Differences in Amino Acid Sequences of Gliadin and Glutenin[1]

J. A. BIETZ and J. A. ROTHFUS, Northern Regional Research Laboratory, Peoria, Illinois 61604

ABSTRACT

Gliadin and glutenin were examined to identify peptides that differentiate the proteins. Pronase-resistant fragments from gliadin and glutenin have average molecular weights of 460 and 640, respectively, and in acid are readily converted to pyroglutamic acid (PGA) peptides. PGA-peptides isolated from pepsin-hydrolyzed Pronase digests contained glutamine (Gln) or glutamic acid; proline, serine, and glycine were other common residues. Several peptides were common to digests of both proteins; but most were unique, demonstrating sequence differences between gliadin and glutenin. Yield data suggested that most unique peptides were from a single protein or only a few different ones. Glycine occurs more frequently in the PGA-peptides from glutenin; and proline, in those from gliadin. That Gln is also positioned differently in the two proteins was evidenced by the enzymatic release of more PGA from glutenin than from gliadin and more PGA-Gln and PGA-Gln-Gln from gliadin than from glutenin.

Some studies (1,2,3) have suggested that glutenin contains gliadin-like subunits joined by disulfide bonds, but others (4–7) have emphasized differences between the proteins. Recently, we showed by column chromatography (8) that enzymatic digestion of gliadin and glutenin produces many similar or identical fragments, as proposed by Ewart (3). The analyses, however, demonstrated that some peptides are characteristic of either gliadin or glutenin. Bietz et al. (9) also found evidence of both unique and similar peptides among isolated gliadin proteins. Their data suggested that minor sequence differences may be responsible for the different properties of gliadin and glutenin and, perhaps, for variability in gluten quality. Because structures of gliadin and glutenin remain unknown, however, the relationship between these proteins is only partially understood.

In further pursuit of information on structural features that differentiate gliadin and glutenin, we have used extensive enzymatic digestion to destroy as many similar sequences as possible, and to degrade dissimilar structures to fragments more easily separated and characterized by existing methods. Ninhydrin-negative PGA peptides from Pronase and Pronase-pepsin digests were isolated and were found to include many sequences unique to gliadin or glutenin, as well as some common to both groups of proteins.

---

[1]Presented at the 55th Annual AACC Meeting, Minneapolis, October 1970. Contribution from the Northern Regional Research Laboratory, Peoria, Ill. 61604. This is a laboratory of the Northern Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

The following abbreviations are used: Ala, alanine; Arg, arginine; Asx, asparagine or aspartic acid; CySSCy, cystine; Gln, glutamine; Glu, glutamic acid; Glx, glutamine or glutamic acid; Gly, glycine; His, histidine, Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; MW, molecular weight; PGA, pyroglutamic acid; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine, TLC, thin-layer chromatography; Tyr, tyrosine; and Val, valine.

MATERIALS AND METHODS

Gliadin and glutenin were isolated from a hard red winter wheat flour (Ponca variety) and gliadin was separated from low-molecular-weight (MW) glutenin as described previously (10,11). Pepsin, twice crystallized, was obtained from Worthington Biochemicals Corp. (Freehold, N.J.) and Pronase (*Streptomyces griseus* protease, B grade) from Calbiochem (Los Angeles, Calif.). Sephadex G-10 and G-25 (fine) were purchased from Pharmacia, Inc. (Uppsala, Sweden); Chromobeads P cation-exchange resin, from Technicon Corp. (Tarrytown, N.Y.); and AG1-X8 (minus 400 mesh, chloride form) anion-exchange resin, from Bio-Rad Laboratories (Richmond, Calif.). AG1-X8 was sized to a particle-size range of 40 to 47 μ (12). Pyridine for chromatographic buffers was refluxed with ninhydrin (0.2% w./v.) for 1 hr. (13) and distilled to remove ninhydrin-positive contaminants. Amino acids were obtained from Mann Research Laboratories (New York, N.Y.), as was Gln-Gln-Gln and Gln-Gln. Gln-Gly and PGA-Pro came from Cyclo Chemicals (Los Angeles, Calif.). PGA-Gln-Gln, PGA-Gln, and PGA-Pro were prepared from Gln-Gln-Gln, Gln-Gln, and Gln-Pro by incubation at pH 1.8 at 40°C. for 24 hr. (see Results and Discussion). Deionized water was used throughout.

Peptides were eluted from Sephadex G-10 (1.9 X 151 cm.) with 1% acetic acid. Chromatography on Sephadex G-25 (1.9 X 149 cm.), AGl-X8 (0.9 X 60 cm.), and Chromobeads P (0.6 X 100 cm.) were as described previously (8); column effluents were automatically monitored by ninhydrin before and after alkaline hydrolysis (14). Peptide maps on paper were prepared by the method of Katz et al. (15) using pH 1.9 electrophoresis buffer (16); location of peptides was by ninhydrin-collidine (17) and modified starch-iodide (18). Digestion with pepsin was described previously (8).

TLC was performed on 0.25 mm. Merck silica-gel plates (Brinkmann

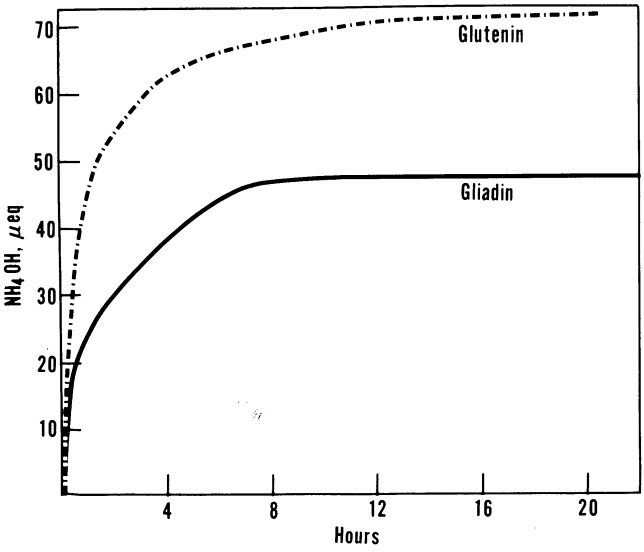

Fig. 1. Rate of digestion of gliadin and glutenin by Pronase.

