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MINI REVIEW

On the Elasticity of Wheat Gluten

P. S. Belton

Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, U.K.

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ABSTRACT

The nature of the interprotein interactions in wheat gluten is discussed with particular reference to the role of the high molecular weight subunits. It is argued that the high molecular weight subunits interact with each other by disulphide bonds and hydrogen bonds. Dough working favours the formation of end to end disulphide bonds in the subunits and this increases the effective molecular weight of the subunit and hence the number of protein–protein interactions. Association of the subunits can also take place by interchain hydrogen bonding. So many hydrogen bonds are formed that not all can be broken simultaneously although there will be unbonded mobile regions (loops) and bonded regions (trains). Stretching extends loops and then causes the proteins to slide over one another. The elastic restoring force is provided by the re-establishment of the loop train equilibrium.

Keywords: gluten, dough, viscoelasticity, functionality.

INTRODUCTION

The role of high molecular weight subunits in the breadmaking quality of flour has been recognised since the pioneering work of Payne and co-workers[1]. A continuing problem in cereal research has been to explain the origins of the role of the HMW subunits; they are known to confer elasticity on the dough but the mechanism by which this is done is still unclear. Over recent years[2], more information has become available about the sequences of these proteins and their possible structures.

A particular feature of the HMW subunits is that they contain very large amounts of glutamine (35 mol%) and significant amounts of glycine (20 mol%) and proline (10 mol%)[3]. They are linear proteins which consist of C and N terminal domains, which are largely alpha helical, separated by long domains of repeat sequences. The C and N termini contain cysteine residues which are able to form disulphide links with residues on other subunits. The repeat domains are characterised by hexapeptide and nonapeptide repeats in the y-type subunits (PGQGQQ and GYYPTSLQQ) and by hexa-nona and tripeptide repeats in the x-type (PGQGQQ, GYYPTSPQQ and GQQ)[3].

The structure of these repeat domains are the subject of active investigation[3–5]. The evidence from HMW subunits, and proteins with similar sequences, is that they are characterised by high levels of mobility in the presence of water and that they consist of β-turns and β-sheets in proportions which vary with water content. The evidence obtained from spectroscopic results has lead to the suggestion[6,7] that hydrogen bonding between the repeat regions of the HMW subunits is responsible

ABBREVIATIONS USED: HMW = high molecular weight.

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for the elasticity of the gluten. In this paper, the model is considered in more detail and is extended to the role of HMW subunits in whole gluten.

THE ROLE OF HYDROGEN BONDING IN ELASTICITY

The essence of elasticity is that, following extension, a restoring force exists which tends to return the material to its original dimensions. The postulation of the existence of some crosslinking mechanism in doughs may explain the resistance of dough to extension but will not in itself explain elasticity. Thus, the well recognised existence of interprotein disulphide bonds in doughs can explain the plasticity of doughs but not their elasticity. On breaking disulphide bonds by extension there is no mechanism which will tend to restore the original dimensions of the material. New bonds may be formed but they will be the result of random motion and alignment by extension of the entities containing the disulphide groups.

MacRitchie and Lafiandra[8] discuss the effects of polymer/polymer interactions in more general terms by considering the polymer physicists' term 'entanglements'. These authors use a point entanglement model in which it is supposed that there are small regions where the polymers interact separated by extended, non-interacting regions. As the authors point out, in any system where there are partly unextended polymer chains interacting by such entanglements there will be consequences for the rheology of the system in terms of the effects of extension rate on the extensibility of the system. However, entanglements, although a generalisation of disulphide cross links, only explain the effects of extension rate on extensibility; they do not explain the elasticity of gluten. In addition, as pointed out below, the high concentration of polymers in the dough system makes a point entanglement model unlikely.

