Identity of High Molecular Weight Gliadin and Ethanol-Soluble Glutenin Subunits of Wheat: Relation to Gluten Structure[1]

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ABSTRACT

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The exact relationship, origin, and function of high molecular weight gliadin (HMWG) and ethanol-soluble reduced glutenin (ESRG), two heterogeneous wheat protein fractions, are not known. HMWG occurs as small oligomers, soluble in 70% ethanol, whereas ESRG is a disulfide-bonded fraction of glutenin, wheat's highest molecular weight protein class. Upon reduction and alkylation, both fractions are alcohol-soluble, consist of subunits having molecular weights near 44,000 and 36,000, and have similar electrophoretic and chromatographic properties and amino acid compositions. Amino-terminal sequence analyses now prove that many of the same polypeptides comprise HMWG and ESRG. Their N-terminal sequence distribution is H_2N-(Val+Asn+Gln+Met)-(His+Gln+Met)-(Ile+Val)-

(Pro+Val+Gln)-(Gly+Val+Gln+Asn)-(Leu+Pro)-Gln-(Leu+Gly)-(Pro+Gln+Leu)-(Gln+Pro+Val)-Gln-(Gln+Pro)-(Gln+Pro)-Pro-(Gln+Leu)-Pro-Gln-Gln-. These data imply that many of these polypeptides are homologous and that the heterogeneity of HMWG and ESRG results from duplication and mutation of a common ancestral gene. During biosynthesis, kernel maturation, or drying, these polypeptides can combine either with each other to form HMWG or with higher molecular weight ethanol-insoluble polypeptides and with highly aggregating polypeptides to form glutenin. A hypothesis explains how the ethanol-soluble nongliadin polypeptides with 44,000 and 36,000 mol wt contribute to gluten's structure and to dough's unique viscoelastic properties.

Wheat endosperm proteins can be classified as albumins, globulins, gliadins, and glutenins by solubility, but each class is heterogeneous. For example, at least 46 gliadins exist (Mecham et al 1978, Wrigley 1970); most are monomers of 30,000–40,000 mol wt (α, β, and γ gliadins) or 60,000–80,000 mol wt (ω gliadins) (Bietz and Wall 1972, Booth and Ewart 1969, Charbonnier 1974), but Sephadex G-100 chromatography also reveals 5–10% of a 100,000–125,000 mol wt fraction (Beckwith et al 1966, Wright et al 1964), designated as high molecular weight gliadin (HMWG). This fraction is similar to glutenin in that it contains subunits, mostly with molecular weights of about 44,000 and 36,000 (Bietz and Wall 1972), joined by disulfide bonds; it has also been designated a low molecular weight glutenin (Kanazawa and

Yonezawa 1973, Nielsen et al 1968).

Similarly, glutenin is complex, with molecular weights ranging into the millions; it consists of disulfide-bonded subunits with molecular weights of 11,600–133,000 (Bietz and Wall 1972) and also commonly associates noncovalently with other proteins. After reduction and alkylation, glutenin subunits can be fractionated on the basis of solubility in neutral 70% ethanol (Bietz and Wall 1973, Kanazawa and Yonezawa 1973). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) shows that ethanol-soluble reduced glutenin (ESRG) contains primarily 44,000 and 36,000-mol wt subunits; small amounts of higher molecular weight polypeptides are also present in ESRG but are uniquely different from the major high molecular weight ethanol-insoluble subunits of glutenin. This molecular weight distribution is similar to that of reduced HMWG (Bietz and Wall 1972), so we investigated the possibility that HMWG and ESRG are closely related (Bietz and Wall 1973). In fact, both starch gel electrophoresis (SGE) and amino acid analysis did demonstrate close similarity between the two fractions. Concurrently, Kanazawa and Yonezawa (1973) showed that "low molecular

weight glutenin" (equivalent to HMWG) and ESRG had similar SGE and isoelectric focusing patterns.

To further investigate the similarity of these fractions and to reveal their origins, importance, and interrelationships, we performed amino-terminal sequence analysis of HMWG and ESRG by Edman degradation. The results suggest that many HMWG and ESRG polypeptides are identical and show that they can combine with each other to form HMWG but can also interact with high molecular weight ethanol-insoluble polypeptides and with aggregating polypeptides to form glutenin.

MATERIALS AND METHODS

Protein Samples

Glutenin and gliadin were isolated from defatted Ponca hard red winter wheat flour (Bietz and Wall 1972). Whole gliadin was fractionated into HMWG (Fig. 1A, fraction a), low molecular weight gliadin (Fig. 1A, fraction f), and intermediate fractions by gel filtration on Sephadex G-100 (Bietz and Wall 1972). HMWG was clarified by centrifugation (Bietz and Wall 1973).