Instruments, Inc., Westbury, N.Y.) with pyridine-acetic acid-*n*-butanol-water (40:14:68:25) (19). Peptides were isolated by streaking combined fractions on TLC plates, with guide spots at the sides. After chromatography, spots visualized in the guide strips were used to locate desired zones on the plate. Silica gel that contained desired peptides was removed with a vacuum collector, and the peptides were eluted with dilute acetic acid. Repeated chromatography and elution of some samples indicated greater than 90% recovery. Samples were stored frozen.

Semiquantitative analysis of peptide concentration was accomplished by spotting 1 μliter of serial dilutions on TLC plates with Gly, Lys, and Gln standards (1.0 μliter of 1.0, 0.5, 0.25, 0.125, and 0.0625 mg. per ml.). Plates were developed with starch-iodide, and photographed on Polaroid Type 51 high-contrast film to enhance precision and sensitivity of the assay. Concentrations as low as 0.06 μg. per μliter were detected by visual comparison of photographs. Yield estimates obtained in this manner differed from amino acid analysis data by less than 50%.

Samples for amino acid analysis were hydrolyzed for 24 hr. in constant boiling HCl at 110°C. *in vacuo*. Analyses were run on a Technicon TSM analyzer with a five- or tenfold-scale expansion (full scale, 0.1 or 0.2 O.D.); norleucine was the internal standard. Manual integration of peaks was necessary at this sensitivity. As little as 0.005 μmoles of peptide could be analyzed.

RESULTS AND DISCUSSION

Digestion of Gliadin and Glutenin by Pronase

Proteins were digested with Pronase (1% w./w.) at pH 7.2 and 40°C. (20). To determine digestion time, 50.7 mg. gliadin or 71.5 mg. glutenin were dissolved or suspended (glutenin) in 10.0 ml. H_2O and adjusted to pH 7.2 with dilute NH_4OH. Following addition of Pronase, the pH was maintained at 7.2 with 0.0645N NH_4OH by a Radiometer TTTlc titrator equipped with a SBR 2c titrigraph. Uptake of NH_4OH with time is shown in Fig. 1. Digestion was complete in 10 hr. for gliadin and 16 to 18 hr. for glutenin; a standard time of 24 hr. was chosen.

Ninhydrin analyses revealed 1 mole of free amino groups for 460 g. gliadin and 640 g. glutenin. Since complete hydrolysis would give 1 mole of free amino groups for 141 g. gliadin and 138 g. glutenin, gliadin and glutenin contain resistant sequences, as noted by Nomota et al. (20). This higher average MW for glutenin peptides, which is an indication of difference from gliadin, supports evidence obtained earlier with peptic digests (8). When Pronase digests were treated with pepsin, a decrease in free amino groups and an increase in ninhydrin-negative peptides occurred, so that the extent of digestion could not be determined.

Pronase contains several proteases, and has a broad specificity that is only partially characterized (21–24). The large amount of Gln in gliadin and glutenin (25) prompted us to react Gln-Gln-Gln, Gln-Gly, and PGA-Pro with Pronase, which produced no cleavage that could be detected by TLC analysis.

Characterization of Pronase and Pronase-Pepsin Digests

Pronase digests of gliadin and glutenin (9.8 mg.) were first compared on Sephadex G-25 (Fig. 2). Most peptides eluted near the bed volume (V_T) and have a MW of 1,000 or less (26).

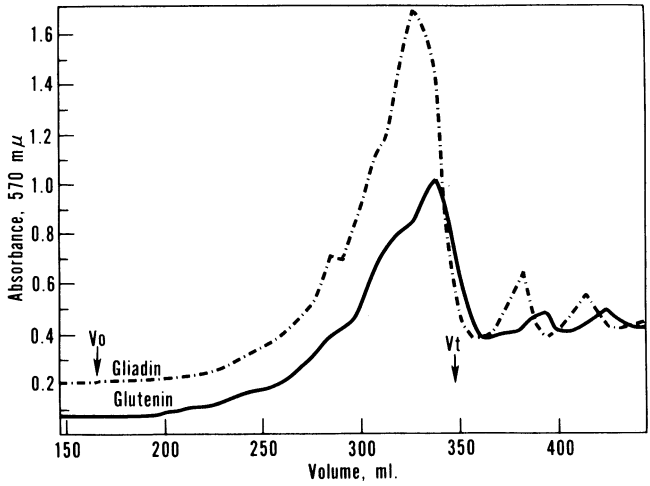

Fig. 2. Chromatographic separations of gliadin and glutenin Pronase digests on Sephadex G-25 (1.9 × 149 cm.). Exclusion volume (V_o) and bed volume (V_T) are indicated. Columns were developed at 0.41 ml. per min. and monitored by ninhydrin after alkaline hydrolysis.

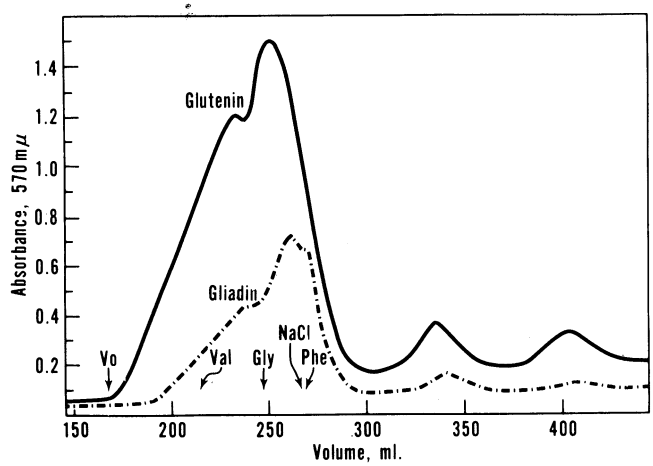

Fig. 3. Chromatographic separations of Pronase digests of glutenin (10.6 mg.) and gliadin (5.3 mg.) on Sephadex G-10 (1.9 × 151 cm.). Flow rates were maintained at 0.41 ml. per min., and peptides were detected by ninhydrin after alkaline hydrolysis. Void volume (V_o) and elution volumes for Val, Gly, NaCl, and Phe are indicated.

