Structure and Function of Gluten Proteins[1]

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ABSTRACT

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Proteins contribute to the nutritional value of foodstuffs, as well as to their physical properties, by forming and stabilizing gels, foams, emulsions, fibers, and doughs. The relations between structural and functional properties are illustrated by examples taken from the cereal proteins. The special position of wheat among the cereals comes from its capability to form gluten by interaction between the less polar prolamins and the more polar glutelins. The rheological properties of gluten are influenced by the proportion of prolamins to glutelins and by the hydrophobicity of the prolamins. Within the glutelin fraction, the very high-molecular, glycine-rich components are of particular importance. High amounts of these proteins are typical of wheat varieties with good baking properties. These high-molecular components are built up of subunits, probably via disulfide bridges, during dough mixing in the presence of oxygen. In the course of a stepwise reduction of the glutelin disulfide bonds, prolamin-like components are liberated first, whereas the components rich in glycine and most characteristic for glutelin remain in a highly aggregated state until the reduction is more complete. Besides disulfide bonds, ionic bonds seem to be of great importance to gluten structure, because rheological properties may be changed very strongly by addition of low-molecular-weight divalent ions. These facts are discussed in connection with different gluten models.

One important concern of chemistry is to understand the relationship between the chemical structure of a compound and its functionality. If we look at cereals, for example, we find that the unique position of wheat within this group depends on its ability to form gluten. Even cereals like rye and barley, which are close phylogenetic relatives, do not have this ability.

Gluten is a cohesive, viscoelastic mass, which can be stretched. Figure 1 shows pictures of gluten obtained by scanning electron microscopy. The gluten was fixed in the relaxed (Fig. 1a) and in the stretched state (Fig. 1b) by freezing in liquid nitrogen and subsequent lyophilization. The stressed state can be easily recognized by the elongated pores (R. Kieffer et al, *unpublished*). The question is, Which structural elements are responsible for these very special properties of gluten?

ROLE OF DISULFIDE BONDS

Many authors have shown that there is a relationship between disulfide bonds and the strength of gluten. Reviews are given among others by Bloksma (1975) and Ewart (1977, 1978). The importance of the high-molecular-weight protein fractions for dough strength and loaf volume is also well established (Pomeranz 1965, Orth and Bushuk 1972, Huebner and Wall 1976).

Reduction of disulfide bonds weaken the gluten. Disulfide reduction is accompanied by a decrease in the amount of protein fractions I and IIa, which have very high molecular weights (Fig. 2). It is remarkable that the decrease of these high-molecular-weight fractions is not linearly related to the percentage of reduced disulfide bonds (Fig. 3): the major changes in the protein content of fractions I and IIa have already occurred when only 4–5% of the total disulfide bonds has been reduced (Seeger and Belitz 1981). These results show that the disulfide bonds are different in their functional importance, some being particularly important for rheological properties and easily reducible.

Oxygen plays an important role during dough preparation: mixing flour from Canadian hard red spring wheat under air leads to stronger doughs (higher resistance, lower extensibility) than mixing under nitrogen (Tsen and Bushuk 1963). The concentration of thiol groups decreases very quickly during mixing under air, down to about 50% of the original value (Sullivan et al 1963), whereas a slight increase was observed under nitrogen (Mecham and Knapp 1966).

If wheat varieties with different rheological and baking properties are milled under nitrogen, and if the gluten is also washed out under nitrogen, the resulting glutens all are relatively weak, as can be seen from their extensigrams (Fig. 4). This is true even for good varieties such as Monopol and Schirokko. The difference between good and poor varieties is evident only if the gluten is washed out under oxygen or in the presence of other oxidizing agents, such as bromate. It is also possible to oxidize the gluten obtained under nitrogen with oxygen or air in a dry state after lyophilization. Such a gluten, oxidized in a subsequent step, exhibits the same rheological properties as a gluten washed out under oxygen. This oxidative strengthening of gluten occurs only with good varieties (Monopol, Schirokko). Poor varieties (Clement, Maris-Huntsman) show virtually no changes by oxidation (Dirndorfer et al 1986). The increase of gluten strength is accompanied by an increase of the protein fraction with the highest molecular weight (Fig. 5).

These results show that the protein fractions responsible for gluten strength occur within the grain in a reduced state or that they are reduced during dough mixing under nitrogen. The oxidative polymerization of the proteins to fractions with very high molecular weights could not have occurred before the dough was mixed in the presence of oxygen. An essential prerequisite for a strong gluten is the occurrence of protein monomers suitable for oxidation. In the case of strong wheat varieties, the oxidative polymerization of these suitable monomers is also possible in lyophilized gluten, i.e., in the nearly complete absence of low-molecular-weight compounds. The role of low-molecular-weight components in connection with gluten formation should therefore not be over estimated: the essential point is the availability of proteins suitable for oxidative polymerization.

