SS-bonds. The assumption has always been that at least two SS-bonds in a given chain would not form intramolecularly, which means that there would be four half-cystine residues available to join with similar residues on three or four other chains, giving the conventional, highly cross-linked network.

It is now proposed that two of the half-SS-bonds will join with similar bonds on one chain only, while the other two bonds combine with a different chain. Thus, a system is created in which there are long linear molecules each consisting of polypeptide chains attached to one another by SS-bonds (Fig. 1). Such strings of polypeptide chains are referred to as *concatenations.*

Concatenations are entangled with one another. The entanglement regions, termed *nodes,* form the cross-links that give gluten its rubber-like elasticity at low extensions (Fig. 2). Nodes will vary in their stability, depending on their structure, the types of polypeptide chains involved, and their relative orientation to one another. As extension increases, nodes are stress-unraveled; the weaker ones tending to go first, although strong ones could fail if the local stress concentrations were unfavorable. The system has, therefore, a capacity for viscous flow which the covalently linked structure has not.

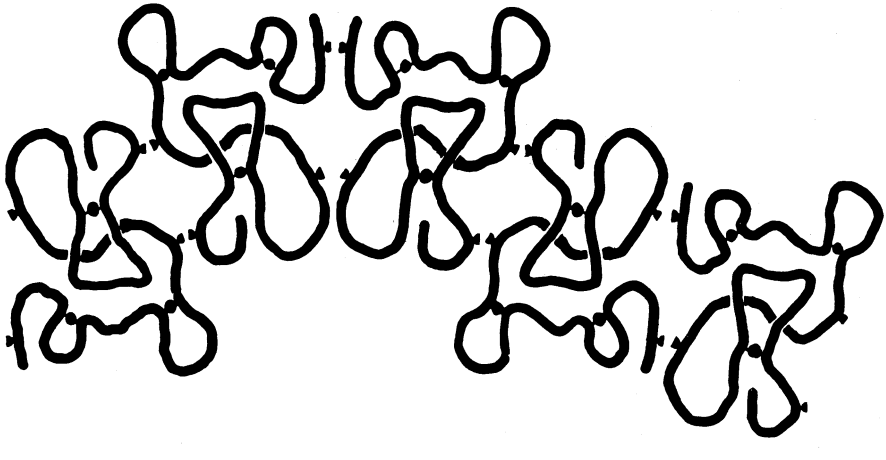

Fig. 1. Proposed model for linear glutenin: fragment of a single concatenation. Five polypeptide chains, in this case of the same kind, are each linked by two SS-bonds to one another. SS-Bonds, shown as dots, are separated in the case of interchain bonds. Polypeptide chains would be much more convoluted and coiled than appears from this stylized picture.

The resistance of dough is rapidly destroyed by SS-bond breaking agents because the concatenations are broken down. If the degree of polymerization (DP) of a concatenation is defined as the number of polypeptide chains in it, then for a sample with a number-average DP of 20, only 5.3% of the joins between chains need be severed to halve the DP of the sample.

The linear concept for glutenin is in accord with its rapid and extensive swelling in water: the nodes may indeed be formed during the swelling process, aided by mixing action.

Tensile Strength and Work Hardening

The tensile strength of glutenin, estimated from that of dough, is of the order of $1 MNm^{-2}$, less than 1% of that of wet wool. This result suggests that secondary forces only may determine the breaking stress of glutenin; the strength of wool depends on covalent bonds as well as secondary forces.

When a covalently cross-linked network is overstressed, even if fracture is incomplete, the test-piece is weaker than before because part of the network has been torn. The linear glutenin model predicts that in the case of dough, concatenations are orientated in the stress direction during extension: the improved organization of the secondary forces, together with the larger number of concatenations brought into play, increases the tensile stress. This phenomenon of work-hardening is well known to occur in dough.

Optimum Work Level and Overmixing

The fact that there is an optimum work maximum in the Chorleywood Bread Process can also be explained on the basis of the linear model. The work input during mixing draws out the concatenations so that they interlace the dough in

