Chromatographic Comparisons of Peptic Digests of Individual Gliadin Proteins[1]

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ABSTRACT

Peptide maps of peptic digests of whole Ponca gliadin and its alpha-, beta-, gamma$_1$-, and gamma$_3$-components are similar. Of these proteins, gamma$_3$-gliadin is most different. Peptide maps from alpha-, beta-, and gamma-gliadins from Red Chief and Comanche varieties also show many similarities both within each variety and between corresponding components. The two gamma-gliadin chromatograms are nearly identical. Definite differences between chromatograms of all components, however, indicate that each protein is unique. Peptide mapping by column chromatography is more sensitive than starch-gel electrophoresis of whole proteins in detecting differences in protein structure. The results support the hypothesis that some amino acid sequences in different gliadin proteins have variable structures but others are the same or very similar. Peptide maps of digests of the reduced and S-aminoethylated proteins suggest that some cysteine residues occur in invariant segments.

Gliadin, the alcohol-soluble protein fraction from wheat gluten, was originally shown to contain four to eight components (1,2), but later studies (3,4,5,6) have indicated that the number of gliadin proteins may be nearer twenty. These components may be separated by sulfoethyl cellulose (SEC) and gel-filtration chromatography (4,5). Gliadins from a single variety and from varieties representing different classes of wheat have been compared by amino acid analyses, ion-exchange chromatography, electrophoresis, and immunochemical studies. Amino acid compositions of Ponca gamma-gliadins are much alike (6), and analyses of gamma$_1$-gliadins from several varieties are almost identical (5). Huebner and Rothfus (5) showed that compositional differences in the gliadin fractions from several wheats were greatest between varieties of different classes and least between varieties of the same class; generally, electrophoretic and chromatographic patterns were similar, but never identical. Doekes (7) compared electrophoretic densitograms of gliadins and was able to arrange 80 varieties into five groups forming a morphological series. Elton and Ewart (8,9), comparing gliadins from several varieties, found both qualitative and quantitative differences in electrophoretic patterns, although many components of similar mobility were detected. Immunological studies (10,11) have demonstrated that a number of wheat varieties contain at least four or five gliadin proteins with close structural resemblance to one another. These comparisons provide indirect evidence of the similarity of gliadins based on properties of the whole proteins.

In another paper (12), fragments from peptic digests of glutenin and gliadin and their derivatives were compared by ion-exchange chromatography and gel filtration. Whereas differences between glutenin and gliadin were apparent, the similarities observed suggested that certain segments of amino acid sequence may be common

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to most or all gluten proteins, as proposed by Ewart (13). This paper describes an initial test of this hypothesis by comparison of fragments from individual gliadin proteins.

MATERIALS AND METHODS

Gliadin was isolated from a gluten dispersion in 0.01M acetic acid by precipitation of the glutenin with alcohol at pH 6.5 as described by Jones et al. (1); low-molecular-weight glutenin was removed by chromatography on Sephadex G-100 (Pharmacia, Inc., Uppsala, Sweden) (14). S-Aminoethylation of proteins was carried out as described by Rothfus and Crow (15).

The Ponca gliadin components used here represented alpha-, beta-, and gamma-gliadins according to the nomenclature suggested by Woychik et al. (2). Alpha-, beta-, gamma$_1$-, and gamma$_3$-gliadins were isolated from SEC chromatographic fractions 7, 5, 1, and 3, respectively, of Huebner et al. (6, Figs. 1 and 2); the alpha-gliadin was the slower of the two major bands in fraction 7, and the others were the major components from their respective fractions. After purification on Sephadex G-50 (4,6), electrophoresis revealed the presence of only trace amounts of other gliadins.

Corresponding gamma-, beta-, and alpha-components obtained by SEC chromatography (5) of Red Chief and Comanche gliadins were used in comparing the two HRW wheats. Red Chief fractions 2, 7, and 12 and Comanche fractions 2, 6, and 11 were chosen. Each gamma-gliadin fraction from these wheats contained a trace beta-component, but the beta- and alpha-gliadin fractions migrated as single components upon electrophoresis.

