Extraction and Structure Studies on Corn Glutelin Proteins[1]

H. C. NIELSEN, J. W. PAULIS, C. JAMES, and J. S. WALL, Northern
Regional Research Laboratory, Peoria, Illinois

ABSTRACT

Various methods were compared for isolating glutelin from the glutelin-starch residue remaining after extraction of salt-soluble and alcohol-soluble proteins from corn endosperm. Glutelin was prepared best by removing starch from the residue either by extraction with 90% dimethyl sulfoxide or by digestion with alpha-amylase. Either procedure yielded glutelin as a residue which was insoluble in even the most potent protein solvents and only became soluble upon disulfide cleavage. Its insolubility and its high cystine content indicate that glutelin is present in corn endosperm in the form of a three-dimensional disulfide cross-linked network. Electrophoresis of the peptide subunits of disulfide-cleaved glutelin shows a range of mobilities between that of zein and the salt-soluble proteins of corn endosperm. Sodium hydroxide (0.1M) extracted almost all the glutelin from the glutelin-starch residue but degraded the protein, as shown by its diffuse electrophoretic pattern and by loss of cystine and lysine. Aqueous 2-chloroethanol extracted only 30% of the glutelin from the glutelin-starch residue, and gel electrophoresis and amino acid analysis showed that the protein was contaminated with zein.

Glutelin is classically defined as the protein of corn endosperm extractable with dilute NaOH after albumins, globulins, and zein have been separated out. It has also been considered the protein remaining in corn endosperm after exhaustive extraction with dilute salt and 70% ethanol. Glutelin constitutes about 35 to 50% of the protein in mature corn endosperm and is important in determining properties and nutritional value of endosperm. Boundy et al. (1) compared amino acid compositions of glutelin and other corn proteins and investigated starch-gel electrophoretic properties of native and reduced-alkylated corn proteins. They concluded that glutelin contains a large number of disulfide-linked subunits related to albumins, globulins, and zein.

Dilute NaOH is not an ideal protein extractant because it slowly destroys cystine and lysine residues (2,3). As an alternative to NaOH extraction, workers at Purdue University (4,5) cleaved the disulfide bonds in corn endosperm proteins with an alkaline sulfite solution containing cupric ions and then isolated endosperm proteins by classic solubility methods. Only 20% of the endosperm protein was in the acid- and alcohol-insoluble fraction, which they termed "glutelin." This protein had a single electrophoretic peak in pH 11.2 glycine buffer. It had three sedimenting components, the smallest of which had a molecular weight of 21,000 to 26,000. These workers postulated that this protein was the basic repeating unit of corn glutelin.

A unique method for preparing glutelin was that of Concon (6). Rather than removing glutelin from starch, he removed starch from glutelin by means of

[1]Presented at the joint AOCS-AACC meeting, Washington, D.C., April 1968. Contribution from the Northern Regional Research Laboratory, Peoria, Ill. 61604. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture.

Copyright © 1970 American Association of Cereal Chemists, Inc., 1821 University Avenue, St. Paul, Minnesota 55104. All rights reserved.

alpha-amylase digestion. Glutelin prepared by this procedure has an amino acid composition quite similar to that of glutelin prepared by more traditional methods.

In this paper, glutelin is considered the protein remaining in corn endosperm after removal of albumins, globulins, and zein by extraction with 0.5M NaCl followed by exhaustive extraction with 70% ethanol. Various methods of isolating glutelin will be compared by means of amino acid analysis, moving-boundary electrophoresis, and starch-gel electrophoresis. Also, evidence will be presented that glutelin is a distinct protein system in corn endosperm characterized by peptide subunits bound into a three-dimensional structure by intermolecular disulfide bonds.

MATERIALS AND METHODS

Isolation of Protein

Starting material was Funk G-83 yellow dent hybrid corn grown in Illinois in 1966 and field-dried. The corn was run through a Beall degerminator, coarse grits were isolated by sieving, bran was aspirated from the air-dried residue, and germ was separated by hand. The endosperm was ground and extracted at 5°C. with hexane to remove lipids according to the procedure of Boundy et al. (1). The endosperm meal was twice extracted with 0.5M sodium chloride for 2 hr. at 4°C. at a 5:1 solvent:meal ratio to remove albumins and globulins. (Globulins were precipitated from the extract by 48-hr. dialysis against distilled water at 4°C.) Zein was next removed from the meal by exhaustive extraction, three times at room temperature, each 4-hr. extraction at a 5:1 ratio of 70% ethanol. The final residue presumably contained only glutelin and starch and is termed the glutelin-containing residue.