Role of Ionic Bonds

Besides disulfide bonds, ionic bonds are of great importance for interactions between the gluten proteins and therefore for gluten strength. This is surprising, because amino acids with acid and basic side chains occur only in low amounts in comparison to amino acids bearing hydrophobic or amide groups (Kasarda et al 1971, Wieser et al 1981, Belitz et al 1982, Wieser et al 1983). This importance of ionic bonds can be demonstrated by the effect of bipolar ions such as amino acids on the rheological properties of gluten (Kieffer et al 1983). Glycine causes a significant increase in gluten strength, as do some other amino acids. The effect of different amino acids depends on the distance between carboxylic and amino groups, and on the size of the side chain (Fig. 6). Dicarboxylic acids also strengthen the gluten, whereas diamines weaken it. These effects are plausible because of the slight positive net charge of gluten at the normal pH range of dough. One can suggest that amino acids as well as dicarboxylic acids act as spacers and form additional ionic bonds that strengthen the protein network because they form additional crosslinks.
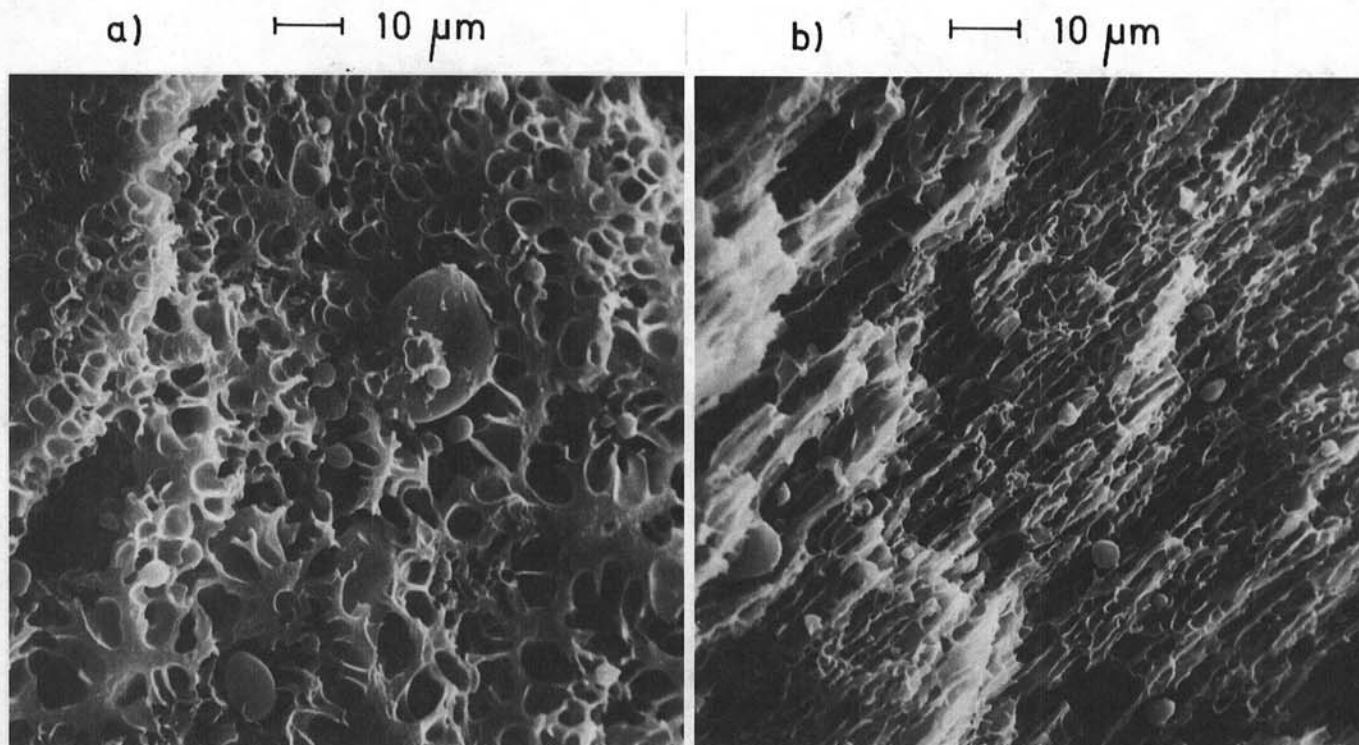
Fig. 1. Scanning electron microscopy of relaxed (**a**) and stretched (**b**) gluten fixed in liquid nitrogen and lyophilized.
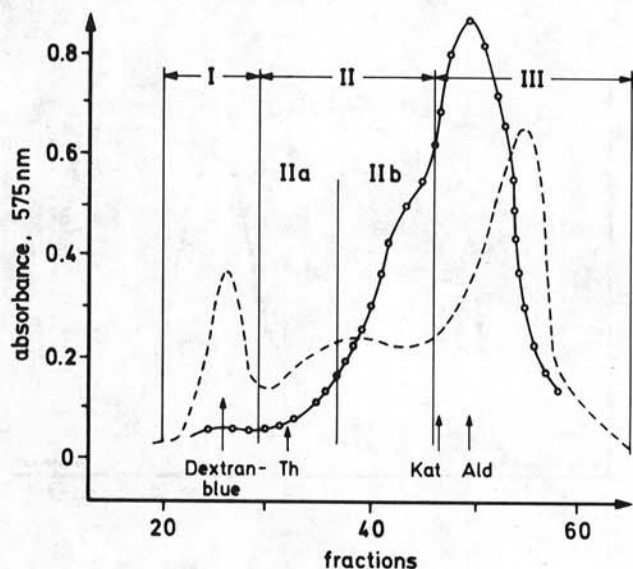