Samples were reduced with β-mercaptoethanol and converted to S-aminoethyl (AE) or S-pyridylethyl (PE) derivatives (Friedman et al 1970, Rothfus and Crow 1968). AE-glutenin and PE-glutenin were divided into ethanol-soluble and ethanol-insoluble fractions; these fractions were previously characterized and compared to whole reduced glutenin (Bietz and Wall 1973).

Electrophoresis

PAGE of ESRG and PE-gliadin fractions a–f (Fig. 1A) was performed in 6% gels with 5% crosslinking, using pH 3.2 aluminum lactate buffer (Kasarda et al 1976b, Mecham et al 1978). For some experiments, urea (1, 3, or 8M), previously deionized in 12M solution using AG501-X8 resin (Bio-Rad), was used. Samples (about 40 μl) contained 100–200 μg of protein. Characterization of these fractions by SDS-PAGE was described previously (Bietz and Wall 1973).

Sequence Determination

Automated Edman degradation was performed on a Beckman model 890C sequencer using a 1M Quadrol program, and phenylthiohydantoin (PTH) derivatives of amino acids were identified by gas-liquid chromatography (GLC) and thin-layer chromatography (TLC) as described previously (Bietz et al 1977, 1979). In addition, high performance liquid chromatography (HPLC) of ethyl acetate phases was performed, using isocratic elution at 60° with acetonitrile/sodium acetate (0.01M), pH 4.5 (35:65) on a 25.0-cm × 4.6-mm Dupont Zorbax octadecylsilane column. Equipment consisted of a Waters WISP model 710 sampler, a Waters model M-6000A pump set at 1.0 ml/min (about 1,000 psi), a Schoeffel SF770 variable wavelength detector adjusted to 254 nm, and a Hewlett-Packard 3385A data system. In this HPLC system, PTH-norleucine, the slowest-eluting PTH-amino acid, elutes at 21–22 min. This system, developed and assembled by A. Eldridge at the Northern Regional Research Center, is similar to that recently described by Gates et al (1979). It is significantly better than any method we previously used to identify and quantitate sequencer fractions: PTH-amino acids difficult to separate by other methods (such as PTH-leucine, PTH-isoleucine, and PTH-norleucine) or difficult to quantitate accurately by GLC (such as PTH-aspartic acid, PTH-asparagine, PTH-glutamic acid, PTH-glutamine, PTH-tryosine, and PTH-tryptophan) are easily and fairly accurately determined. In addition, mild conditions permit detection of labile PTH amino acids such as PTH-serine and PTH-threonine, which is difficult or impossible even by amino acid analysis; multiple peaks result for these derivatives, however, and recoveries are typically low. HMWG and ESRG were sequenced both as PE-derivatives and AE-derivatives, with essentially identical results. Each analysis was repeated at least four times for 30–40 cycles; 14–16 mg of protein was examined in each experiment.