Sephadex G-10, with a fractionation range of MW 0 to 700 (26), was then used to study these digests (Fig. 3). Void volume (V_o), determined with glutenin, and elution volumes of Val (26), Phe (26), Gly, and NaCl are indicated. The chromatograms indicate similar size distributions, but differences are apparent. Results with G-10 and G-25 (Fig. 2) indicate that higher MW peptides (eluted near V_o) occur in digests of glutenin.

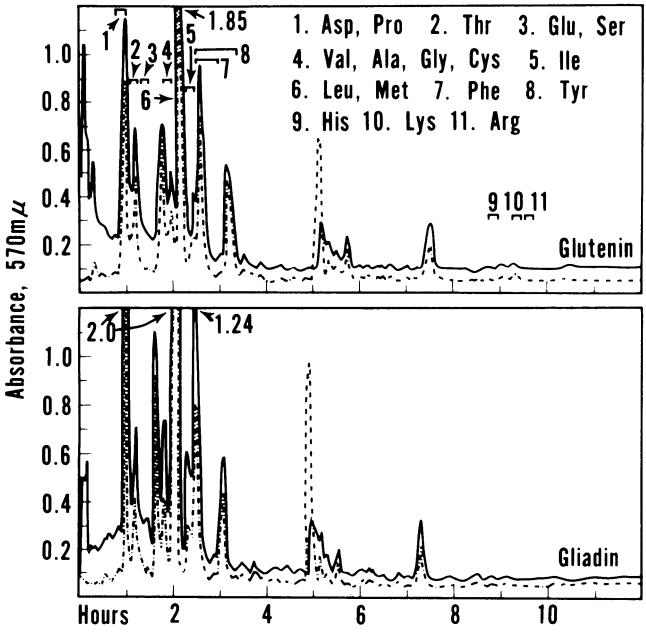

Fig. 4. Chromatographic separations of Pronase digests of gliadin and glutenin on Chromobeads P (100 X 0.6 cm., 0.50 ml. per min.), analyzed by ninhydrin with (solid line) and without (broken line) alkaline hydrolysis. Elution positions of amino acids are indicated, as well as maximum absorbance of peaks off the graph.

Cation-exchange chromatograms of Pronase-gliadin (8.2 mg.) and Pronase-glutenin (8.3 mg.) are presented in Fig. 4. The chromatograms are nearly identical and suggest that differences between basic peptides in the digests are relatively minor. Most peaks between 0.75 and 3.25 hr. correspond to elution positions of free amino acids. Peptides eluted during the first hour are incompletely resolved, however, and were examined further.

Collected effluent containing these peptides is referred to as Fraction 1. Two-dimensional paper peptide maps of Pronase-gliadin and -glutenin and of their Fractions I are reproduced in Fig. 5. Maps of the whole digests have several identical major spots, some of which correspond to positions for Gly, Ala, Glu, Asp, Tyr, Leu, Phe, Lys, His, or Arg. Maps of Fractions I, however, are not alike, and contain newly formed ninhydrin-negative peptides not present in the whole digests. These modified peptides do not migrate during electrophoresis at pH 1.9, but separate by chromatography. This behavior is characteristic of PGA-peptides, which form from N-terminal Gln-peptides under acidic conditions by deamidation and ring closure (see ref. 27 for review). The dissimilarity of Fractions I suggests that they contain peptides which differentiate gliadin and glutenin.

Although Pronase has pepsin-like activity (28), further degradation of the Pronase digests with pepsin produced mixtures that looked more alike than Pronase digests when compared on Sephadex G-10. The glutenin digest, however, still contained higher MW fragments than the gliadin digest. Changes owing to

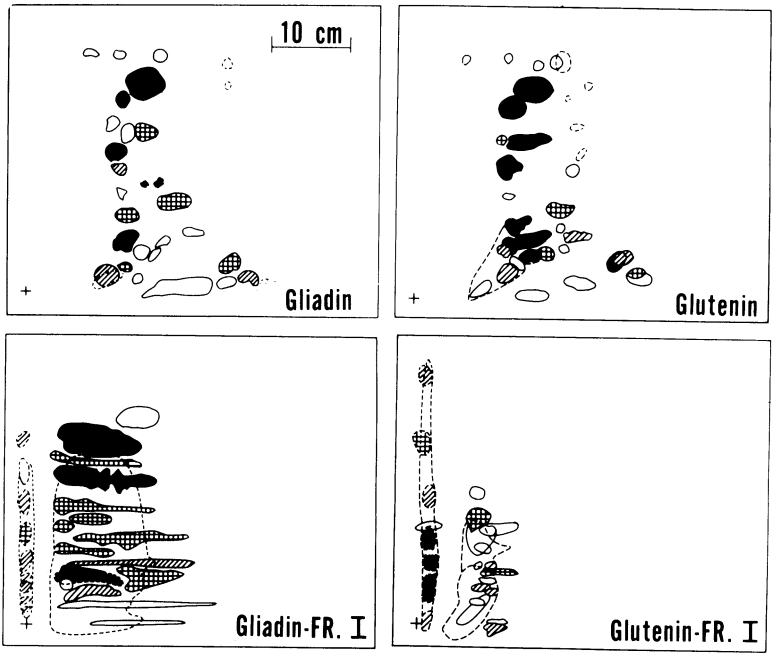

Fig. 5. Peptide maps of Pronase digests (1 to 3 mg. each) of gliadin, glutenin, and Fractions I of gliadin and glutenin. Electrophoresis at pH 1.9 horizontally from the origin (+) was followed by descending chromatography in *n*-butanol-acetic acid-water (4:1:5, upper phase). Peptides were first detected with ninhydrin (solid line); starch-iodide (broken line) revealed additional ninhydrin-negative peptides. Shading indicates relative spot intensities.