Fig. 2. Separation of succinylated gluten (variety Kolibri) on Sepharose 6B-Cl before (----) and after (o-o-o-o) total reduction of disulfide bonds with dithiothreitol. Elution with 0.5% triethylamine/acetic acid, pH 7.5; the protein content of the fractions was determined by reaction with ninhydrin after alkaline hydrolysis; molecular weights of marker proteins: thyroglobulin (**Th**) 800 kDa, catalase (**Kat**) 240 kDa, aldolase (**Ald**) 145 kDa.
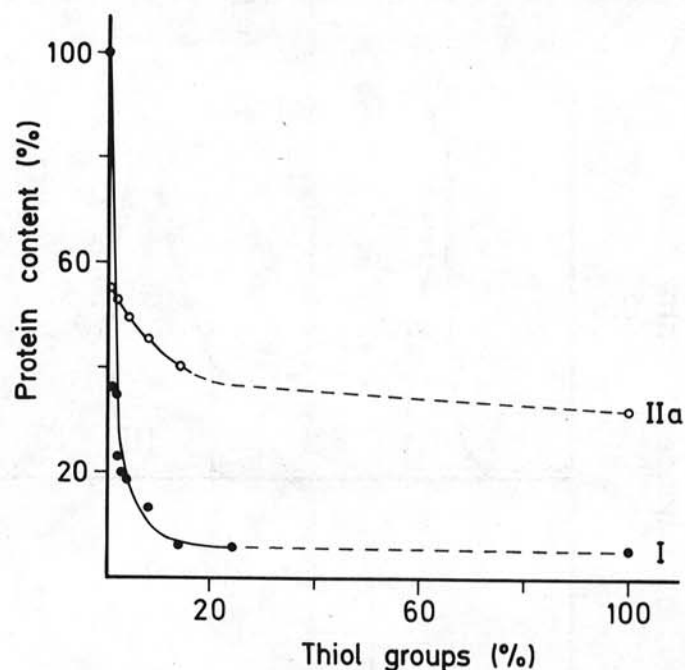

Fig. 3. Decrease in the amount of the high-molecular-weight protein fractions I and IIa after stepwise reduction of succinylated gluten (variety Kolibri) with mercaptoethanol.

Role of Interactions Between Prolamins and Glutelins

The formation of gluten when flour is wetted is mainly caused by a specific interaction between two protein fractions: the apolar prolamins and the more polar glutelins. The average hydrophobicities of these two protein fractions from different cereals, calculated from their amino acid compositions, are given in Table I. Usually the elastic properties of gluten are ascribed to the glutelin fraction, whereas the viscous properties come from the prolamin fraction (Kasarda et al 1971). To simplify matters, the prolamins can be understood as a solvent for the glutelins.