Preparative cation-exchange chromatography of peptides was performed on Aminex AG50W-X2 (200- to 325-mesh, 1.9 × 150 cm., 40°) at 2.0 ml. per min.; chromatograms were developed with a pyridine-acetate gradient from 0.2M (pyridine), pH 3.1 to 2.0M, pH 6.5 (16). Other methods including peptic digestions, peptide mapping on paper, amino acid analyses, and automated peptide analysis involving cation-exchange chromatography of peptides on a sulfonic acid resin, Chromobeads P, or anion-exchange chromatography of peptides on a strongly basic resin containing quaternary ammonium groups, AG1-X8, with a Technicon peptide analyzer were as described previously (12). A constant flow rate of 0.5 ml. per min. was maintained during all chromatographic separations with Chromobeads P and AG1-X8.

RESULTS

Amino Acid Compositions

Amino acid compositions of Ponca alpha- and beta-gliadins from duplicate 24-hr. hydrolysates are given in Table I. For comparison, the compositions of gamma$_1$- and gamma$_3$-gliadin as reported by Huebner et al. (6) are included.

Alpha- and beta-gliadin have unique compositions. They differ most from the gamma-components in their contents of glutamic acid, proline, half-cystine, isoleucine, and tyrosine, and to a lesser degree in the amounts of several other amino acids. Like gamma$_3$-gliadin, alpha-gliadin contains one residue of lysine; beta-gliadin, like gamma$_1$-gliadin, has no lysine.

TABLE I. COMPARATIVE AMINO ACID COMPOSITIONS OF PONCA GLIADINS

Amino Acid	Residues per Minimal Molecular Weight[a]			
	Alpha	Beta	Gamma$_1$[b]	Gamma$_3$[b]
Lysine	1	0	0	1
Histidine	2	2	2	2
Arginine	2	2	2	2
Aspartic acid	3.5	3	4	3
Threonine	2	2	3	3
Serine	5-6	6	8	7
Glutamic acid	40	48	56	62
Proline	17	19	21	29
Glycine	3	2	3	4
Alanine	3	4	5	5
1/2-Cystine	2	1.3	3	3
Valine	5	6	6	7
Methionine	1	1	1	2
Isoleucine	5	5	7	7
Leucine	8	9	10	10
Tyrosine	3	3	4	1
Phenylalanine	4	4-5	5	9
Tryptophan	...[c]	...[c]	0	1

[a]On the basis of one lysine in alpha- and gamma$_3$-gliadin and one methionine in beta- and gamma$_1$-gliadin.

[b]Data from Huebner et al. (6).

[c]Not determined.

Cation-Exchange Chromatography

Chromatograms of peptic digests of Ponca gliadin and its alpha-, beta-, gamma$_1$-, and gamma$_3$-components (2 to 3 mg. each) are shown in Fig. 1. Chromatograms are reproducible, and recovery of peptides is quantitative on the basis of nitrogen determinations (12). Variations in resin condition or gradient composition occasionally caused elution times to differ slightly, but separation and order of elution of peaks were unaffected. The peptide analyzer readily detects peaks with an absorbance 0.001 to 0.002 above base line. Examination of chromatographic fractions by chromatography and electrophoresis on paper indicates that individual peptides are eluted in narrow zones. Cation-exchange chromatograms have an increasing base line, characteristic of the gradient, upon which peptide peaks are superimposed. Integration reveals that the ammonia peak, eluted between 5 and 6 hr., usually represents less than 10% of the total amide content of the sample, indicating that only limited acid hydrolysis of asparagine and glutamine could occur during the relatively mild digestion (12). Furthermore, blank analyses indicate that most or all ammonia is an atmospheric contaminant.

The elution patterns shown in Fig. 1 are similar, and contain nearly the same number of peaks. Each pattern begins with three peaks, at 0.15, 0.45, and 0.8 hr., in a region characterized by large, acidic or neutral, and ninhydrin-negative peptides. These peptides are not retarded by the cation-exchange resin at pH 3.1 (12). Other similarities are apparent in the region after the first hr. and before ammonia. Two major peaks or double peaks occur between 2.5 and 4.5 hr. in chromatograms of digests of gliadin and its alpha-, beta-, and gamma$_1$-components.