The simplest way of explaining a restoring force in crosslinked polymer networks is to invoke the phenomenon of rubber elasticity. In rubber, the equilibrium conformation of the chains is supposed to be a random coil; stretching the rubber results in the extension and ordering of the chains and thus a loss in entropy. This entropy loss provides the restoring force in the rubber since the contraction resulting from the reformation of the coil will restore the entropy lost on extension. Ewart[9] evoked this mechanism in a discussion of the gas-holding properties of dough, but failed crucially in his discussion to recognise that such a network would be entangled. The problem with rubber elasticity is that it assumes that the polymer units do not interact with each other in any chemical way and that there are no solvent–polymer interactions to consider. Both of these factors, which will be both enthalpic and entropic, are of major importance in proteins. Thus, when considering proteins it is important to bear in mind that it is the Gibbs free energy, that is the balance between enthalpy and entropy, that determines the equilibrium state, not the entropy alone.

Consideration of the repeat sequence structures of HMW subunits show that they contain a very high level of glutamine. In subunit 1Dx5[2], the ratio of glutamine to other amino acids is about 1·1:1. The glutamines occur 102 times as pairs of residues, 90 times separated by a single amino acid, 69 times separated by two amino acids and 19 times separated by seven amino acids. The repeat sequences are thus very hydrophilic along their whole length and, because of the structure of glutamine, will have a very high capacity to form both intra- and inter-molecular hydrogen bonds. In the dough, the levels of hydration of the protein are low. A typical dough contains about 0·8 g of water per gram of dry flour. Assuming that the water is equally distributed between protein and starch there will be the same amount of water per gram of protein. Since the mean molecular weight of gluten is about 126·5 Daltons per residue, there will be about six molecules of water available per residue. This represents a very concentrated system in which interprotein contacts will be very significant.

The starting point for a model to explain the elasticity of the HMW subunits assumes that an isolated protein may be represented as a long chain made 'sticky' by the high density of polar hydrogen bonding groups. The globular ends contain cysteine residues. In the absence of water the chains will tend to hydrogen bond to each other to form a dense mass [Fig. 1(a)]. As water is added there will an increase in the number of water–protein hydrogen bonds formed, but the number of interchain hydrogen bonds will ensure that it is very unlikely that all the interchain hydrogen bonds will break simultaneously [Fig. 1(b),(c)]. Thus there will always be a balance between the residues involved in interchain hydrogen bonds and those which are hydrated. A similar situation occurs in the absorption of poly-

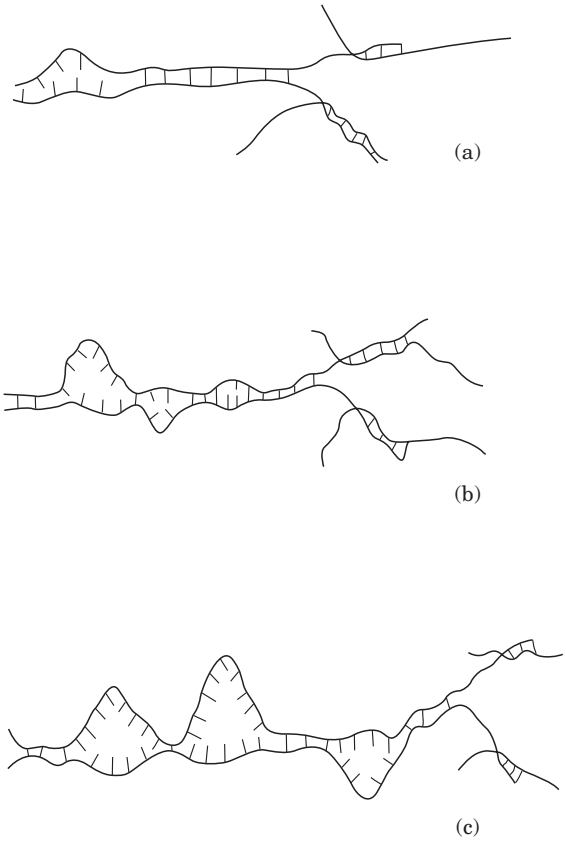

Figure 1 The effects of hydration on the loop and train behaviour of HMW subunits. For simplicity, interactions between two chains are shown. In reality many chains may interact. (a) Low levels of hydration – hydrogen bonds are mainly interchain. (b) Intermediate levels of hydration – some loops are formed. (c) High levels of hydration – the system contains many loops but sufficient interchain bonds exist to maintain interchain contacts.