RESULTS

Electrophoresis

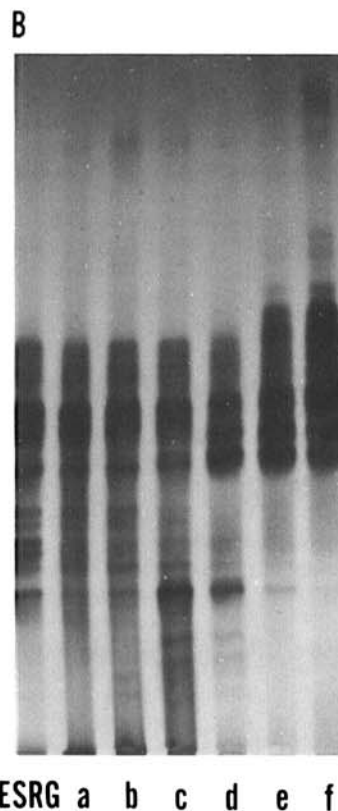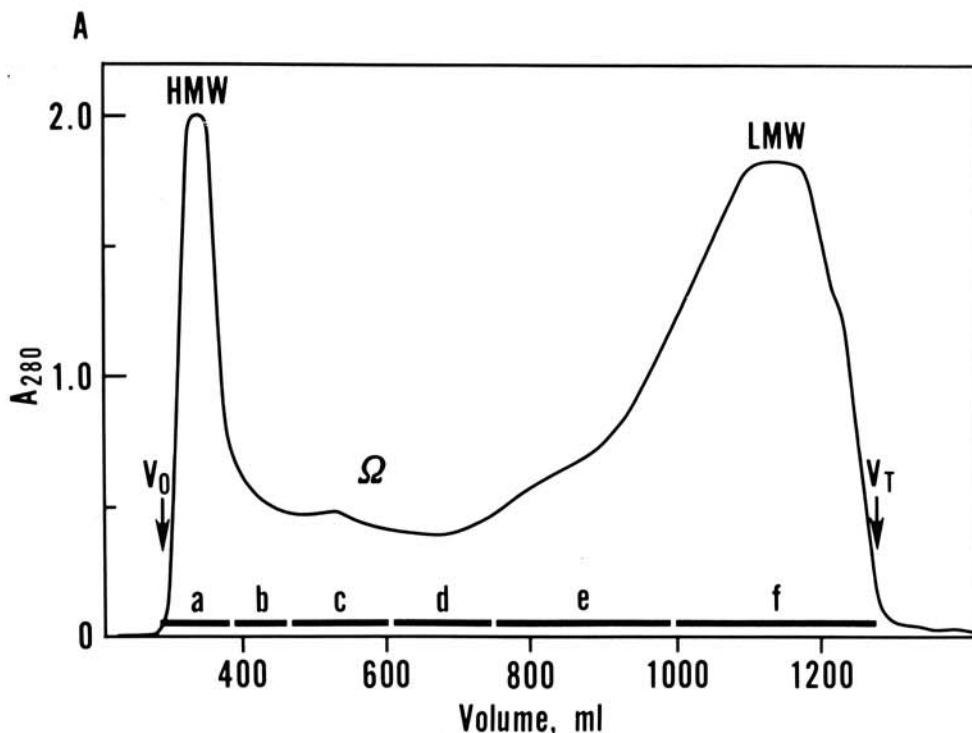SDS-PAGE and SGE previously revealed considerable

Fig. 1. A, Gel filtration of whole Ponca gliadin on Sephadex G-100, showing high molecular weight (HMW), low molecular weight (LMW), and intervening fractions; B, pH 3.2 aluminum lactate polyacrylamide gel electrophoresis of pyridylethyl-ethanol-soluble glutenin (ESRG) and pyridylethylated gliadin G-100 fractions a–f.

similarity of PE-HMWG and PE-ESRG in size and charge distribution (Bietz and Wall 1973). Because newer PAGE methods have higher resolution than our previous SGE method, protein fractions were reexamined by PAGE in pH 3.2 aluminum lactate buffer. In 4 M urea, this method revealed 20–25 bands in ethanol-soluble PE glutenin, but a somewhat blurred pattern was obtained (Bietz 1979). PAGE in 1, 3, or 8 M urea did not improve band sharpness or separation for ESRG or PE-HMWG (results not shown).

In the absence of urea, however, improved separations of ESRG and HMWG resulted (Fig. 1B); approximately 20 fairly sharp bands resolved in each fraction. PE-ESRG and PE-HMWG are nearly identical, both qualitatively and quantitatively, confirming and strengthening our previous SGE observations (Bietz and Wall 1973).

Figure 1 also includes patterns of other pyridylethylated gliadin G-100 fractions. Low molecular weight gliadin (Fig. 1B, fraction f) contains 20–30 bands mainly in the α-gliadin and β-gliadin regions (Mecham et al 1978, Wrigley and Shepherd 1973) and differs from HMWG or ESRG in two major ways. First, many of the major gliadin bands do not correspond in mobility to bands of HMWG or ESRG. This agrees with the different molecular weight distribution observed for most ESRG and HMWG subunits (mainly 44,000-mol wt, with lesser amounts of 36,000-mol wt subunits) as compared to low molecular weight gliadins, most having molecular weights near 36,000 (Bietz and Wall 1972, 1973).

The second major difference between HMWG or ESRG and low molecular weight gliadin is that several bands of lower mobility, largely between γ-gliadins and ω-gliadins in underivatized gliadin (Mecham et al 1978), are present in ESRG and gliadin G-100 fractions a–d but are absent in low molecular weight gliadin (Fig. 1B, fractions e–f). Most of these bands have greater mobilities than do the characteristic ω-gliadins coded by chromosome 1D (Shepherd 1968) (the slowest bands in Fig. 1, fractions c–d). They probably differ from most monomeric γ-gliadins as well because they occur in fractions e–f (Fig. 1) of pyridylethylated low molecular weight gliadin, where monomeric γ-gliadins are known to elute to only a slight extent.