Several procedures were tried to extract glutelin from the glutelin-containing residue. The procedures are described below and are summarized in Table I.

1. Extractions for 4 hr. with 0.1M NaOH at 25°C. at a 5:1 solvent:solid ratio, after which the extracts were dialyzed against water at 1°C. and freeze-dried.
2. Same as 1, except the extract was neutralized to pH 5.9 with 10% acetic acid before dialysis.
3. Same as 2, except extraction was done at 1°C.
4. Extraction with 0.2M dimethylaminoethanol (7); other conditions were the same as in 1.
5. Extraction for 3 hr. with 8M urea at pH 8; other conditions the same as in 1.
6. Extraction for 2 hr. with 30% 2-chloroethanol at 25°C. at a 5:1 solvent:solid ratio followed by overnight dialysis at 1°C. and freeze-drying.
7. Same as 6, but with 70% 2-chloroethanol-0.01M HCl (8).
8. Same as 6, but with 100% 2-chloroethanol.
9. Extraction with 8M urea-0.075M mercaptoethanol at pH 8 for 3 hr. at 25°C. at a 5:1 solvent:solid ratio, after which the extract was made 1.2M with acrylonitrile to alkylate sulfhydryl groups, followed by overnight dialysis at 1°C. and freeze-drying.
10. Extraction of starch from the glutelin-containing residue with 90% dimethyl sulfoxide (DMSO) (9) at 25°C. for 2 hr. at a 10:1 solvent:solid ratio followed by centrifugation for 1 hr. at 59,000 X g (Spinco type 21 rotor at 21,000 r.p.m.). The precipitate which contained glutelin plus some gelled starch was saved and the starch-containing supernatant discarded.
11. Removal of starch by digestion with alpha-amylase. The glutelin-containing residue (30 g.) was dispersed in 1,500 ml. of pH 7.5 phosphate buffer and heated to 90°C. to gel the starch. After cooling, 1 g. of alpha-amylase (Nutritional Biochemicals control No. 4978) was added and digestion was allowed to proceed overnight at 25°C. Digestion of starch was monitored by

TABLE I. AMOUNTS AND COMPOSITION OF CORN GLUTELIN ISOLATED BY VARIOUS PROCEDURES

Extractant	Conditions	Glutelin Extracted[a] %	Nitrogen in Glutelin Preparation %
Alkaline extractants			
0.1M NaOH	4 hr. at 25°C., extract not neutralized	86	8.6
0.1M NaOH	4 hr. at 25°C., extract neutralized	76	13.7
0.1M NaOH	4 hr. at 1°C., extract neutralized	70	12.9
0.2M Dimethylaminoethanol	4 hr. at 25°C., extract not neutralized	12	4.4
Other protein extractants			
8M Urea pH 8	3 hr. at 25°C.	6	1.3
30% 2-Chloroethanol	2 hr. at 25°C.	30	4.7
70% 2-Chloroethanol-0.01M hydrochloric acid	2 hr. at 25°C.	32	2.0
100% 2-Chloroethanol	2 hr. at 25°C.	18	1.4
Extraction with disulfide-bond cleavage			
8M Urea-0.075M mercaptoethanol	3 hr. at 25°C.,then alkylation of sulfhydryl with acrylonitrile	16	2.7
Removal of starch from glutelin			
90% Dimethyl sulfoxide	2 hr. at 25°C.; glutelin ppt. removed by centrifugation	70	2.2
Alpha-amylase	Digest heat-gelled starch overnight with enzyme; glutelin ppt. removed by centrifugation	74	5.9

[a]Based on percent of nitrogen isolated from the glutelin-containing residue.

disappearance of blue color formed with I_2 in KI solution. The digestion mixture was centrifuged and the precipitate washed with water and freeze-dried.