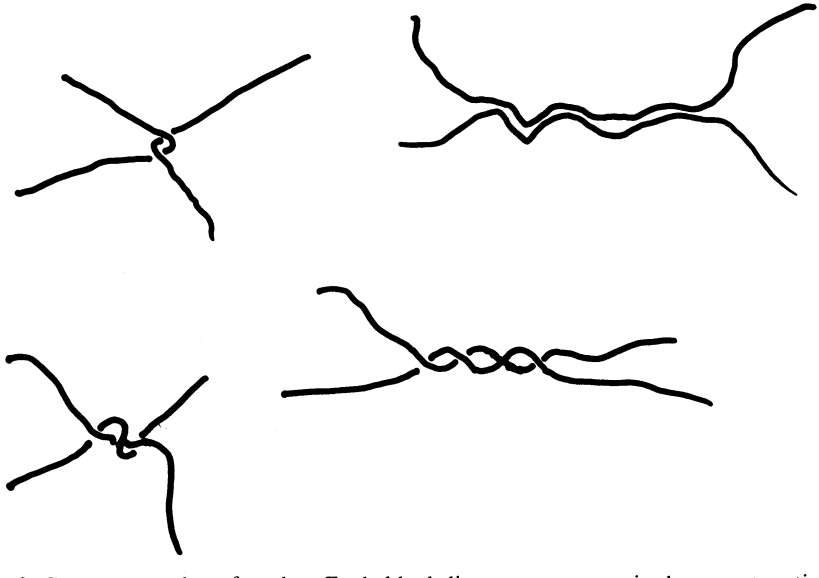

Fig. 2. Some examples of nodes. Each black line represents a single concatenation of polypeptide chains joined together by SS-bonds.

all directions, instead of being in the compact assemblies laid down during synthesis. If mixing continues for too long extension of the individual polypeptide chains in the orientated concatenations takes place. The native state of these chains is a coiled and folded conformation, stabilized by secondary forces, and the tendency when unfolded, to return to the native state of lower free energy is responsible for the elastic restoring force of the dough (Fig. 3). Extension of these chains increases the resistance to mixing still further. Stress unraveling of nodes becomes more widespread, and local stress concentrations cause covalent bonds to fail. On energetic considerations, the interchain SS-bonds are the ones which do so, lowering the DP, and giving two free radicals, S·, which then revert to SH by hydrogen abstraction from water. These SH-groups relieve adjacent stressed concatenations by SS-interchange. The dough loses resistance as the DP falls and stress is relieved rapidly. Overmixing produces a weak sloppy dough, which having had its glutenin DP drastically reduced, and lost many of its secondary cross-links, behaves like a viscous liquid.

Dronzek and Bushuk (3) have produced evidence that free radicals are produced in dough during mixing, and three other groups of workers (4–6) have observed increases in the SH content of dough on mixing under nitrogen.

Lability of SS-Bonds During Stress

Evidence has been adduced that stressed SS-bonds in keratin show increased reactivity (7). Whether this results from activation or merely from improved accessibility, similar behavior can reasonably be imputed to glutenin. Therefore, it is postulated that when SS-bonds between chains are exposed by stress (Fig. 4), they may be severed by interchange with thiols, either those naturally present or created by mechanical scission.

Stress Relaxation and Improvers

This mechanism together with the return of polypeptide chains to the folded

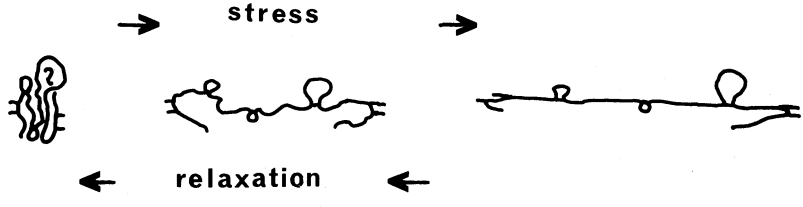

Fig. 3. Unfolding of a polypeptide chain in a glutenin concatenation under stress. The tendency of the unfolded chain to return to the compact native form of lower free energy is thought to be mainly responsible for the elastic restoring force in dough.

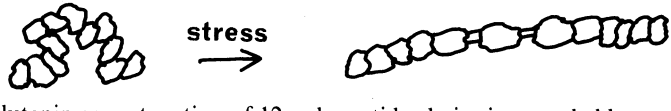

Fig. 4. A glutenin concatenation of 12 polypeptide chains is extended by stress exposing SS-bonds.

state govern the decay of dough stress with time. Stress relaxation can be controlled by oxidizing improvers which lower the thiol concentration and, therefore, the probability that a stressed link will be severed. Stress relaxation has then to depend much more on the slower process whereby Brownian movement enables chains to slip past one another in order to recover their individual native conformations.