incubation with pepsin were more apparent in cation-exchange chromatograms of pepsin-Pronase-gliadin and -glutenin (Fig. 6). Comparison to the Pronase digests (Fig. 4) revealed a significant increase in peptides eluted during the first hour (Fraction I). To determine if pepsin produced these changes, Pronase-glutenin was incubated at pH 1.8 as in pepsin hydrolysis, but with no enzyme. The chromatogram of the product was identical to that of pepsin-Pronase-glutenin (Fig. 6). The major effect of the incubation was probably a nonenzymatic conversion of Gln- to PGA-peptides, even though changes in gel filtration patterns suggest that there was limited proteolysis by pepsin. Since pepsin digestion facilitated the isolation and purification of these peptides which elute in Fractions I, it was continued in further studies.

Paper peptide maps of Fractions I from the pepsin-Pronase digests of gliadin and glutenin showed several differences among ninhydrin-negative peptides and contained no free amino acids other than glutamic acid as PGA. The remaining portions of each digest were nearly identical and contained free amino acids.

Fractions I were extremely hygroscopic, and accurate direct measurements of yield could not be made. Recoveries of peptides eluted after the first hour indicated that about 40% each of gliadin and glutenin could have been present in Fractions I. The hygroscopic nature of peptides in Fractions I is especially significant in view of

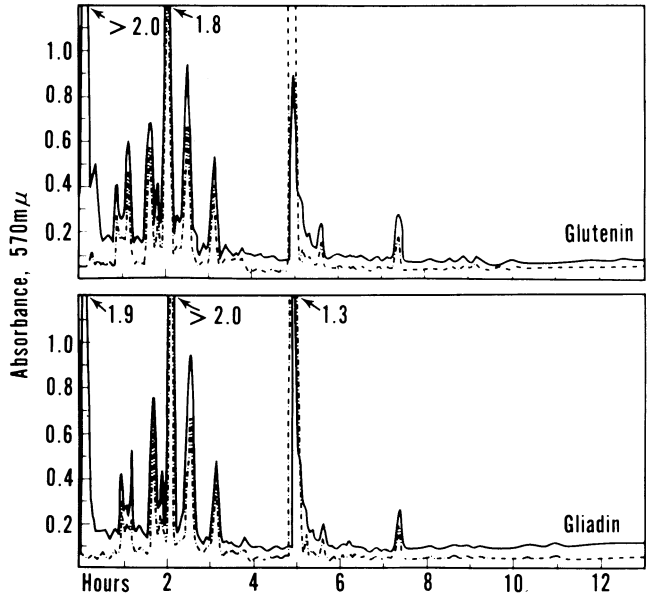

Fig. 6. Separations on Chromobeads P (100 X 0.6 cm., 0.50 ml. per min.) of gliadin and glutenin digested both with Pronase and pepsin. Effluents were analyzed by ninhydrin with (solid line) and without (broken line) alkaline hydrolysis. Maximum absorbancies for peaks off the graph are indicated.

the affinity of PGA peptides for water (29). In terms of this property, the structure postulated for these peptides seems appropriate.

AG1-X8 chromatograms of Fractions I of pepsin-Pronase-gliadin (8.5 mg.) and -glutenin (9.6 mg.) are shown in Fig. 7. Peptides eluted after the first hour are ninhydrin-negative, but react with starch-iodide or with ninhydrin after alkaline hydrolysis, and are thought to be PGA-peptides. Major peaks in these two chromatograms coincide, but those at 6.0, 10.1, 18.7, and 20.3 hr. in the gliadin chromatogram and 5.5, 8.4, 10.4, 12.1, 13.2, 16.4, 16.8, 17.8, 18.4, 19.7, and 20.9 hr. in that of glutenin are unique.

Peptides from Gliadin and Glutenin

Preparative separations of Fractions I from 196.8 mg. of pepsin-Pronase-gliadin and 236.3 mg. of pepsin-Pronase-glutenin were performed on the 0.9 X 60 cm. AG1-X8 column in the same manner as analytical separations (Fig. 7); elution patterns were not altered by the increased sample load. Tubes of 4.6 ml. of column effluent were collected every 10 min. and lyophilized. After the contents of each tube were redissolved in 0.2 ml. H_2O, 2 μl were analyzed by TLC. Results are shown in Fig. 7. Most tubes contained mixtures, but a limited number of major peptides were present. Sharp resolution is revealed by the narrow zones in which peptides were eluted. Although the glutenin digest appears more complex than that of gliadin (Fig. 7), analyses of larger quantities revealed traces of additional gliadin peptides, as in glutenin. Only major peptides will be considered further.