According to the theory of entropic elasticity, an increase in the amount of solvent should weaken the elastic properties of gluten by decreasing the number of cross points. Figure 7 shows extensigrams of gluten to which prolamins of different cereals were added (R. Kieffer et al, *unpublished*). As expected, the prolamins of wheat, rye, and barley weaken the gluten, but the maize prolamin zein, which is significantly more hydrophobic than the other prolamins, has a contrary effect: it strengthens the gluten. Obviously the increase in "solvent" hydrophobicity strengthens the ionic bonds, which are of great importance for gluten elasticity, as
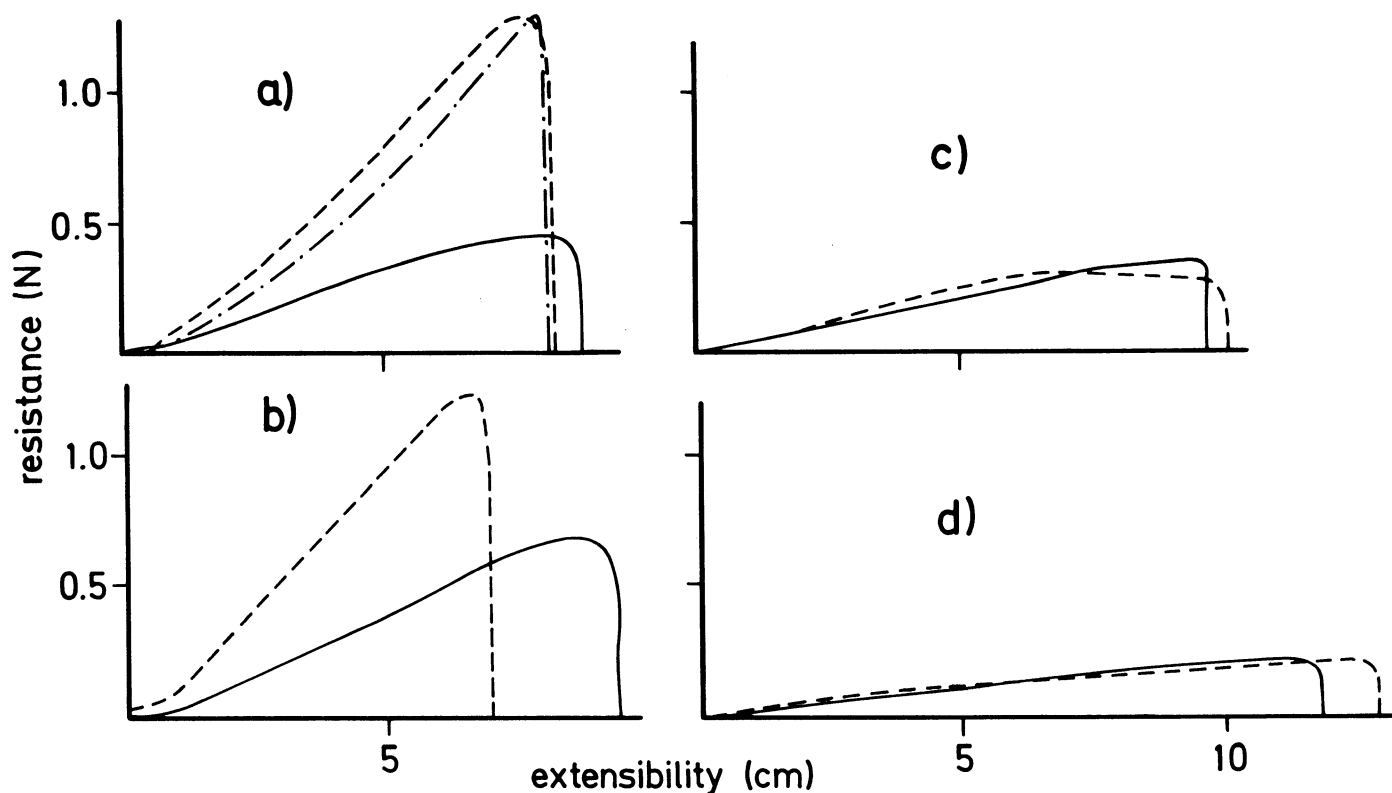
Fig. 4. Extensigrams of glutens from different wheat varieties: **a,** Monopol; **b,** Schirokko; **c,** Clement; and **d,** Maris-Huntsman. Load-extension tests were performed according to Kieffer et al (1981). Glutens were washed out under air (----), under N_2 (——), or under N_2 in the presence of bromate (-·-··-).
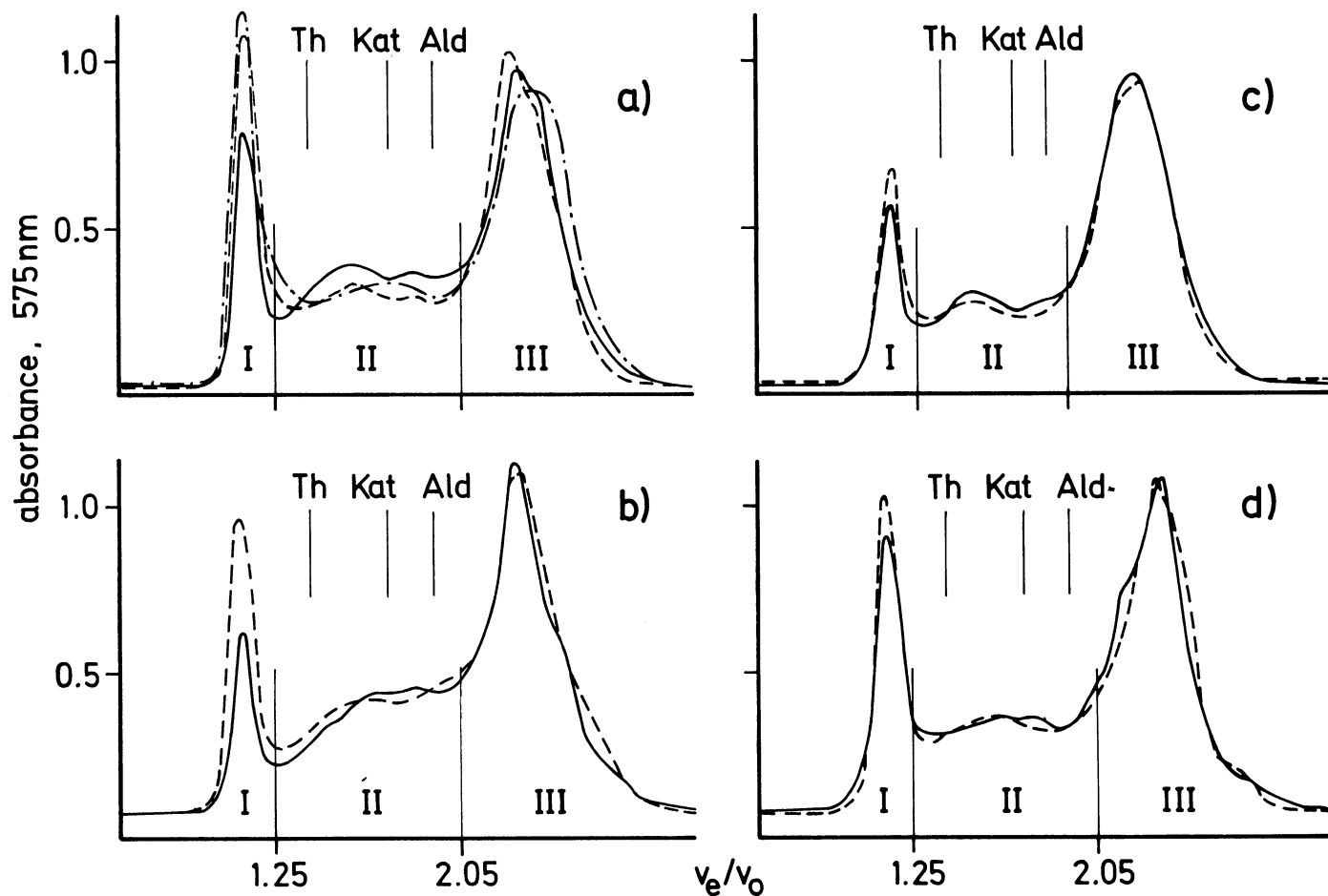