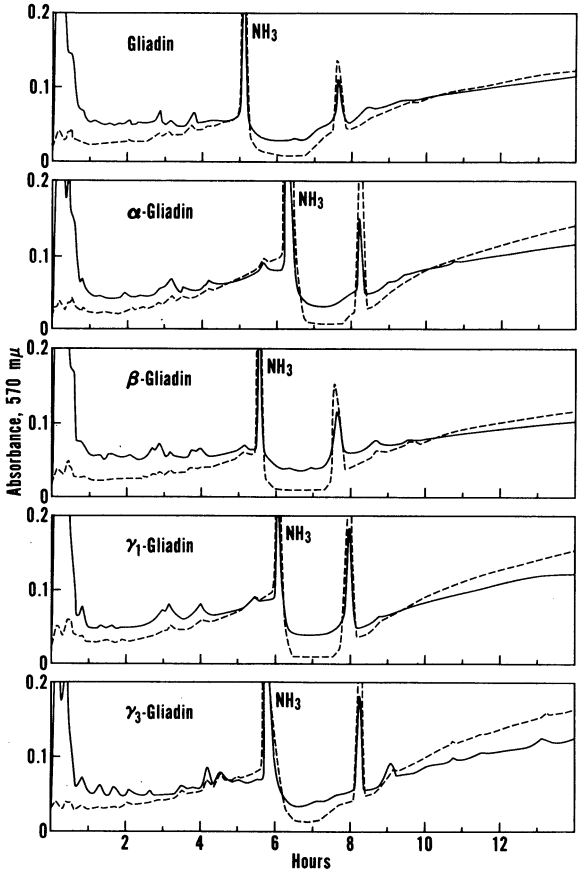

Fig. 1. Chromatographic separations of peptic fragments from Ponca gliadin and its alpha-, beta-, gamma$_1$-, and gamma$_3$-components on Chromobeads P. Solid line represents ninhydrin reaction after alkaline hydrolysis; broken line represents reaction with ninhydrin without hydrolysis. This convention is used in all figures.

A smaller central peak is also present in all except gamma$_1$-gliadin. Digests of the three components also yield fragments eluted about 0.5 hr. before ammonia, at 7.5 to 8 hr. and at 8.5 to 9 hr. The material eluted at 7.5 to 8 hr. has not been identified. It may represent free amino acids or small peptides, as indicated by the small hydrolyzed-to-unhydrolyzed ratio. The chromatogram of gamma$_3$-gliadin is least like the others in Fig. 1 with respect to position and intensity of peaks. This is particularly apparent between 2.5 and 5 hr. and in the basic region after 9 hr., where several unique peptides are present. Gamma$_3$-gliadin resembles gamma$_1$-gliadin, however, in that both contain peptides eluted at 1.3, 1.7, and 2.0 hr.

The significant differences which exist in each chromatogram show that each protein is unique. For example, each differs in the basic region after 8.5 hr.: alpha-gliadin has peaks at 8.75, 9.5, and 10.75 hr.; beta-gliadin, at 8.75 and 9.5 hr.;

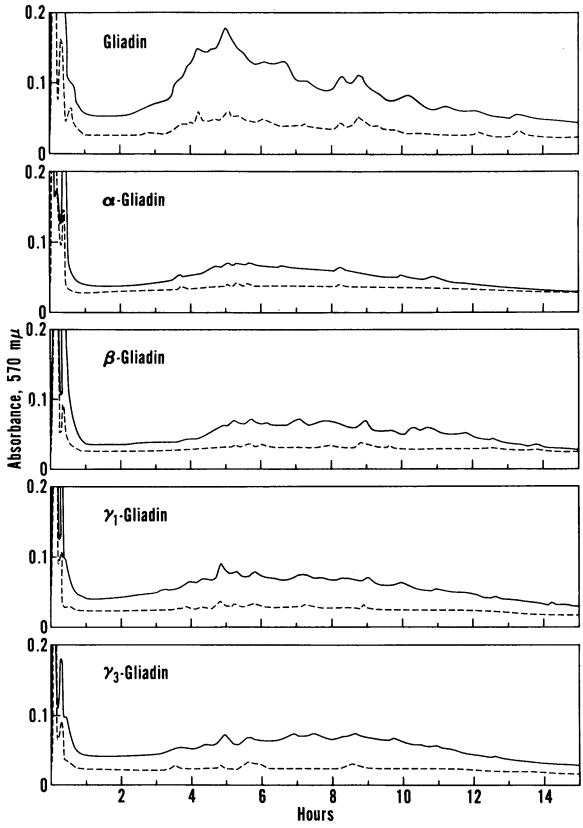

Fig. 2. Chromatographic separations of peptic fragments from Ponca gliadin and its alpha-, beta-, gamma$_1$-, and gamma$_3$-components on AG1-X8.