Amino Acid Analyses

Protein samples were hydrolyzed 24 hr. by refluxing 50 mg. protein in 100 ml. constant-boiling HCl. After HCl was removed from the hydrolysates by rotary evaporation under partial vacuum and they were dissolved in an appropriate buffer to a given volume, their amino acid compositions were determined by the procedure of Benson and Patterson (10) with a Phoenix Model K-8000 analyzer. The results were calculated according to procedures developed by Cavins and Friedman (11). Cystine was determined as cysteic acid and methionine as methionine sulfoxide on separate samples after oxidation with performic acid (12).

Electrophoresis

Moving-boundary electrophoretic measurements were made with a Spinco Model H instrument. Protein samples were dissolved in 8M urea-0.2M lactic acid at 0.4% protein concentration and then dialyzed overnight against electrophoresis

buffer, consisting of 6M urea-0.2M lactic acid-0.0167M aluminum lactate, pH 3.3. The buffer is quite similar to that used by Jones et al. (13) except for the addition of urea, which is necessary to keep glutelin proteins in solution. Current was adjusted to 2 ma., which was one-fourth the current that could be used in a buffer containing only dilute salts with the same conductivity. This low current controlled the tendency of the urea-containing system to convect.

Starch-gel electrophoresis was carried out in gels at pH 3.1 containing 8M urea, 0.0083M aluminum lactate (Z. D. Gilman Inc.), 0.52M lactic acid, and 16% starch (Connaught) (14), and in the apparatus described by Beckwith et al. (15). Samples were inserted on paper strips immersed in solution containing 5% protein in electrophoresis buffer. To facilitate electrophoretic migration in starch gel, the disulfide bonds in many of the samples were cleaved by reduction with mercaptoethanol followed by alkylation with acrylonitrile. Reduction was done by adding a 100-fold excess of mercaptoethanol to the sample dissolved at 1% protein concentration in 8M urea buffered to pH 7.5 with 0.01M phosphate, and stirring 16 hr. at 25°. Alkylation was done by adding twice as much acrylonitrile as mercaptoethanol to the reaction mixture and stirring for 1 hr., and was followed by dialysis against dilute acetic acid and freeze-drying (16).

RESULTS AND DISCUSSION

Isolation of Protein

Since NaCl (0.5M) extracted an average of 6% of the endosperm protein, albumins and globulins must constitute only a minor portion of corn-endosperm protein. An average of 44% of the endosperm protein was removed after three extractions with 5 vols. of 70% ethanol (zein). The remaining 50% of the corn-endosperm protein we consider glutelin, on the basis of the definition of glutelin as the corn-endosperm protein not soluble in dilute salt solution nor in neutral 70% ethanol.

All the glutelin isolation experiments described here were performed on the endosperm residue remaining after this exhaustive extraction with dilute saline and 70% ethanol. The various conditions used to isolate glutelin are summarized in Table I, along with amounts of protein isolated and nitrogen contents of the isolated proteins. These procedures can be divided into four classes: (a) extraction with alkaline solvents, (b) extraction with other protein solvents, (c) extraction with gentle cleavage of disulfide bonds, and (d) removal of starch from the glutelin-containing material.

Extraction with dilute NaOH is the traditional method for isolating glutelin from corn endosperm. Extraction with 0.1M NaOH at 25°, followed by dialysis, removed 86% of the glutelin present as measured by the amount of nitrogen removed from the glutelin-containing residue. Since much of the glutelin so obtained was soluble in distilled water, the extraction treatment evidently altered the protein. When the NaOH extract was neutralized to pH 5.9 with acetic acid after the 4-hr. extraction period, 76% of the protein was isolated. Upon neutralization a strong mercaptan odor was noted. Extraction with 0.1N NaOH at 1°C. for 4 hr., followed by neutralization to pH 5.9 with acetic acid, yielded about 70% of the glutelin from the meal residue. Little glutelin was soluble in neutral

aqueous solution. Again, upon neutralization a mercaptan odor was noted. The mercaptan odor strongly indicated that alkaline cleavage of disulfide bonds was taking place (2,3). Dimethylaminoethanol (0.2M) extracted only 12% of the glutelin present, and after neutralization the familiar mercaptan odor was noticeable, which indicated that this reagent also extracted glutelin by means of alkaline cleavage of disulfide bonds.