Fig. 5. Separation of succinylated glutens from different wheat varieties on Sepharose 6B-C1. The glutens were washed out under air (----), under N_2 (——), or under N_2 in the presence of bromate (-·-··-). Varieties: **a,** Monopol; **b,** Schirokko; **c,** Maris-Huntsman; and **d,** Clement. Elution conditions and marker proteins as in Fig. 2.

mentioned before.

The shear modulus G of dough and gluten can be measured by high-pressure capillary viscosimetry according to the following equation (Kieffer et al 1982):

$$\ln \eta = \ln \eta_0 - \frac{1}{G} \cdot \tau,$$

where τ = shear stress, η = viscosity, G = shear modulus, and $1/G$ = elastic deformability.

Figure 8 shows that an increasing amount of prolamin causes a decrease in the shear modulus of gluten (R. Kieffer et al, *unpublished*). These results are in accordance with the load-extension
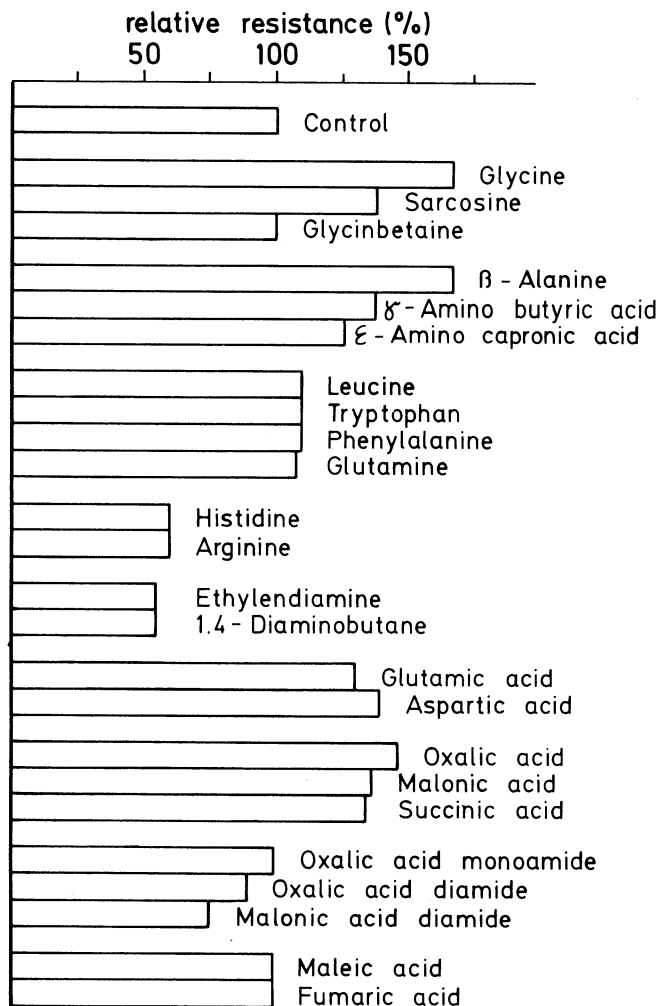
Fig. 6. Relative resistance (% of control) of wheat gluten (variety Kolibri) after addition (3.7%, w/w) of different amino acids, diamines, and dicarboxylic acids. The resistance values are the maximum heights of the extensigrams obtained according to Kieffer et al (1981).
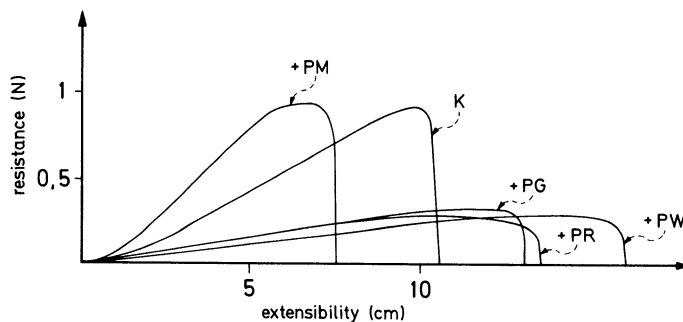