Origin of Glutelins

Normally, mutational changes are observed infrequently because of the rigid structural conditions to which proteins must conform in order to function properly in metabolism. Storage proteins in seeds have much less stringent structural requirements to fulfill since their object seems only to be digested; there is no evidence that they play any other part in metabolism or in maintaining plant structure. During evolution, storage proteins may have mutated rapidly, therefore, as presumably all the mutations were preserved. Eventually, a mutation would lead to a major change of conformation—the newly synthesized polypeptide chain as it came off the polyribosome would no longer fold into the conformation that prevailed before the last mutation and brought the SH-groups of cysteine residues into juxtaposition so that they could easily be oxidized to SS-bonds. The new conformation would be such that one or two SS-bonds could not form, their component cysteine residues being widely separated (Fig. 5). These exposed cysteine residues could, however, be oxidatively joined to their counterparts on other polypeptide chains. In this way, cross-linked, macromolecular structures, known as glutelins, were created. The hypothesis put forward here is that in the cases of viscoelastic cereals, wheat, rye, and barley, if there are four exposed cysteine residues they will be in two pairs, and those in each pair will not be far enough apart to be attached to different polypeptide chains. This material will ensure linear concatenations.

No restriction is placed on chains of different kinds joining the same concatenation. Large and small chains could be mixed; such a distribution might enable the concatenation to form stronger nodes. Conversely, it is not unlikely

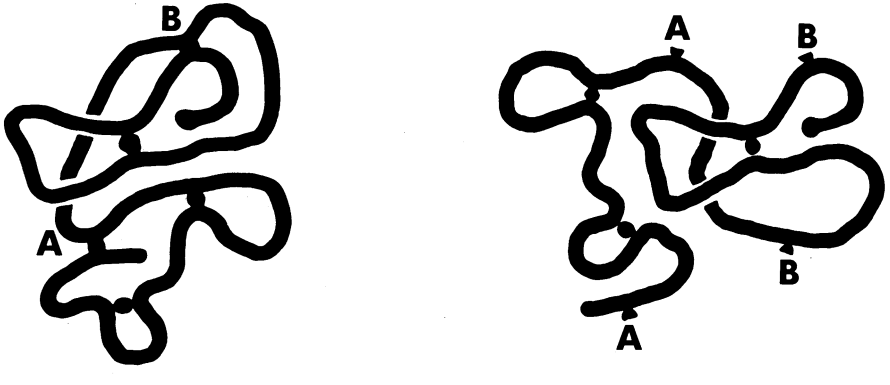

Fig. 5. Stylized impression of how an ancestral glutenin protein (**a**) changes after a mutation so that two of the SS-bonds, A and B, are no longer able to form intramolecularly (**b**). The thick black line represents the polypeptide chain.

that polypeptide chains, accumulating in the neighborhood of the polyribosome synthesizing them, will tend to polymerize among themselves. Until more is understood about glutelin synthesis and structure, the question whether or not glutelin concatenations are homogeneous as regards type of chain is an open one.

Possibility of Some 3-Dimensional Cross-Linking

If concatenations were rigidly homogeneous as regards the type of chain, this possibility would conceivably permit some kinds of chain to polymerize trifunctionally (*i.e.*, attached to 3 different chains), thus going to 3-dimensional networks. The remaining linear polymers of glutenin would then be responsible, as before, for the rheological behavior of the dough which would be merely modified by the presence of 3-dimensionally cross-linked glutenin polymers and other dough constituents. It is emphasized that this is only a possibility. If, as seems likely, some interlinking between chains of different kinds occurs during and after synthesis, it would require only one kind to be trifunctional to cross-link the whole of the glutenin in the 3-dimensional or branching node.

Species Differences

The amino acid sequences of the chains must ensure sufficient polarity for the glutens to swell; *i.e.*, protein-water rather than protein-protein contacts must be preferred, but not so much that the chains become soluble, which would militate against effective nodes.

Differences in rheological behavior between wheat, rye, and barley glutelins may be governed, at least in part, by their relative positions between the two extremes of complete solubility, and complete inability to swell in water.

Observational Support

Bernardin and Kasarda (8,9) have observed under the microscope viscoelastic filaments being extruded from flour particles after wetting with water. Their observations are not incompatible with the model described here. Osmotic pressure will develop in an assembly of protein molecules as it is permeated by water and this pressure could force viscous material from the particles. Certainly the excellent photomicrographs these authors have produced suggest that endosperm protein has a fibrous nature. By analogy with the fact that known fiber-forming high polymers possess long linear molecules, this evidence supports the idea that linear macromolecules are predominant. From rheological studies Smith and Tschoegl (10) have concluded that there are no covalent cross-links in dough. Experimental evidence has been obtained (11) to suggest that there are two labile SS-bonds per glutenin polypeptide chain of mol wt 44,000. In this context labile means that they react with SO_3^{2-} at about 0°C in the absence of protein denaturing agents. Such SS-bonds are believed to be interchain. Intrachain bonds are more stabile because well organized secondary forces assist in holding adjacent parts of the chain in a folded conformation. It is significant that only a fraction of the total SS bonds are involved in dough resistance (12).

The hypothesis, discussed more fully elsewhere (13–16), is compatible with the observed facts and with such experimental evidence as is available.

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