Thus, PAGE indicates that ESRG and HMWG are nearly identical. Most of their polypeptides differ from low molecular weight gliadins, but they do contain some subunits intermediate in mobility to γ- and ω-gliadins.

Sequence Analysis

HMWG and ESRG are very complex mixtures (Fig. 1), but studies have shown that useful sequence information can be obtained from mixtures of homologous proteins (Autran et al 1979; Bietz et al 1977, 1979; Kasarda et al 1974). Sequencer analyses of

these fractions are summarized in Table I. Identifications were made by HPLC, GLC, and TLC; quantitative data are from both HPLC and GLC. Little variability occurred between replicate analyses, so averaged results, expressed as nmoles per milligram of sample, are given. If 44,000 is the molecular weight of ESRG and HMWG subunits, 1 mg contains 22.7 nmol. Our results indicate a total of 8.78–9.16 nmol of major PTH amino acids per milligram at cycle 1, representing initial yields of 39–40%. These yields are somewhat lower than for other cereal protein fractions (Bietz et al 1977, 1979) but are not uncommon. In part, they may also indicate other minor constituents or subunits lacking free amino groups in ESRG and HMWG.

On the basis of these data, a distribution of amino acids in the amino-terminal sequences of the subunits common to HMWG and ESRG is proposed (Fig. 2). Heterogeneity of the fractions did not permit determining individual sequences or establishing longer sequences; isolation and sequence analysis of single polypeptides is a goal of future research. These results (Fig. 2) are the first sequence data for HMWG or for any glutenin subunit. Most of the same major amino acids were identified at corresponding positions of HMWG and ESRG (Table 1), although numerous minor quantitative differences, some variability in recoveries, and a few
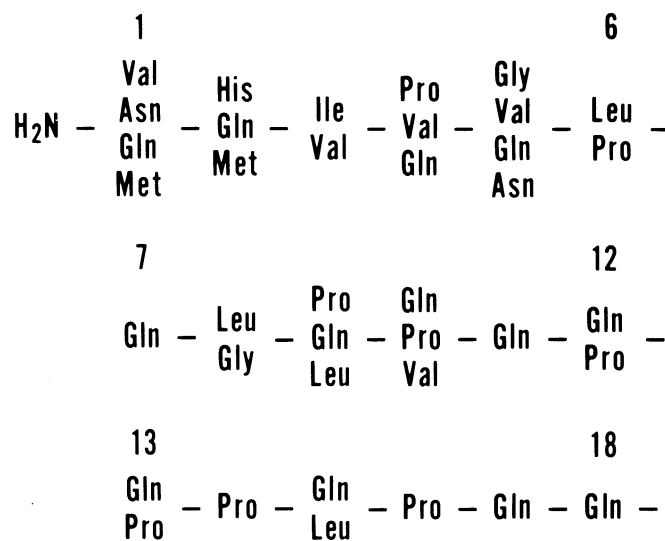

Fig. 2. Distribution of major amino acids in the amino-terminal sequences of the major polypeptides common to high molecular weight gliadin and ethanol-soluble reduced glutenin.

TABLE I
Quantitative N-Terminal Sequence Analysis of Ethanol-Soluble Reduced Glutenin (ESRG) and High Molecular Weight Gliadin (HMWG)

Cycle	ESRG	HMWG
1	V(2.71),[a] N(2.56), Q(2.37), M(1.52)	Q(2.70), N(2.20), V(2.04), M(1.84)
2	H,[b] Q(2.68), M(1.16)	H,[b] Q(1.57), M(0.82)
3	I(2.98), V(1.71), Q(1.22)	I(1.88), V(0.46)
4	P(2.92), V(1.33), Q(0.55), L(0.34)	P(2.08), Q(1.42), A(0.77), L(0.77), V(0.73), F(0.29)
5	G(1.75), V(1.67), Q(1.09), N(1.00)	G(1.78), N(1.18), Q(1.17), V(1.15)
6	L(3.08), P(2.27), I(0.99)	L(2.42), P(0.69), I(0.59)
7	Q(4.30)	Q(1.84)
8	L(1.21), G(1.16)	L(0.52), G(0.52)
9	P(1.90), Q(1.21), L(1.00)	Q(1.65), P(1.21), L(0.79)
10	Q(1.77), P(1.36), V(0.90), F(0.30)	Q(1.74), V(0.96), P(0.65), F(0.39)
11	Q(2.25)	Q(2.03)
12	Q(4.21), P(0.98)	Q(3.67), P(1.03)
13	Q(2.21), P(1.29)	Q(2.31), P(1.50)
14	P(1.46)	P(0.55), A(0.49)
15	Q(1.95), L(1.04)	Q(1.76), L(1.17)
16	P(0.76)	P(0.46)
17	Q(0.64)	Q(1.01)
18	Q(2.26), P(0.73), F(0.61)	Q(2.09), P(0.80), F(0.44)