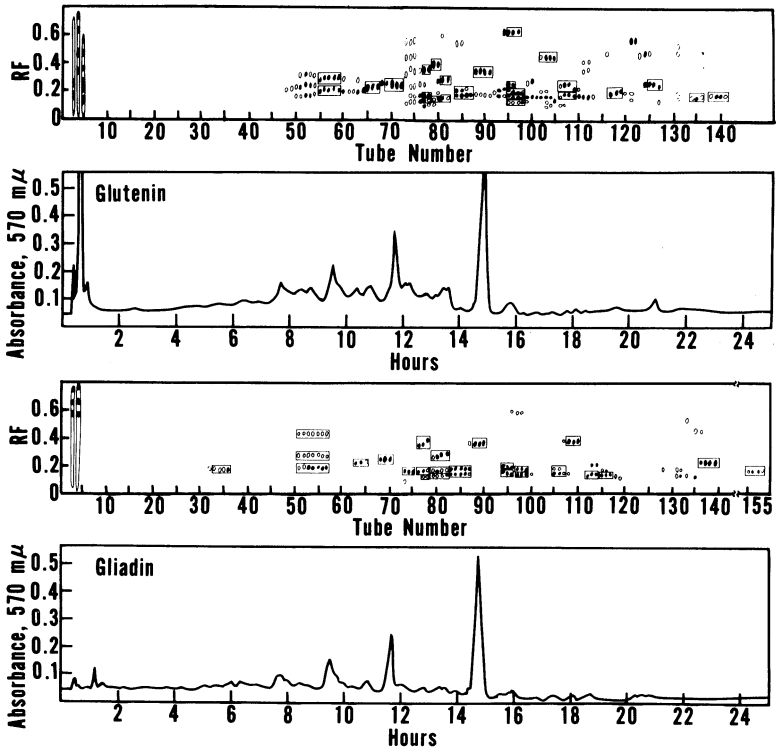

Fig. 7. AG1-X8 chromatograms of Fractions I from gliadin and glutenin digested with Pronase and pepsin. Flow rates were maintained at 0.46 ml. per min., and effluents were analyzed by ninhydrin after alkaline hydrolysis. Portions from individual tubes of identical separations were chromatographed on silica-gel thin-layer plates, as shown; dark shading indicates high spot intensity. Very faint spots were omitted. The peptides described in Tables I and II are indicated by boxed spots.

Peptides from gliadin (A1–A25, Table I) and from glutenin (B1–B25, Table II) were isolated by TLC of pooled samples (Fig. 7). R_f and yield of each peptide are listed in the tables, with amino acid analysis data. Probable compositions represent the best stoichiometry for the data; the relative lack of other residues in amino acid chromatograms indicated purity and allowed unambiguous determination of peptide compositions in most cases.

Glu is the major constituent in acid hydrolysates of all peptides in Tables I and II. Since these peptides form spontaneously under acid conditions and react with starch-iodide but not with ninhydrin, one residue of the Glu most be N-terminal PGA. Additional Glu in the acid hydrolysate may represent Glu or Gln in the peptide. Since Gln is much more prevalent than Glu in gliadin and glutenin (25) and NH_3 values (Tables I and II) frequently correspond to non-N-terminal Glu, it is likely that most Glu found after hydrolysis is actually Gln in the peptides. In a few (A18, A23, A24, B3, B11, B16, B20, and B23), however, NH_3 values and delayed emergence from AG1-X8 indicate that Glu is present. Glu and Gln cannot be

TABLE I. AMINO ACID ANALYSES AND PROBABLE COMPOSITIONS OF GLIADIN PEPTIDES

Peptide	Tubes[a]	R_f	Yield[b]	Amino Acid Analyses[c]						Probable Composition
				Glu	Pro	Gly	Ser	NH_3	Other	
A1	33–36	0.17	0.45	1.00	0.44	0.16	0.19	2.63		PGA-(Glx, Pro)
A2	51–57	0.19	14.73	1.00				1.40		PGA-Gln-Gln
A3	51–57	0.28	6.93	1.00	0.79			1.09		PGA-(Glx_x, Pro_x)[d,e]
A4	51–57	0.43	0.85	1.00	0.49	0.07	0.07	0.83	Leu: 0.22	PGA-(Glx_3, Pro_2, Leu)
A5	63–65	0.23	3.66	1.00	0.57	0.17	0.50	1.10		PGA-(Glx, Pro, Ser)
A6	68–70	0.25	30.69	1.00				0.95		PGA-Gln
A7	73–75	0.16	2.26	1.00	0.57			0.62		PGA-(Glx, Pro)
A8	76–78	0.12	0.58	1.00	0.61	0.09	0.26	0.74		PGA-(Glx_2, Pro_2, Ser)
A9	76–78	0.16	2.18	1.00	0.45		0.07	0.63		PGA-(Glx, Pro)
A10	76–78	0.36	2.45	1.00	0.15		0.14	0.82	Thr: 0.45	PGA-(Glx, Thr)
A11	79–82	0.13	0.45	1.00	0.70		0.24	0.82		PGA-(Glx_3, Pro_3, Ser)
A12	79–82	0.16	0.44	1.00	0.54		0.13	0.76		PGA-(Glx_2, Pro_2)
A13	79–82	0.28	3.03	1.00	0.18	0.46	0.40	0.76		PGA-(Glx, Gly, Ser)
A14[f]	83–87	0.14+0.18	9.26	1.00	0.43		0.07	0.46		PGA-(Glx, Pro)
A15	88–90	0.36	50.75	1.00				0.20		PGA
A16	94–96	0.15	1.36	1.00	0.22			0.84		PGA-(Glx_3, Pro)
A17	94–96	0.20	1.78	1.00	0.42			0.51		PGA-(Glx, Pro)
A18	97–99	0.14	7.22[g]	1.00				0.40		PGA-(Glx_x)[e]
A19	97–99	0.18	2.56	1.00	0.39			0.48		PGA-(Glx, Pro)
A20[f]	105–107	0.15+0.18	1.24	1.00	0.36	0.09	0.08	0.46		PGA-(Glx_2, Pro)
A21	108–110	0.44	3.25	1.00	0.46			0.40	Ile: 0.34	PGA-(Glx_2, Pro, Ile)
A22	112–114	0.14	9.36[g]	1.00				0.70		PGA-(Glx_x)[e]
A23	115–117	0.14	6.70[g]	1.00				0.40		PGA-(Glx_x)[e]
A24	136–139	0.22	15.55[g]	1.00				0.45		PGA-(Glx_x)[e]
A25	155–158	0.16	2.71[g]	1.00				0.70		PGA-(Glx_x)[e]

[a] "Tubes" refers to tube numbers given in Fig. 7.