gamma$_1$-gliadin, a single peak at 8.75 hr.; and gamma$_3$-gliadin, peaks at 9, 10.75, 11.5, and 13.25 hr.

Anion-Exchange Chromatography

The acidic peptides not resolved on cation-exchange resins can be separated by anion-exchange chromatography (12). Thus, peptide maps from chromatography of peptic digests of Ponca gliadin (10 mg.) and its alpha-, beta-, gamma$_1$-, and gamma$_3$-components (3.0 mg. each; Fig. 2) on an anion-exchange resin were used to compare acidic portions of the proteins. The anion-exchange chromatograms, like those obtained by cation-exchange chromatography, show many similarities and some differences among the individual gliadin proteins. Nearly every peak present in the pattern from whole gliadin is represented by a counterpart in at least one of the tracings from the individual proteins. The more basic peptides and ammonia were eluted during the first 0.5 hr. Thereafter, prominent similarities include three peaks in each unhydrolyzed tracing between 4.5 and 6.0 hr. and peaks at 3.5 to 4.0, 4.5 to 5.0, 5.5 to 6.0, 6.6 to 7.2, and 11.0 hr. in each hydrolyzed

tracing. Several other peaks appear in the hydrolyzed tracings from more than one of the individual gliadins.

The alpha- and beta-components show less similarity than when chromatographed on a cation-exchange resin (Fig. 1); this condition suggests that acidic areas of the proteins may be less similar than basic regions. Alternatively, the greater similarity of anion-exchange chromatograms of gamma$_1$- and gamma$_3$-gliadin digests, as compared to the cation-exchange chromatograms (Fig. 1), suggests that the opposite is true for these components.

Comparison of S-Aminoethylated Proteins

Reaction of ethylenimine with reduced glutenin in buffered solution specifically modifies cysteine residues (15). By amino acid analyses, we confirmed that similar treatment modifies only cysteine in reduced gliadin. Resulting S-aminoethylcysteine peptides are more basic than corresponding peptides from unmodified proteins and, depending upon structure, may be retarded more on cation-exchange resins than the parent cysteine peptides. Thus, it was possible to observe both the effect of disulfide cleavage and modification of cysteine residues when peptic digests of reduced and S-aminoethylated (AE) gliadin and components were chromatographed on Chromobeads P (Fig. 3). Chromatograms of peptides from AE-gliadin components are nearly identical with respect to number, elution time, and intensity of individual peaks. For example, every peak in the alpha-gliadin chromatogram, except that at 6.75 hr., has a counterpart eluted at about the same time in chromatograms of the other components. Nonetheless, the uniqueness of each protein is revealed by peaks at 6.75 hr. in alpha-gliadin, 9.25 hr. in beta-gliadin, 6.25 hr. in gamma$_1$-gliadin, and 4.3, 11.1, and 11.7 hr. in gamma$_3$-gliadin. Reduced absorbance at 0.1 hr. is an additional unusual feature of the beta-gliadin chromatogram.

If the AE-gliadin chromatograms (Fig. 3) are compared to those of the corresponding unmodified proteins (Fig. 1), further structural information may be obtained. Six or seven new or increased peaks are present after aminoethylation, and five (at 0.75, 1.75 to 2.0, 2.5 to 2.75, 8 to 8.5, and 8.7 hr.) occur in each chromatogram.

It is significant that two to four new or increased peaks occur in the basic region (after 8 hr.) of each AE-gliadin chromatogram. These probably represent AE-cysteine peptides. Figure 4 shows an elution pattern from preparative chromatography on Aminex AG50W-X2 of an AE-gliadin digest (102.3 mg.). Although more than 40 peaks are present, only nine contain AE-cysteine by the method of Rothfus (17). On paper, peptide maps of these nine fractions contain an average of 12 spots, but only one to three in each map are present in significant amounts.