[a] Amino acid abbreviations: A = alanine, F = phenylalanine, G = glycine, H = histidine, I = isoleucine, L = leucine, M = methionine, N = asparagine, P = proline, Q = glutamine, V = valine. Values in parentheses represent nmoles obtained per milligram of sample.
[b] Thin-layer chromatography indicated that phenylthiohydantoin-histidine was the major residue in this fraction; it was not determined by other methods.

qualitative differences (possibly representing unique polypeptides) did occur. Nevertheless, these results, considered with all other comparisons of these fractions, strongly suggest that many of the same polypeptides occur both in HMWG and in ESRG.

Small amounts of high-molecular weight polypeptides occur in both ESRG and HMWG, but these either are not identical to high molecular weight ethanol-insoluble reduced glutenin subunits or do not contribute to the observed sequences (Fig. 2). Repeated attempts to determine N-terminal sequences of ethanol-insoluble reduced glutenin or of polypeptides isolated from that fraction have failed; most high molecular weight ethanol-insoluble glutenin subunits apparently lack free amino groups on their N-terminal amino acids.

One to six major amino acids were identified at each degradative cycle (Table I), and usually a maximum of three were identified. Because each fraction contains at least 20 polypeptides (Fig. 1), the data suggest that many or most of these polypeptides are homologous and originate from one or only a few ancestral genes, as has been observed for low molecular weight gliadins and for zeins (Bietz et al 1977, 1979). During wheat's evolution, one ancestral diploid gave rise to species represented by the A, B, and D genomes. Gene duplication must also have occurred, with mutation increasing the number of translated gene products. These three diploid species then combined into hexaploid *Triticum aestivum* (AABBDD), containing 20 or more genes coding the homologous HMWG or ESRG polypeptides.

HMWG and ESRG sequences (Fig. 2) can be compared to those of the low molecular weight gliadins (Bietz et al 1977). Many major amino acids in HMWG and ESRG do not occur at the same positions in any known α, β, or γ gliadin; examples are Met_1, His_2, Gln_2, Ile_3, Gly_5, and Leu_5. Similarly, many low molecular weight gliadin residues do not correspond to ESRG or HMWG; examples are Arg_2 and Asn_{12} in the α's and β_5 gliadins; Pro_1, Ile_2, Gly_3, and Gln_3 in γ_2 gliadin; and Pro_1, Gly_3, Gln_3, and Trp_7 in γ_3 gliadin. Some sequence similarity also exists between HMWG (or ESRG) and the low molecular weight gliadins. For example, Val occurs at positions 1 and 5 and Gln at positions 9 and 11 of all fractions, and individual α, β, or γ polypeptides exhibit further similarity to HMWG (or ESRG). This apparent homology may be coincidental, caused by similar amino acid compositions (Bietz et al 1977, Bietz and Wall 1973) and heterogeneity of the fractions. Overall, however, the sequence analyses and PAGE results suggest that many or most low molecular weight gliadins are not constituents of HMWG or ESRG.