Fig. 7. Extensigrams of wheat gluten after adding prolamins (50% related to dry gluten) from different cereals. The extension tests were performed according to Kieffer et al (1981). **K,** Control; **PW,** wheat prolamin; **PR,** rye prolamin; **PG,** barley prolamin; and **PM,** maize prolamin.
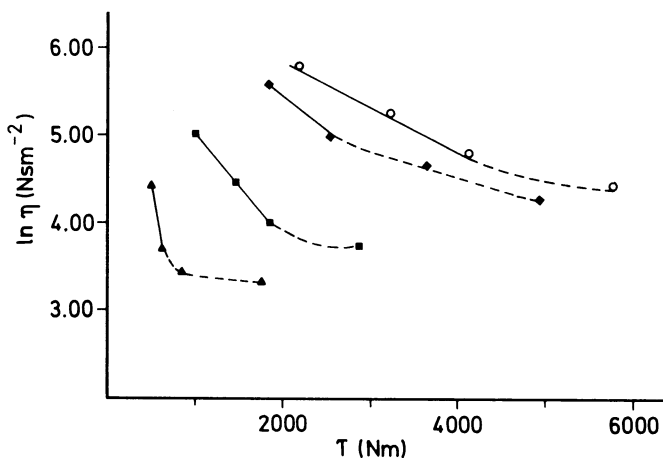

Fig. 8. High-pressure capillary viscosimetry of wheat gluten after addition of different amounts of gliadin. The measurements were performed according to Kieffer et al (1982). Control, O---O; 20%, ◆----◆; 30%, ■----■; and 50%, ▲----▲; gliadin related to dry gluten.

TABLE II
Amino Acid Composition (mol %) of High-Molecular-Weight Peptide Fractions from Rye (GR), Barley (GG), and Wheat (GW) Glutelins[a]

Amino Acid	GR 100a	GG 100	GW										
			100	1,000	1,001	1,002	1,003	1,004	1,005	1,006	1,007	1,008	1,009
Glutamic acid	48.0	42.3	49.9	54.3	53.0	47.2	46.1	42.1	45.6	43.9	44.9	40.5	43.9
Proline	32.1	40.2	19.8	12.8	13.7	15.2	16.8	17.2	29.8	32.7	30.3	32.0	33.2
Glycine	3.1	0.8	19.6	29.0	30.7	28.0	27.0	26.9	3.6	2.0	2.7	2.1	3.4
Phenylalanine	6.7	6.2	3.3	0.0	0.0	0.0	0.0	0.0	9.7	10.1	10.4	10.5	9.2
Leucine	2.4	0.0	1.7	1.9	0.0	0.7	0.6	0.0	4.5	3.8	3.4	3.7	2.5
Others[b]	7.7	10.5	5.7	2.0	2.6	8.9	9.5	13.8	6.8	7.5	8.3	11.2	7.8

[a]The fractions GR 100a, GG 100, and GW 100 were obtained from chymotryptic hydrolysates of the corresponding glutelins by subsequent chromatographies on Sephadex G25, Dowex 50 WX 2 and Durrum DA X 2-20. The fractions GW 1,000 to 1,009 were obtained from fraction GW 100 by RP-HPLC (ODS, elution system: triethylammonium formiate 0.01 mol/1, pH 6/acetonitrile: 95+5→60+40, v/v).
[b]Sum of other amino acids.

tests of Figure 7 and demonstrate that the prolamin content of gluten really changes the elastic deformability.