Resin costs prohibited the use of Chromobeads P for preparative work. Under conditions used in the comparative analyses, Aminex AG50W-X2 gave very poor resolution.

Gliadin Proteins from Different Wheat Varieties

Comanche and Red Chief, HRW wheats of good and poor breadmaking quality respectively, yield gliadins that show only a few differences in chromatographic and

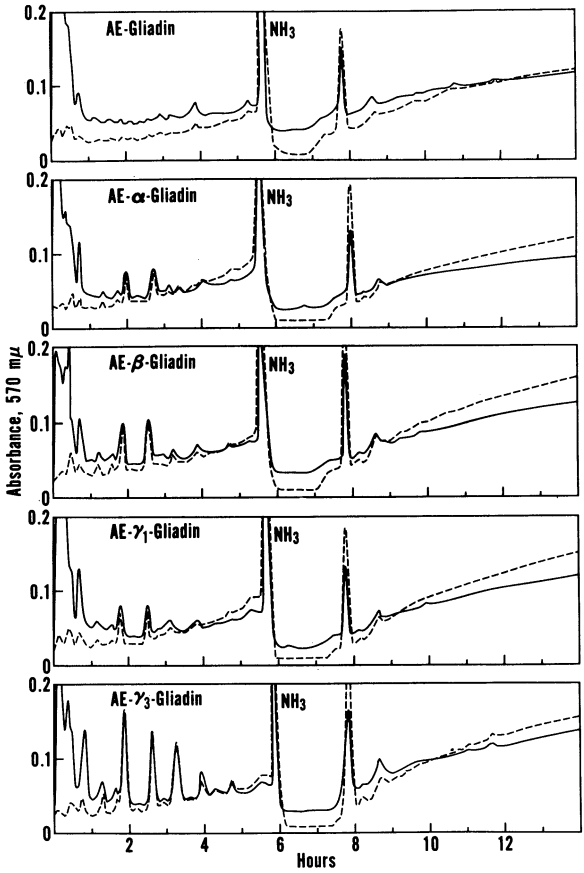

Fig. 3. Chromatographic separations on Chromobeads P of peptic fragments of Ponca aminoethyl(AE)-gliadin and its alpha-, beta-, gamma$_1$-, and gamma$_3$-components.

electrophoretic properties (5). This observation suggests that differences in gluten quality might be attributed to a few unique proteins in each variety. However, if gluten proteins contain large segments which have similar properties, chromatography and starch-gel electrophoresis of the intact proteins may not detect subtle differences in structure that also could be responsible for variations in gluten quality. Comparisons of fragments from analogous Red Chief and Comanche proteins produce evidence of differences that are not readily apparent when the whole proteins are compared.

Chromatograms of peptic digests of alpha-gliadin fractions from Red Chief and Comanche varieties are reproduced in Fig. 5. The chromatograms include many peaks with identical elution times. Definite differences, however, show that the two proteins are not identical; for example, Red Chief alpha-gliadin contains several basic peptides absent in Comanche alpha-gliadin. The broad peak between 3 and 4 hr. in the Comanche alpha-gliadin chromatogram, and also in beta- and

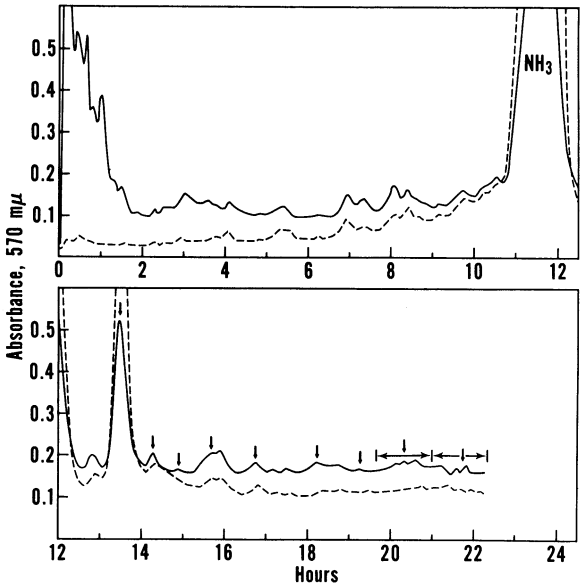

Fig. 4. Preparative chromatographic separation of an AE-gliadin peptic digest on Aminex AG50W-X2. Arrows indicate AE-cysteine peptides.