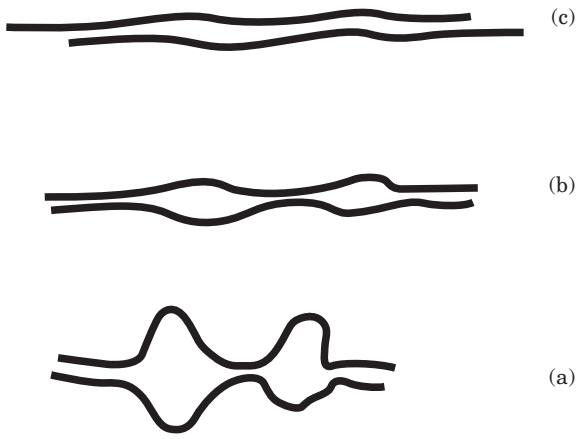

Figure 2 The deformation of polymers resulting from extending the network. (a) The equilibrium configuration. (b) Small extension – only the loops are deformed. (c) Large extension loops are flattened and the interchain hydrogen bonds are broken so that the chains slip over each other.

mers on surfaces: although the interactions of each individual residue with the surface may be weak the number of interactions ensures that when a polymer is absorbed it is very unlikely to desorb because the statistical probability of all the surface polymer interactions terminating simultaneously is very low. This effect leads to the formation of 'loops and trains'. That is, regions where there are groups of polymer surface interactions and regions where there are groups of polymer solvent interactions.

The train region is probably associated with β-sheet formation. There is evidence[10], from infrared spectroscopy, that on hydration from the dry state the quantity of β-sheet first increases then decreases. The increase is associated with ordering resulting from the transition from the glassy to rubbery region. As hydration proceeds, the amount of train region in the β-sheet conformation is reduced and the hydrated loop region increases. This is identified with the formation of extended hydrated β-turn structures[10]. NMR results suggest that the loop regions are associated with mobile polymer regions[5]. In general, it would be expected that any one chain will form trains with a number of other polymers and thus an interconnected network will form. On stretching, the network will first deform by deformation of the loops and then by the trains being pulled apart (Fig. 2). The structure will relax by returning to the equilibrium of loops and trains. The restoring force will consist of an entropic term associated with the conformational entropy of the loops and with the enthalpy of hydrogen bond formation in the trains, the entropy loss resulting from the interchain hydrogen bond formation will, in part, be compensated by the increased entropy of the hydrogen bonded water released.

It is interesting to compare this model with the proposals of Ewart[12,13], which also suggest the existence of loops in the gluten network[12]. The major difference in the Ewart model was the idea that the loops were formed by a crosslinking disulphide bridge. Specifically, Ewart proposed that when the chain was under tension the disulphide bridge took the strain and preserved the loop. In addition, it was supposed that the 'glutenin molecule' consisted of a linear concatenation of

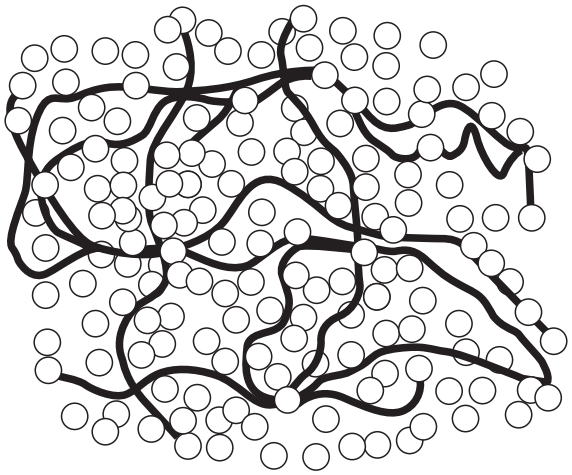

Figure 3 A model for the molecular structure of gluten. HMW subunits are approximated by linear polymers, interchain disulphide links are not shown. Other polymers are approximated by spheres.

loops which formed an extended chain[12,13]. This, clearly, is a very different approach to the one proposed here in which the fundamental linear molecule is the high molecular weight subunit.