ROLE OF GLYCINE-RICH SEQUENCES

It is well known from the literature that high-molecular-weight subunits from reduced wheat glutenin contain remarkably high amounts of glycine (Huebner et al 1974, Shewry et al 1984). The results described here and many others reported indicate the very special role of the high-molecular-weight protein fraction for the functional properties of wheat gluten. Therefore it is of interest to look for the structural difference between this fraction and the corresponding fractions from other cereals, for example, rye or barley.
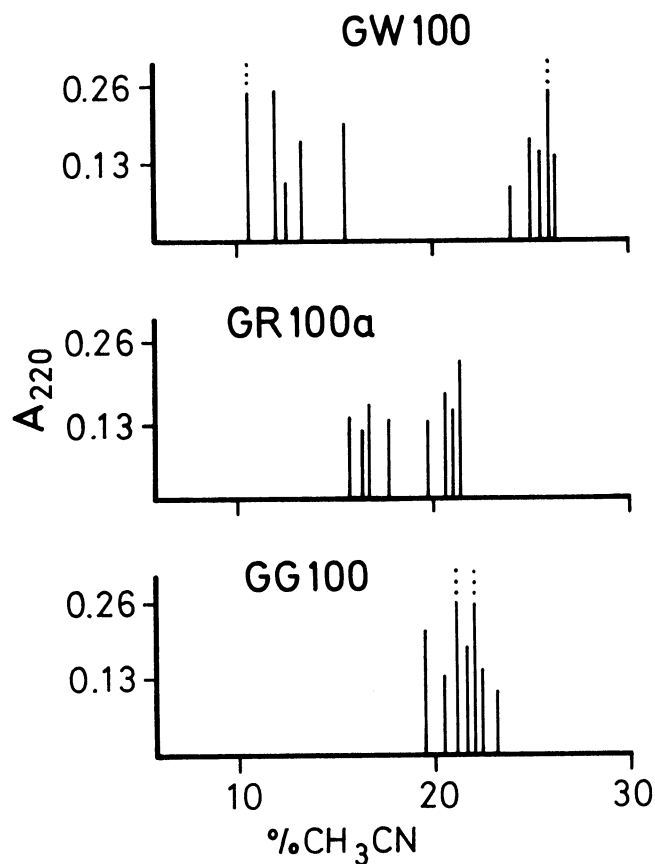

Fig. 9. Peptide patterns obtained by separation of high-molecular-weight chymotryptic peptide fractions from the glutelins of wheat (**GW 100**), rye (**GR 100a**), and barley (**GG 100**) using reverse-phase high-performance liquid chromatography on ODS-Hypersil. The peptide fractions GW 100, GR 100a, and GG 100 were obtained by subsequent chromatographies of the chymotryptic hydrolysates of the corresponding glutelins on Sephadex G25, Dowex 50 WX 2, and Durrum DA X 2-20. Elution with triethylamine formiate buffer pH 6.0/acetonitrile, linear gradient (according to Belitz et al 1984).

OQPAQGQQGQQPGQGQQGQQPGQGQ..   SQQQPPFSQQGQQ−

YPTSPQQSGQGQQPGQGQ−   SPLQPGQGQPGQ−

YPTSPQQPGQGQQPGQL−   YPTSPQQPGQQL−

VQGQGQIQPQQPAQL−   GSIQTPQQQPQ−

YPISPQQPGQGQQSG−   OGQGQQGQQPG..

YPTSPQQPGQGQQL−   GQQPQQQQL−

Fig. 10. Amino acid sequences of glycine-containing peptides from a chymotryptic hydrolysate of wheat glutelin. One-letter code for amino acids: A, alanine; G, glycine; I, isoleucine; L, leucine; O, pyroglutamic acid; P, proline; Q, glutamine; S, serine; T, threonine; V, valine; Y, tyrosine; and . . , incomplete sequence.

We have tried to answer this question by investigating typical peptide fragments from the glutelins of different cereals (Belitz et al 1984). The fragments were obtained by chymotryptic hydrolysis and separation of the partial hydrolysates by several subsequent steps (gel chromatography, cation and anion exchange chromatography, and reversed-phase high-performance liquid chromatography). Only wheat had a high-molecular-weight peptide fraction (GW 100) containing high amounts of glycine (Table II). Corresponding fractions from rye (GR 100a) and barley (GG 100) had completely different amino acid compositions. Further separation by reversed-phase high-performance liquid chromatography (Fig. 9) resulted only in the wheat fraction being split into two peptide groups of different polarity. As expected, the amino acid compositions of these two peptide groups were very different: the peptides of the relatively apolar group (GW 1005−1009) are closely related to the prolamines, whereas the more polar peptides (GW 1000−1004) are marked by a very high glycine content (Table II). Such peptides occur only in wheat glutelin and were not observed in any of the prolamin or glutelin fractions of other cereals. Figure 10 shows the amino acid sequences of several peptides of this type (Wieser et al 1984, H. Wieser et al, *unpublished*), using one-letter symbols for the amino acids. Characteristic tripeptide sequences and their relative frequencies (in percentages of total tripeptide sequences) as reported by Wieser et al (1984) are QGQ, 7.1; GQG, 9.5; GQQ, 6.0; QPG, 8.3; PGQ, 8.3; and QQP, 7.1% of 84 sequences. These are also predominant in a partial amino acid sequence of a wheat glutelin postulated by Thompson et al (1983) and based on the nucleic acid sequence of the corresponding gene: QGQ, 10.0; GQG, 9.2; GQQ, 7.9; QPG, 5.9; PGQ, 5.0; and QQP, 3.8% of 239 sequences.