DISCUSSION

The present data, considered with our previous studies and the work of others, may largely explain the interactions, properties, and origin of wheat gluten proteins. Research has previously suggested that many different classes of polypeptides are incorporated into glutenin and gluten and that both associative and covalent bonds may be important in maintaining gluten structures (Kasarda et al 1976a, Khan and Bushuk 1978, Wall 1979). By integrating this previous information with our present results, we now put forward a proposal attempting to emphasize the function and probable importance of ethanol-soluble nongliadin polypeptides as well as of other wheat proteins in forming gluten structures. Some possible interactions of these types of polypeptides are diagrammed in Fig. 3. These interactions are consistent with most available data, but we emphasize that, because wheat proteins are so heterogeneous and because many have been
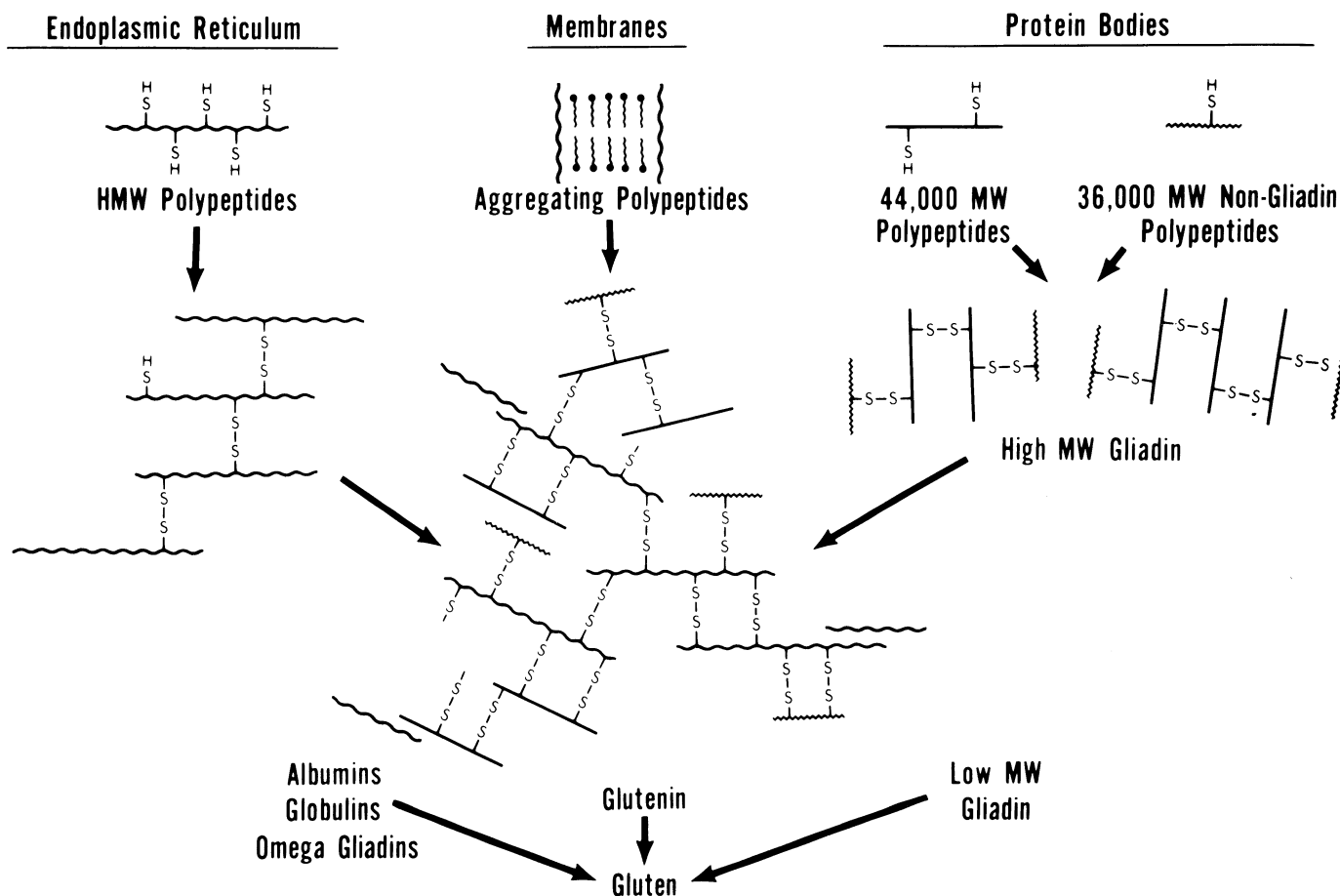

Fig. 3. Some possible interactions of wheat proteins.

only partially characterized, this scheme is a hypothesis, meant to provide a basis for future investigation.

Types of Polypeptides

We propose that at least six major types of polypeptides contribute to wheat gluten and that each has distinct structures, properties, and origins.

High Molecular Weight Polypeptides. One group of wheat endosperm polypeptides is found only as subunits of glutenin and has unusually high apparent molecular weights of 80,000–133,000. These polypeptides differ from lower molecular weight glutenin subunits or from gliadins in amino acid composition and are insoluble in neutral 70% ethanol. The different size, composition, and reactivity of these polypeptides suggest that they have an independent origin or synthetic mechanism, such as the endoplasmic reticulum; Simmonds (1972) previously suggested the possibility of separate particulate origins for different glutenin subunit fractions. Cysteine percentages in these polypeptides are equal to or less than those of other wheat proteins, but their larger molecular size makes sufficient cysteine available for formation of extensive disulfide-linked networks of glutenin by interacting with each other and with other polypeptides.