[b] Expressed as μmole peptide per gram gliadin.

[c] Expressed as mole percent of Glu.

[d] A3 is not PGA-Pro, since the R_f of PGA-Pro is 0.94.

[e] The subscript "x" indicates an unknown number of residues.

[f] Mixture.

[g] Total recovery of Glu from peptide.

TABLE II. AMINO ACID ANALYSES AND PROBABLE COMPOSITIONS OF GLUTENIN PEPTIDES

Peptide	Tubes[a]	R_f	Yield[b]	Amino Acid Analyses[c]						Probable Composition
				Glu	Pro	Gly	Ser	NH_3	Other	
B1	55–59	0.21	4.47	1.00	0.16	0.08	0.07	0.50		PGA-Gln-Gln
B2	55–59	0.29	5.23	1.00	0.42	0.48	0.08	1.35		PGA-(Glx, Pro, Gly)
B3	65–67	0.23	2.92	1.00		0.24	0.19	0.37		PGA-(Glx_3, Gly, Ser)
B4	69–72	0.25	20.43	1.00		0.04		0.55		PGA-Gln
B5	77–78	0.13	0.95	1.00	0.38	0.47	0.10	0.85		PGA-(Glx, Pro, Gly)
B6	77–78	0.17	1.29	1.00	0.46			0.55		PGA-(Glx, Pro)
B7	77–78	0.35	3.09	1.00				0.69	Thr: 0.70	PGA-(Glx, Thr)
B8	79–80	0.14	0.64	1.00	0.33	0.49	0.07	0.63		PGA-(Glx_2, Pro, Gly)
B9	79–80	0.40	0.53	1.00	0.10			0.70	Ala: 0.67	PGA-(Glx_2, Ala_2)
B10	81–82	0.15	1.03	1.00	0.28	0.38	0.09	0.65		PGA-(Glx_2, Pro, Gly)
B11	81–82	0.29	3.08	1.00		0.41	0.53	0.35		PGA-(Glx, Gly, Ser)
B12[d]	84–87	0.17+0.22	5.10	1.00	0.49	0.06		0.42		PGA-(Glx, Pro)
B13	88–91	0.34	117.54	1.00				0.16		PGA
B14	95–98	0.12	0.47	1.00	0.25	0.48	0.23	1.81		PGA-(Glx, Gly)
B15	95–98	0.16	1.00	1.00		0.25	0.05	0.62		PGA-(Glx_2, Gly)
B16	95–98	0.20	1.49	1.00	0.33	0.14	0.03	0.32		PGA-(Glx_2, Pro)
B17	95–98	0.25	2.19	1.00	0.64	0.06		0.48		PGA-(Glx, Pro)
B18	95–98	0.64	4.22	1.00		0.08		0.70	Leu: 1.02	PGA-Leu
B19	102–105	0.45	2.32	1.00	0.55	0.10		0.57	Ile: 0.34	PGA-(Glx, Pro, Ile)
B20	106–109	0.17	8.23[e]	1.00		0.12		0.42		PGA-(Glx_x)[f]
B21	106–109	0.23	1.83	1.00	0.57			0.46		PGA-(Glx, Pro)
B22	117–119	0.20	4.27[e]	1.00		0.10	0.08	0.47		PGA-(Glx_x)[f]
B23	125–127	0.24	17.71[e]	1.00		0.04	0.06	0.19		PGA-(Glx_x)[f]
B24	134–136	0.16	0.39	1.00	0.18	0.21	0.13	1.19	Asx: 0.43	PGA-(Glx, Asx)
B25	138–141	0.16	2.04[e]	1.00				1.29		PGA-(Glx_x)[f]

[a] "Tubes" refers to tube numbers given in Fig. 7.

[b] Expressed as μmole peptide per gram glutenin.

[c] Expressed as mole percent of Glu.

[d] Mixture.

[e] Total recovery of Glu from peptide.

[f] The subscript "x" indicates an unknown number of residues.

differentiated solely by amino acid analyses, because some peptides appear to contain more NH_3 than Glu.

Pro, Gly, and Ser are the other amino acids most frequently found in these peptides. Leu, Ile, and Thr occur less frequently, and Ala and Asx are in only two glutenin peptides. No other amino acids were detected in significant amounts.

Eight unique gliadin peptides (A1, A3, A7/A9, A12, A14, A16, A17/A19, and A20) and five unique glutenin peptides (B6, B12, B16, B17, and B21) appeared to contain only Glx and Pro even though they exhibited different chromatographic properties. At least four PGA-(Glx, Pro) peptides might be expected *a priori*. Several other variants containing multiple residues, carboxyl-terminal substituents, or both, are also possible.

Several identical peptides occur in both digests. Specifically, the following correspond: A2-B1 (PGA-Gln-Gln); A6-B4 (PGA-Gln); A7/A9-B6 [PGA-(Glx, Pro)]; A10-B7 [PGA-(Glx, Thr)]; A13-B11 [PGA-(Glx, Gly, Ser)]; A14-B12 [PGA-(Glx, Pro)]; A15-B13 (PGA); and A17/A19-B17 [PGA-(Glx, Pro)]. These peptides are the first shown to occur in both gliadin and glutenin, as suggested previously (3,8). PGA, PGA-Gln, and PGA-Gln-Gln were identified by TLC and AG1-X8 chromatographic comparisons with standards.

Gliadin peptides A1, A3–A5, A8, A11, A12, A16, A18, and A20–A25 and glutenin peptides B2, B3, B5, B8–B10, B14–B16, and B18–B25 are unique to gliadin or glutenin and demonstrate sequence differences between the proteins.