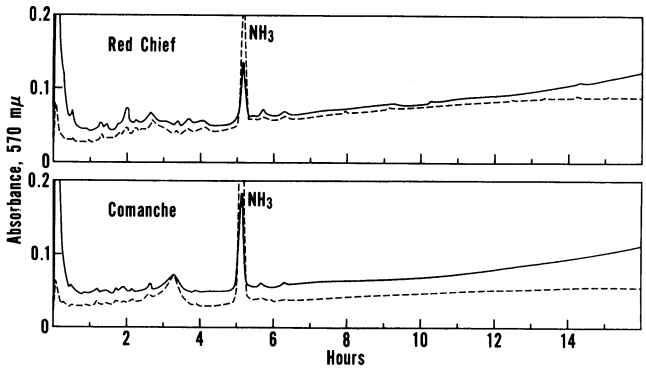

Fig. 5. Chromatographic separations of Red Chief and Comanche alpha-gliadin peptides on Chromobeads P.

gamma-gliadin chromatograms (Figs. 6 and 7), varies in size between duplicate chromatograms and, occasionally, obscures adjacent peaks. Because of such behavior, we suspect this peak may represent, in part, an artifact or nonpeptide material.

Although chromatograms of Red Chief and Comanche beta-gliadin digests in Fig. 6 are also quite similar, significant differences, such as at 1.0, 6.8, and 8.3 hr. in the Comanche beta-gliadin chromatogram, again show that the two proteins are not identical.

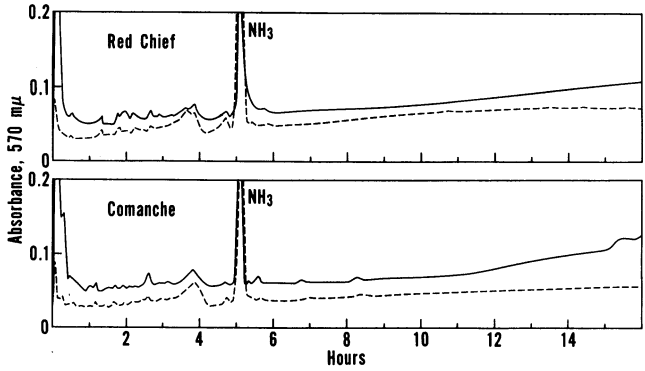

Fig. 6. Chromatographic separations of Red Chief and Comanche beta-gliadin peptides on Chromobeads P.

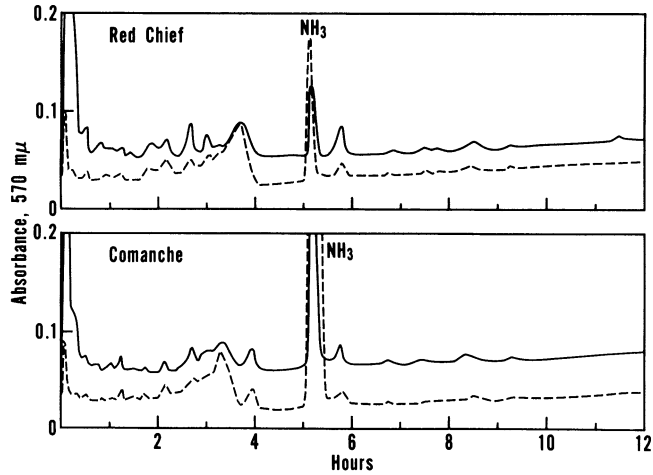

Fig. 7. Chromatographic separations of Red Chief and Comanche gamma-gliadin peptides on Chromobeads P.

Chromatograms of Red Chief and Comanche gamma-gliadin digests in Fig. 7 are more similar than those of the corresponding alpha- and beta-gliadins. The peak between 3 and 4 hr. has shifted and, in the Comanche chromatogram, may obscure the peak at 3.25 hr. in the Red Chief chromatogram. Changes in peak intensity are apparent at 1.4 and 1.8 hr., and unique peaks occur at 7.75 and 3.9 hr. in the Red Chief and Comanche chromatograms, respectively. Other than these differences, the two chromatograms are almost identical.