Aggregating Polypeptides. After treating glutenin with hydrophobic bond-breaking solvents such as SDS or sodium dodecanoate in the absence of reducing agent, gel filtration chromatography on Sepharose CL-4B separates a variety of lower molecular weight proteins from the high molecular weight glutenin peak (Huebner and Wall 1980). If glutenin is reduced and alkylated, these same polypeptides may also be isolated by gel filtration upon Sephadex G-200; high-molecular weight and low-molecular weight glutenin subunits elute as peaks B and C, but peak A contains polypeptides that remain highly aggregated under the gel filtration conditions used (Huebner and Wall 1974). SDS-PAGE revealed that peak A contains polypeptides having molecular weights of approximately 64,000–70,000, 40,000, 30,000, and lower. The amino acid composition of fraction A polypeptides is more like those of albumins and globulins than of any other glutenin fraction (Huebner et al 1974); indeed, a recent study demonstrates that the 68,000-mol wt band usually observed in reduced glutenin, coded by chromosome 4D, is actually a NaCl-soluble high molecular weight globulin.[3] A membrane origin for these highly-aggregating polypeptides is proposed.

44,000-mol wt Polypeptides. Another group of polypeptides is, after reductive cleavage of disulfide bonds, soluble in neutral 70% ethanol. In solubility, therefore, these polypeptides resemble gliadin; unlike gliadin, however, they have molecular weights near 44,000. The 44,000-mol wt polypeptides seem to occur in mature wheat only in HMWG or as subunits of glutenin; therefore their cysteine contents or locations probably do not permit formation of stable molecules through intramolecular disulfide bonding alone. Rather, disulfide bonding must be both intermolecular and intramolecular. The capacity of 44,000-mol wt polypeptides to form intermolecular disulfide bonds must be limited, however, because HMWG molecules have average molecular weights of only 100,000–125,000. The 44,000-mol wt polypeptides may originate in protein bodies (Miflin and Shewry 1979).

36,000-mol wt Nongliadin Polypeptides. SDS-PAGE of reduced glutenin or HMWG reveals a major band having a molecular weight of approximately 36,000. This molecular weight is similar to that of most low molecular weight gliadins, but PAGE (Fig. 1) suggests nonidentity of the polypeptides; for this reason, the term "36,000-mol wt nongliadin polypeptides" is used to designate this fraction. In addition, although low molecular weight gliadins seem to exist only as monomers, the 36,000-mol wt nongliadin polypeptides occur as intermolecularly-disulfide-bonded subunits of oligomeric HMWG or of glutenin.

Albumins, Globulins, and ω-Gliadins. Many albumin and globulin proteins exist in wheat endosperm, and some adsorb or associate tenaciously but noncovalently with other wheat proteins. For example, β-amylase binds to glutenin (Rothfus and Kennel 1970), and fraction A of PE-glutenin (Huebner et al 1974) seems to

consist of strongly aggregating albumin or globulin polypeptides. Some indication exists that albumins and globulins may affect dough development and strength through disulfide interchange involving endogenous thiol groups.[4]

ω-Gliadins are unique from other gliadins in having molecular weights of 60,000–80,000 and in lacking cysteine and methionine (Bietz and Wall 1972, Booth and Ewart 1969, Charbonnier 1974). Therefore, ω-gliadins probably interact with other proteins only noncovalently; little is known of their origin or importance.

Low Molecular Weight Gliadins. Low molecular weight gliadins are a very heterogeneous but homologous group of polypeptides having molecular weights near 36,000. Most of these are classified as α-gliadins or β-gliadins on the basis of their electrophoretic mobilities. Sequence analysis, however, shows that γ_1 gliadin is also a homologous member of this group, thus demonstrating the weakness of nomenclature systems based upon electrophoretic mobility alone (Bietz et al 1977). Low molecular weight gliadins are apparently synthesized by ribosomes associated with the endoplasmic reticulum, and they accumulate in particulates called protein bodies (Morton et al 1964). Disulfide bonding in native low molecular weight gliadins seems to be entirely intramolecular, so that they do not form covalent bonds with each other or with other polypeptides. These proteins, however, have very strong associative tendencies and often contaminate extracted glutenin preparations (Bietz and Wall 1975).