One major difference involves Gly, which occurs in a single gliadin peptide (A13) but in eight peptides from glutenin (B2, B3, B5, B8, B10, B11, B14, and B15). Because Gly is more abundant in glutenin than in gliadin (78 vs. 25 mmoles per 100 g.) (25) and Gln-Gly is resistant to Pronase, it is likely that Gly occurs adjacent to Gln more often in glutenin than in gliadin. This structural difference could contribute to different properties of the proteins. Similarly, Pro occurs more frequently in the gliadin peptides than in those isolated from glutenin (15 vs. 10 peptides). Pro is also more abundant in gliadin than in glutenin (148 vs. 114 mmoles per 100 g.) (25) and Gln-Pro is likewise resistant to Pronase. Thus Gln-Pro may occur more frequently in gliadin than in glutenin, and may also contribute to different properties of the proteins.

In view of the generally recognized disruptive effects of Gly and Pro on ordered secondary and tertiary protein structures, differences that we observe between glutenin and gliadin may occur at especially important regions within the proteins. Whereas evolutionary replacement of Pro by Gly or *vice versa* would be conservative with respect to function, such subtle variation occurring between segments of ordered structure could produce significant variation in properties of the proteins. An example of such a variation in properties may be the high-Gly fraction of glutenin that is especially susceptible to aggregation and precipitation at low ionic strength (7).

Another difference is that 2.32 times as much PGA (or Gln) results from digestion of glutenin as of gliadin, indicating that more Gln in glutenin occurs in peptide bonds labile to Pronase or pepsin. Gliadin, however, yields 1.5 times as much PGA-Gln and 3.3 times as much PGA-Gln-Gln as does glutenin; thus, Gln distribution may be a major difference between the two protein fractions. Other differences are the Leu and Ile peptides (A4 and A21) in gliadin and the Ala, Leu,

Ile, and Asx peptides (B9, B18, B19, and B24) in glutenin. Conceivably, further differences between gliadin and glutenin exist, but they could have been destroyed by the enzymatic treatment.

In regard to peptide yields, we point out that if the MW of gliadin proteins is 18,000 (30) and that of glutenin subunits is 20,000 (31), about 50 μmoles of protein chains are present per g. If there are 20 to 40 major gliadin subunits (32,33) and a like number of glutenin subunits, approximately 1.2 to 2.5 μmoles of each unique subunit could be present per g. Thus, yields of identical peptides from the same locus in each subunit should be near 50 μmol. per g., but yields of peptides unique to a subunit should not be much higher than 2.5 μmol. per g. Yields of most PGA-peptides (Tables I and II) are much nearer 2.5 than 50 μmol. per g. Our data suggest that these peptides occur in one or only a few different proteins, but additional evidence is needed to prove this possibility[2]. It is also conceivable that these peptides could have resulted from partial degradation of one or more very unusual proteins. This seems unlikely, however, considering the similarity between isolated gliadin proteins and whole gliadin (9).

In an earlier study (8), mixtures of peptides from pepsin digests of gliadin and glutenin contained up to 60% Glx and 18% Pro. Other studies (34,35,36) which have also shown hydrolysates of gliadin peptides to be rich in Glu or in Glu and Pro suggest that extended sequences of Glu and Gln might exist. Since Gln-Gln-Gln and poly-Glu (24) are resistant to Pronase, such peptides should be readily apparent. The peptides consisting only of Glu and Gln, which were isolated here in greatest yield, include PGA, PGA-Gln, and PGA-Gln-Gln, so it is likely that no more than three Glu or Gln residues are adjacent in most gliadin or glutenin proteins. Glu and Gln are probably distributed randomly throughout the proteins. Until now, the unusual sequence Gln-Gln-Gln has been found only in tobacco mosaic virus coat protein (37), and as the PGA-tripeptide, fastigiatine, from the alga *Pelvetia fastigiata* (38).

The PGA peptides described here may be of fundamental and applied interest in other areas. The extreme hygroscopic nature of PGA and PGA peptides has found useful applications (39); uncontrolled conversion of glutaminyl to PGA-peptides or proteins, however, may be a potential problem. PGA-peptides from gliadin have also been implicated in celiac disease (34,36); a better understanding of their compositions, as presented here, may be useful in further study of this disease, and may suggest modifications of gliadin to alter its effect on sensitive individuals.

Acknowledgment

We gratefully acknowledge the assistance and cooperation of James Cavins in conducting the amino acid analyses.

Literature Cited

1. WOYCHIK, J. H., HUEBNER, F. R., and DIMLER, R. J. Reduction and starch-gel electrophoresis of wheat gliadin and glutenin. Arch. Biochem. Biophys. 105: 151 (1964).

[2]Note added in proof: More recent studies (Bietz, J. A., and J. S. Wall. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem., in press) reveal that most gliadin proteins have MW near 36,500 and that most glutenin subunits have MW between 36,000 and 133,000. With these values, however, the yield data still indicate that most of the isolated peptides occur in only a few polypeptide chains.