Cation-exchange chromatograms of digests of Ponca alpha-, beta-, gamma$_1$-, and gamma$_3$-gliadins (Fig. 1) resemble one another closely. This relationship also exists among Red Chief and among Comanche components (Figs. 5 to 7). Nevertheless, significant differences between chromatograms of the alpha-, beta-, and gamma-gliadin fractions from each variety emphasize the uniqueness of each protein.

Limited quantities of individual Red Chief and Comanche gliadin components restricted these comparisons to cation-exchange chromatography. Although acidic portions of the proteins are only partially resolved, it is doubtful that comparisons of anion-exchange chromatograms would deviate from the pattern established for Ponca components, where anion-exchange chromatograms indicated both similar and dissimilar fragments.

Since the gliadin fractions used for comparison of Red Chief and Comanche do not correspond to the alpha-, beta-, gamma$_1$-, and gamma$_3$-gliadins from Ponca, no comparison can be made between Ponca and either Red Chief or Comanche varieties from these data.

DISCUSSION

In another study (12), it was concluded that similarities between gliadin and glutenin were due to the presence of common segments of amino acid sequence in most or all gluten proteins as proposed by Ewart (13). The data presented here on the similarities between fragments in digests of gliadin components from a single variety or different varieties of wheat support this hypothesis. Our data emphasize the possibility that minor structural changes are important determinants of variability in gluten quality. Automated comparison of enzymatic digests of proteins appears to be a more valid method for detecting differences in structure than comparing the intact proteins.

At least five new or increased peaks occurred in corresponding positions in each AE-gliadin chromatogram (Fig. 3), making these peptide maps more alike than those of the unmodified proteins. These new or increased peaks could contain AE-cysteine peptides, but their number suggests that some are probably due to changed digestibilty following disulfide cleavage. Nine peaks containing AE-cysteine peptides were detected in the basic region of an AE-gliadin chromatogram (Fig. 4). These peptides, if they do not represent different stages of digestion, are too numerous to be present in each gliadin component. Peaks at 0.75, 1.75 to 2.0, and 2.5 to 2.75 hr. (Fig. 3) seem to be peptides released only after disulfide cleavage. Their presence indicates a significant difference in conformation between native and AE-gliadin.

Since the cation-exchange resins used for analytical and preparative chromatography of peptides from AE-gliadin both contain the same active group, the order of elution of peptides from both resins should be the same. It therefore is likely that the peaks occurring at 8 to 8.5 and 8.7 hr. in each AE-gliadin chromatogram (Fig. 3) correspond to the two most prominent AE-cysteine-containing peaks at 14.3 and 15.7 hr. in Fig. 4. Other new basic peaks may correspond to other AE-cysteine peptides. These chromatograms, then, suggest that gliadin proteins have common sequences around at least two cysteine residues, but that other cysteine sequences are probably unique to each protein.

In general, the number of peaks in peptide maps of a particular gliadin is approximately (±15%) equal to its content of leucine, tyrosine, and phenylalanine (Table I), residues at which pepsin is most active in the hydrolysis of proteins (18). Thus, compositional differences might account for some of the variation between peptide maps. The fact that any one chromatogram still resembles the others (Figs.

1 to 3) is evidence that differences among these gliadin proteins are due, however, to relatively minor sequence changes.

Economically important wheats are the result of hybridization of three species, which evolved from the same ancestor (19). In view of this common origin, it is understandable how gliadin proteins could be as similar as our data suggest. The similarity of other proteins from genetically related sources is already well established (20). The concept that gluten proteins contain substantial portions of similar or identical amino acid sequences, as indicated by our data and by observations of Ewart (10,13) and of Elton and Ewart (9), may simplify studies on the chemical basis for the unique properties of gluten. Whereas precedent has stressed advantages of conducting structural studies on thoroughly purified proteins, the occurrence of common or similar sequences may help elucidate much gluten protein structure on the basis of whole gliadin or glutenin. Instances in which substantial deviation from average behavior exists would merit extensive purification of individual components.

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