THE VISCOELASTICITY OF GLUTEN

The explanation so far only accounts for the behaviour of HMW subunits, it does not account for the behaviour of gluten or the importance of disulphide bonds in gluten viscoelasticity. A very simplified model of gluten at the molecular level is shown in Figure 3. Only two classes of protein are considered: linear proteins and globular proteins. The linear proteins interact with each other via the loop and train mechanism and by disulphide bonding. In the diagram, for simplicity, disulphide bonds are not shown. As a first approximation, the chains are imagined to interact with the globular proteins by non-bonding forces such as Van de Waals interactions. The number of linear–linear protein interactions as well as the number of linear–globular protein interactions will depend on the effective length of the linear proteins. If the proteins are joined end to end by disulphide linkages at their N-terminal and C-terminal regions their effective length will be much greater than if no such bonds exist. Initially, before any mechanical energy input into the dough, all possible arrangements of interchain disulphide linkages may exist. There may, for example, be side-to-side dimers or cyclic polymers. Repeated elongation combined with the making and breaking of disulphide linkages will cause alignment of the linear proteins during elongation and favour the formation of end-to-end polymers. This effectively increases the molecular weight and will result in the number of polymer–polymer interactions increasing. Since each extended polymer will be able to interact with a greater part of the total matrix, the resistance to deformation and the restoring force after deformation will be increased. Thus, there will be a tendency for repeated elongation to cause work hardening. Clearly, if the system is strained sufficiently to totally disrupt the structure, loss of strength will occur and strain thinning will be observed. Thus, it is possible to see how a maximum in dough strength arises in the dough mixing process.

The model proposed has some clear and testable predictions to make (in addition to those made previously[7]) about the effects of the nature of the proteins on gluten rheology. Gliadins form a matrix within which the critical deformation of the long polymers takes place. They contribute to resistance to extension by forming a viscous environment, any displacement of the long polymers must displace some globular polymers. If the interaction between the globular polymers is increased, their viscous resistance will be increased and there will thus be an increased resistance to extension. There should also be a slowing down of elastic recovery since the re-establishment of the loop-train equilibrium will depend on the rates of polymer segmental motion, which will be dependent on environmental viscosity.

Resistance to extension will also be dependent on the loop-train ratio. It is to be expected that the energy required to deform loops will be less than that required to 'unzip' trains. An increase in the train content of the system will therefore increase the resistance to extension. Since trains are associated with β-sheet formation, it would be expected that the resistance to extension would be dependent on the relative proportion of the HMW subunits in the β-sheet conformation. The loop-train model also implies, assuming loops are more easily deformed than trains, that at deformations small enough to only deform the loops, the energy required per unit length extension will be less than that required at greater extensions where the trains are disrupted.

The length and nature of the repeat region in the HMW subunit will play a role in the viscoelastic properties. If the number of proteins remains con-

stant, increasing the length of the repeat unit will result in more polymer–polymer interactions and thus increased resistance to extension and a higher degree of extension before the limit case of disulphide bond breaking is reached. The presence of a disulphide link in the repeat sequence, such as in the 1Dx5 subunit, would act rather like the effects of vulcanisation on rubber. The degree of extension before a considerably increased resistance was experienced would be reduced and the elastic recovery of the system would be enhanced, as the distance for diffusion of the chain segments before restoration of equilibrium would be reduced.

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