Polypeptide Interactions

As indicated in Fig. 3, wheat endosperm polypeptides can interact in several ways to form HMWG, glutenin, and gluten. The ethanol-soluble 44,000 and 36,000-mol wt nongliadin polypeptides, which make up a substantial portion of glutenin on a quantitative basis (Bietz and Wall 1973), can interact through intermolecular disulfide bonds with each other to form a series of small oligomers known as HMWG, having an average molecular weight of 100,000–125,000. Similarly, the same 44,000 and 36,000-mol wt nongliadin polypeptides can form additional intermolecular disulfide bonds with the high molecular weight polypeptides and can associate strongly with the aggregating polypeptides to form glutenin. Figure 3 indicates that oligomeric forms of high molecular weight polypeptides unite with HMWG to form glutenin; further research is necessary, however, to ascertain whether these interactions involve oligomeric or monomeric forms, or both, of the high molecular weight, 44,000-mol wt and 36,000-mol wt nongliadin polypeptides.

The interactions diagrammed in Fig. 3 occur during synthesis, maturation, or desiccation of the endosperm; they are probably largely random in nature and result from juxtaposition of individual particulates and organelles. Ethanol-soluble protein classes differing from monomeric prolamins may occur in all cereals; alcohol-soluble glutelin subunits have been identified in corn (Paulis and Wall 1971), sorghum (Jambunathan and Mertz 1973), and barley (Lontie and Voets 1959) as well as in wheat.

Upon rehydration (as during dough formation), glutenin can interact with low molecular weight gliadin and with albumins, globulins, and ω-gliadins to form gluten in at least three ways: 1) disulfide interchange may promote more stable configurations, while simultaneously incorporating other polypeptides; 2) covalent interactions may occur between bonding sites previously inaccessible; and 3) proteins may associate noncovalently, as through hydrophobic or hydrogen bonding.

Most low molecular weight and ω-gliadins probably associate noncovalently with glutenin; low molecular weight gliadins can be removed from glutenin by reprecipitation from neutral 70% ethanol (Bietz and Wall 1975), and ω-gliadins apparently have no cysteine. Thus, these gliadins probably affect dough properties by diluting or modifying interactions of other gluten constituents. Certain γ-gliadins, however, may be associated with bread-baking quality (Sozinov 1978) or with durum viscoelasticity (Damidaux et al 1978); their contribution to wheat properties and quality obviously needs further investigation. Low molecular weight gliadins may also be covalently incorporated into gluten by

disulfide interchange, however, since reoxidation of reduced gliadin under concentrated conditions can lead to intermolecular disulfide bond formation (Beckwith et al 1965).

These protein interactions (Fig. 3) may give some insight into wheat quality and how it varies. For example, if synthesis of polypeptides centrally important to formation of a desirable gluten network were suppressed through environmental factors (Yoshino and McCalla 1966) or modified through mutation, glutenin size distribution could vary and affect wheat strength or mixing time (Bietz et al 1973, Huebner and Wall 1976). Similarly, changing the relative proportions of each type of protein would change the properties of the resulting dough.

Obviously, this hypothesis leaves many unanswered questions, and alternate explanations exist for some phenomena (although we consider the present hypothesis to be most consistent with the available data). For example, is glutenin only an artifact of isolation, formed during rehydration? We do not think so; the size distribution of isolated native glutenin seems to be constant for any variety and is also related to quality. Can the 44,000-mol wt and 36,000-mol wt nongliadin polypeptides occur in situ as high molecular weight, alcohol-insoluble polymers, as well as in HMWG and in glutenin? Previous studies of corn (Wolf and Khoo 1975) and sorghum (Seckinger and Wolf 1973) show proteinaceous material remaining in protein bodies after alcohol extraction; in corn, this material can then be extracted by a combination of alcohol and reducing agent.[5] What are the actual origins of wheat proteins? Low molecular weight gliadins and possibly 44,000-mol wt and 36,000-mol wt nongliadin polypeptides occur in protein bodies (Miflin and Shewry 1979), but we really have little specific information regarding other protein classes. Obviously, these questions can only be answered by isolating discrete particulate fractions from immature wheat endosperm and characterizing their proteins; preliminary studies along these lines are now under way at our laboratory.

CONCLUSION

Our results demonstrate that a group of ethanol-soluble nongliadin wheat endosperm polypeptides having molecular weights of about 44,000 and 36,000 can form small oligomers known as HMWG or can interact, either as monomers or oligomers, with ethanol-insoluble high molecular weight polypeptides and with highly aggregating polypeptides to form glutenin. A hypothesis is proposed explaining how these polypeptides interact with each other and with other wheat polypeptides to form gluten, giving wheat dough its unique viscoelastic properties.

[5]M. J. Wolf, 1976. Unpublished observations.

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