Other protein solvents tried were 8M urea at pH 8 and 2-chloroethanol at three concentrations. The urea system extracted only 6% of the glutelin. It also dissolved considerable starch, as evidenced by the low nitrogen content of the glutelin extracted. Each of the aqueous 2-chloroethanol systems extracted about 30% of the glutelin present, but the isolated proteins were low in nitrogen, apparently because the solvent was also extracting starch. Pure 2-chloroethanol extracts less protein and more starch.

At this point it seemed that good protein solvents like 2-chloroethanol and strong urea did not effectively extract glutelin, whereas strong alkali extracted glutelin efficiently, presumably because the alkali released the protein by means of disulfide cleavage. Therefore, it was decided to extract glutelin with 8M urea plus 0.075M mercaptoethanol, the purpose of mercaptoethanol being to specifically cleave enough disulfide bonds to solubilize the glutelin protein in urea. Urea plus mercaptoethanol extracted more than twice as much glutelin as urea alone, although the amount of glutelin extracted was still low. This small amount extracted was in part a result of the starch's swelling in the strong urea solution and forming a large mass which occluded some of the protein that would otherwise have been in the extract.

Instead of removal of glutelin from the glutelin-containing residue, experiments were tried in which starch was removed. In the first experiment the glutelin-containing residue was extracted with 90% DMSO for 2 hr. at room temperature and then centrifuged. Most of the starch was solubilized, and glutelin was left as a yellowish precipitate contaminated with some gelled starch. The precipitate contained 70% of the glutelin in the residue. The glutelin precipitate was low in nitrogen owing to the presence of gelled starch.

After swelling by heating to 90°C., starch was also removed from the glutelin-containing residue by digestion with alpha-amylase in phosphate buffer. After the treated slurry was centrifuged and washed to remove enzyme and soluble carbohydrate, 74% of the nitrogen in the glutelin-containing residue was recovered. The incomplete recovery of nitrogen may be due either to mechanical loss, or to the release of occluded soluble proteins and peptides in the glutelin residue, or possibly to some protease activity in the alpha-amylase preparation.

The glutelin obtained by the DMSO and the alpha-amylase procedures was insoluble in 8M urea, 6M guanidine hydrochloride, 90% DMSO, and the like. It only became soluble in these solvents after cleavage of disulfide bonds. This insolubility would strongly indicate that glutelin subunits in mature corn endosperm are cross-linked by disulfide bonds to form a three-dimensional network.

Amino Acid Composition

In Table II are given amino acid analyses on glutelin prepared by various methods. Theoretical calculations like those made by Tkachuk (17) show that these

TABLE II. AMINO ACID COMPOSITION OF CORN GLUTELIN PREPARED BY VARIOUS METHODS[a]

	Method of Preparation					
Amino Acid	0.1M NaOH[b]	Dimethylamino-ethanol	30% 2-Chloroethanol	Urea + SH	Dimethyl Sulfoxide	Alpha-Amylase
Lysine	14	14	13	18	26	19
Histidine	23	39	12	31	29	28
Ammonia	167	223	240	212	186	173
Arginine	27	24	18	28	29	28
Aspartic	50	33	49	37	46	44
Threonine	35	34	30	33	35	35
Serine	54	48	51	48	45	48
Glutamic	168	169	213	158	183	162
Proline	132	124	68	107	84	126
Glycine	56	63	38	68	62	61
Alanine	97	74	110	81	76	88
1/2-Cystine	19	26	10	46	...	37
Valine	50	52	47	51	56	55
Methionine	20	16	17	30	21	20
Isoleucine	29	24	33	23	32	31
Leucine	119	88	124	83	83	101
Tyrosine	31	25	33	33	31	30
Phenylalanine	32	22	39	22	32	28

[a]mM per 17.5 g. N or mM per 100 g. protein.

[b]Four-hour extraction with 0.1M NaOH without neutralization.

glutelin preparations when pure would contain 17.5% nitrogen; hence, the amino acid compositions are expressed as mmoles of amino acid per 17.5 g. of nitrogen, which expression is equivalent to residues per 100,000 g. of protein.