It is interesting to note that the molecular basis of the elastic properties of elastin, a protein of connective tissue, is attributed to the occurrence of sequences rich in glycine (Urry et al 1983): the characteristic conformational feature is a β-spiral, that is an α-helix that contains β-turns. The β-turns are linked by glycine residues, which for steric reasons cannot be replaced by any other amino acid residue (Fig. 11). Glycine-rich sequences of wheat glutelin can be arranged in a similar manner (Fig. 12). This structural relationship between elastin and glutelin suggests the possibility that the glycine-rich sequences in wheat are responsible for the elastic properties (Tatham et al 1984, 1985). Comparisons of the chymotryptic hydrolysates of the glutelin fractions from different wheat varieties (Wieser et al 1985) show that glycine-rich peptides occur in significantly higher amounts in strong varieties (Fig. 13). It is remarkable that a sample of durum wheat included in these investigations shows a peptide pattern in which the glycine-rich fraction is nearly absent.

CONCLUSION

There are many indications to the crucial structural elements of the gluten proteins: disulfide bonds, ionic bonds, glycine-rich
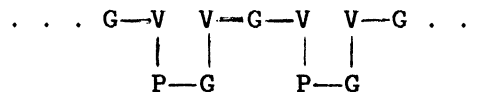

Fig. 11. The characteristic section of the elastin chain contains β-turns linked by glycine residues.
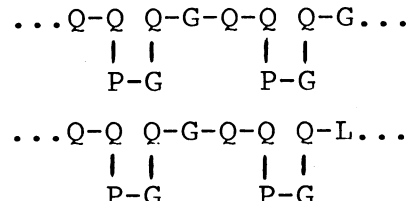

Fig. 12. Glycine-rich sequences of wheat gluten can be arranged in a similar manner as those of elastin: top, sequence from Thompson et al (1983), and bottom, from Wieser et al (*unpublished*).
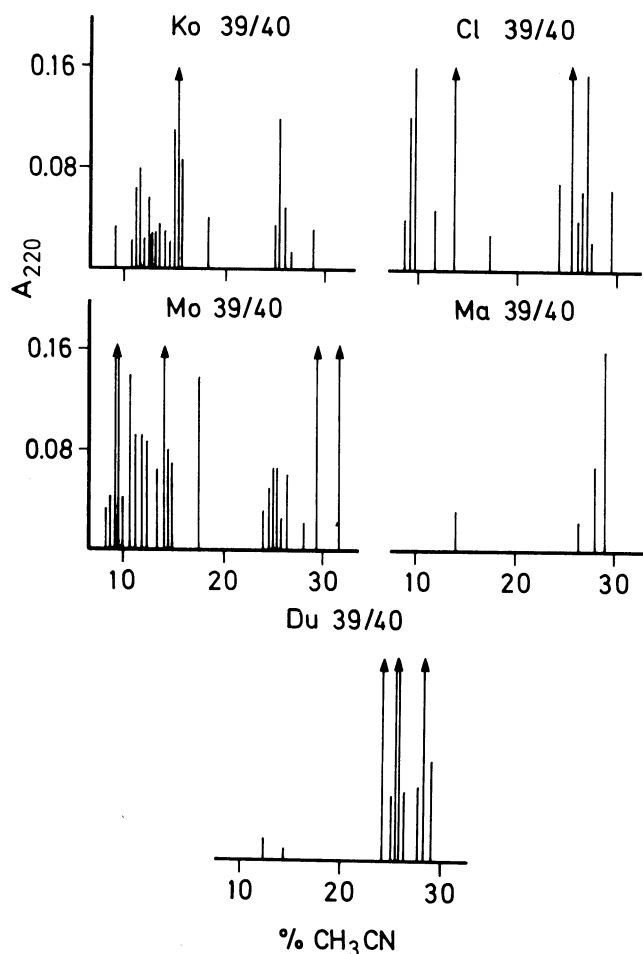
Fig. 13. Peptide patterns obtained by reversed-phase high-performance liquid chromatography of high-molecular-weight peptide fractions from the glutelins of different wheat varieties: Ko, Kolibri; Cl, Clement; Mo, Monopol; Ma, Maris-Huntsman; and Du, Durum wheat. The peptide fractions 39/40 were obtained by chymotryptic hydrolysis and gel chromatography on Sephadex G50. Elution conditions were as in Fig. 9; the ordinate values are percent acetonitrile. Glycine-rich peptides are eluted between 8 and 18% acetonitrile. Arrows indicate $A_{220} > 0.16$.

sequences, and so on. Based on this knowledge, it is now necessary to look in more detail at the properties of individual proteins or groups of proteins, especially at their redox behavior. The differences in the protein composition of varieties with different rheological properties may be helpful in selecting components having special functional importance out of the large number of similar proteins present.

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