2. ROHRLICH, M., and SCHULZ, W. B. TH. Untersuchungen über den Verlauf des enzymatischen Abbaus der Weizenmehlproteine. Naturwissenschaften 47: 181 (1960).
3. EWART, J. A. D. Fingerprinting of glutenin and gliadin. J. Sci. Food Agr. 17: 30 (1966).
4. BECKWITH, A. C., and WALL, J. S. Reduction and reoxidation of wheat glutenin. Biochim. Biophys. Acta 130: 155 (1966).
5. EWART, J. A. D. Amino acid analyses of glutenins and gliadins. J. Sci. Food Agr. 18: 111 (1967).
6. ESCRIBANO, M. J., KEILOVA, H., and GRABAR, P. Gliadin and glutenin after reduction or oxidation. Biochim. Biophys. Acta 127: 97 (1966).
7. ROTHFUS, J. A., and CROW, M. J. A. Aminoethylation and fractionation of glutenin. Evidence of differences from gliadin. Biochim. Biophys. Acta 160: 404 (1968).
8. BIETZ, J. A., and ROTHFUS, J. A. Comparison of peptides from wheat gliadin and glutenin. Cereal Chem. 47: 381 (1970).
9. BIETZ, J. A., HUEBNER, F. R., and ROTHFUS, J. A. Chromatographic comparisons of peptic digests of individual gliadin proteins. Cereal Chem. 47: 393 (1970).
10. JONES, R. W., TAYLOR, N. W., and SENTI, F. R. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. 84: 363 (1959).
11. BECKWITH, A. C., NIELSEN, H. C., WALL, J. S., and HUEBNER, F. R. Isolation and characterization of a high-molecular weight protein from wheat gliadin. Cereal Chem. 43: 14 (1966).
12. BIO-RAD LABORATORIES. Particle sizes and settling rates. Price list U (1969).
13. TECHNICON CORPORATION. Assembly and operating instructions. Peptide analyzer. Instruction manual T-67-101 (1967).
14. CATRAVAS, G. N. Automatic method for separation, hydrolysis, and detection of peptides. Anal. Chem. 36: 1146 (1964).
15. KATZ, A. M., DREYER, W. J., and ANFINSEN, C. B. Peptide separation by two-dimensional chromatography and electrophoresis. J. Biol. Chem. 234: 2897 (1959).
16. WERNER, G., and WESTPHAL, O. Stofftrennungen durch Hochspannungs-Papierelektrophorese. Angew. Chem. 67: 251 (1955).
17. LEVY, A. L., and CHUNG, D. Two-dimensional chromatography of amino acids on buffered papers. Anal. Chem. 25: 396 (1953).
18. PAN, S. C., and DUTCHER, J. D. Separation of acetylated neomycins B and C by paper chromatography. Anal. Chem. 28: 836 (1956).
19. WEILAND, T., and GEORGOPOULOS, D. Zweidimensionale Auftrennung von Peptiden (Fingerprinttechnik) auf Dunnschichtplatten. Biochem. Z. 340: 476 (1964).
20. NOMOTA, M., NARAHASHI, Y., and MURAKAMI, M. A proteolytic enzyme of *Streptomyces griseus*. VI. Hydrolysis of protein by *Streptomyces griseus* protease. J. Biochem. 48: 593 (1960).
21. OUCHI, T. Studies on proteolytic enzymes of *Streptomyces griseus*. Part I. Separation and purification of peptidase and proteinase from culture media of *Streptomyces griseus* ATCC 3463. Agr. Biol. Chem. 26: 723 (1962).
22. NARAHASHI, Y., and YANAGITA, M. Studies on proteolytic enzymes (Pronase) of *Streptomyces griseus* K-1. I. Nature and properties of the proteolytic enzyme system. J. Biochem. 62: 633 (1967).
23. NARAHASHI, Y., SHIBUYA, K., and YANAGITA, M. Studies on proteolytic enzymes (Pronase) of *Streptomyces griseus* K-1. II. Separation of exo- and endopeptidases of Pronase. J. Biochem. 64: 427 (1968).
24. TROP, M., and BIRK, Y. The specificity of proteinases from *Streptomyces griseus* (Pronase). Biochem. J. 116: 19 (1970).
25. WU, Y. V., and DIMLER, R. J. Hydrogen ion equilibria of wheat glutenin and gliadin. Arch. Biochem. Biophys. 103: 310 (1963).
26. PHARMACIA FINE CHEMICALS, INC. Sephadex-gel filtration in theory and practice (1966).
27. BLOMBÄCK, B. Derivatives of glutamine in peptides. Methods Enzymol. 11: 398 (1967).
28. NOMOTA, M., NARAHASHI, Y., and MURAKAMI, M. A proteolytic enzyme of *Streptomyces griseus*. VII. Substrate specificity of *Streptomyces griseus* protease. J. Biochem. 48: 906 (1960).

29. LADEN, K. and SPITZER, R. Identification of a natural moisturizing agent in skin. J. Soc. Cosmet. Chem. 18: 351 (1967).
30. HUEBNER, F. R., ROTHFUS, J. A., and WALL, J. S. Isolation and chemical comparison of different gamma-gliadins from hard red winter wheat flour. Cereal Chem. 44: 221 (1967).
31. NIELSEN, H. C., BABCOCK, G. E., and SENTI, F. R. Molecular weight studies on glutenin before and after disulfide-bond splitting. Arch. Biochem. Biophys. 96: 252 (1962).
32. HUEBNER, F. R., and ROTHFUS, J. A. Gliadin proteins from different varieties of wheats. Cereal Chem. 45: 242 (1968).
33. WRIGLEY, C. W. Protein mapping by combined gel electrofocusing and electrophoresis: application to the study of genotypic variations in wheat gliadins. Biochem. Genet. 4: 509 (1970).
34. BRONSTEIN, H. D., HAEFNER, L. J., and KOWLESSAR, O. D. Enzymatic digestion of gliadin: the effect of the resultant peptides in adult celiac disease. Clin. Chim. Acta 14: 141 (1966).
35. FINLAYSON, A. J. The compositions of some peptides produced by an enzymic hydrolysis of wheat gliadin. Can. J. Biochem. 42: 1133 (1964).
36. BISERTE, G., and HAN, K. Residual peptides in the enzymic hydrolysis of wheat gliadin. Bull. Soc. Chim. Biol. 47: 597 (1965).
37. DAYHOFF, M. O. Atlas of protein sequence and structure, 1969. National Biomedical Research Foundation: Silver Spring, Maryland (1969).
38. DEKKER, C. A., STONE, D., and FRUTON, J. S. A peptide from a marine alga. J. Biol. Chem. 181: 719 (1949).
39. LADEN, K. Hygroscopic salts of carboxylic acids as humectants. U.S. Patent 3,235,457 (Feb. 15, 1966).

[Received November 30, 1970. Accepted June 22, 1971]