In all of the glutelin preparations, the amount of ammonia is almost equal to the sum of the glutamic and aspartic residues, so both of these amino acids are primarily present as residues of the amides, glutamine and asparagine. Glutamine and asparagine constitute about 20% of the amino acid residues in corn glutelin; proline constitutes a little less than 15% of the residues; and hydrophobic residues such as leucine and alanine constitute a little over 40% of the residues. Amperometric titration (18,19) showed that none of the glutelin preparations contained sulfhydryl groups, i.e., cysteine.

Glutelin prepared by the alpha-amylase procedure contains 37 half-cystine residues per 100,000 g. of protein. This amount of cystine is substantially higher than that in glutelin prepared by extraction with NaOH or dimethylaminoethanol. This is expected, since alkali degrades cystine into a complex of products (2,3). The large amount of cystine in glutelin is more than enough to allow formation of a three-dimensional disulfide network, which is postulated as the reason for glutelin's not being extractable from corn endosperm without cleavage of disulfide bonds.

There are significant variations in the amino acid composition of glutelin prepared by various procedures. With the exception of cystine which is destroyed under alkaline conditions and lysine which reacts with one of the degradation products of cystine (2,3), these differences in amino acid composition indicate that the glutelins prepared by various procedures are different in protein composition. Since the alpha-amylase and DMSO procedures isolate most of the protein in the

glutelin-containing residue without chemical degradation, the amino acid composition of these preparations should closely represent the glutelin system present in intact corn endosperm.

Electrophoresis

Moving-boundary and starch-gel electrophoresis were carried out with similar buffers so that results of the two methods would be comparable, with two major exceptions:

1) Resolution in starch-gel is better than in moving-boundary. For example, a protein that moves as one peak in moving-boundary electrophoresis will often be resolved into two or more bands on starch-gel electrophoresis.

2) Extremely large protein molecules will not migrate into a starch gel and those of intermediate size are retarded on starch with attendant streaking. Thus it is necessary to reduce large protein molecules to their subunits via disulfide cleavage prior to starch-gel electrophoresis. Resolution of large intact protein molecules is possible in moving-boundary electrophoresis.

Moving-boundary electrophoretic patterns of various corn-endosperm proteins are reproduced in Fig. 1. Starch-gel patterns of these proteins in both their native and reduced-alkylated forms are given in Fig. 2. Albumins and globulins exhibit several peaks with mobilities ranging between 2 and 10 Tiselius units, whereas gel patterns contain a large number of fast-moving bands.

Zein has a moving-boundary pattern (Fig. 1) consisting of one peak with a mobility of only 0.75 Tiselius unit. The starch-gel electrophoretic pattern of native zein (Fig. 2) is characterized by a pair of slow-moving bands, corresponding to the single peak seen in moving-boundary electrophoresis, plus streaking caused by high-molecular-weight components. Streaking is not present in reduced-alkylated zein.

The glutelin sample shown in Fig. 2 was prepared by the alpha-amylase procedure. The native material was not soluble in the electrophoresis solvent, hence gave no gel-electrophoretic pattern. The peptide subunits in the reduced-alkylated material yielded several bands with mobilities mainly between those of zein and those of albumins and globulins.

Moving-boundary and starch-gel electrophoretic patterns of glutelin prepared by various procedures are given in Figs. 1 and 3, respectively. Several of the glutelin samples were reduced and alkylated before starch-gel electrophoresis to facilitate migration into the gel. Glutelin prepared by the 90% DMSO and the alpha-amylase procedures had to be reduced and alkylated before moving-boundary or starch-gel electrophoresis to make it soluble.

Glutelin extracted with 0.1M sodium hydroxide at 1°C. has a moving-boundary electrophoretic pattern (Fig. 1) containing a wide distribution of components with two peaks at mobilities of 2 and 5 Tiselius units. The starch-gel electrophoretic pattern (Fig. 3) of this material shows most of the protein at the origin, which position is indicative of high molecular weight. Glutelin extracted by 0.1M sodium hydroxide at 25°C. has a complex moving-boundary electrophoretic pattern (Fig. 1). Its starch-gel electrophoretic pattern (Fig. 3) is characterized by streaking. Upon reduction and alkylation, this material has a starch-gel electrophoretic pattern characterized by a large number of bands. It appears that 0.1M NaOH at room

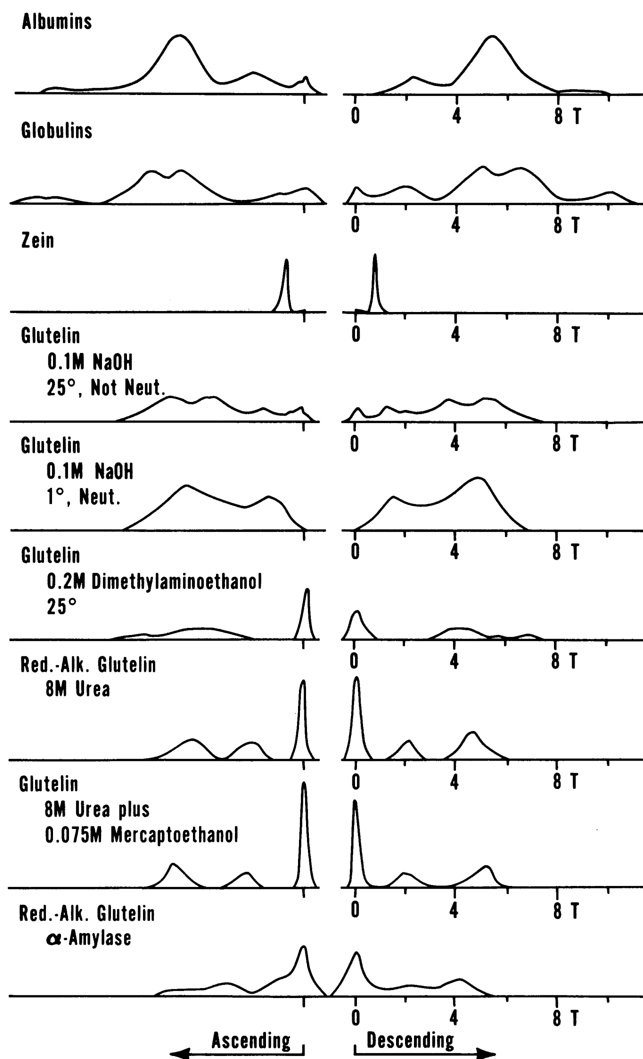

Fig. 1. Moving-boundary electrophoretic patterns of corn glutelin prepared by various methods plus other corn proteins obtained by classic extraction procedures. Solvent is 6M urea-0.2M lactic acid-0.0167M aluminum lactate, pH 3.3.

temperature has cleaved disulfide bonds in glutelin and has produced other degradative changes which result in a complex electrophoretic pattern.

Only a small amount of glutelin is extracted with 0.2M dimethylaminoethanol, and this material has a complex moving-boundary electrophoretic pattern (Fig. 1). The starch-gel electrophoretic pattern of the material (Fig. 3) in both its native and reduced alkylated forms is characterized by streaking plus a number of bands.

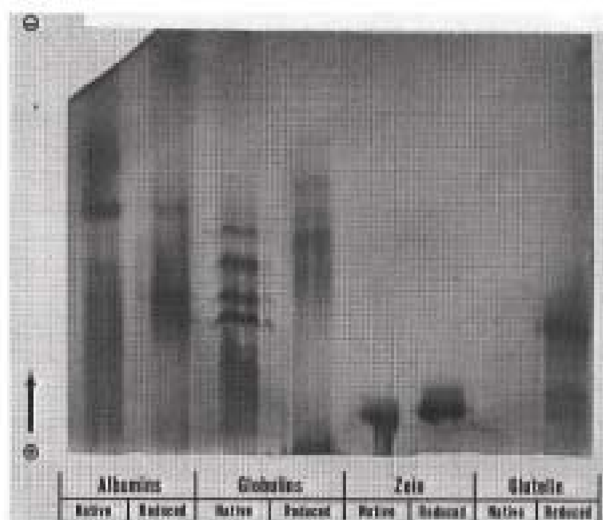

Fig. 2. Starch-gel electrophoretic patterns of major classes of corn-endosperm proteins. Albumins, globulins, and zein were isolated by solubility procedures and glutelin was isolated by the alpha-amylase procedure. Buffer is 8M urea-0.52M lactic acid-0.008M aluminum lactate, pH 3.1.

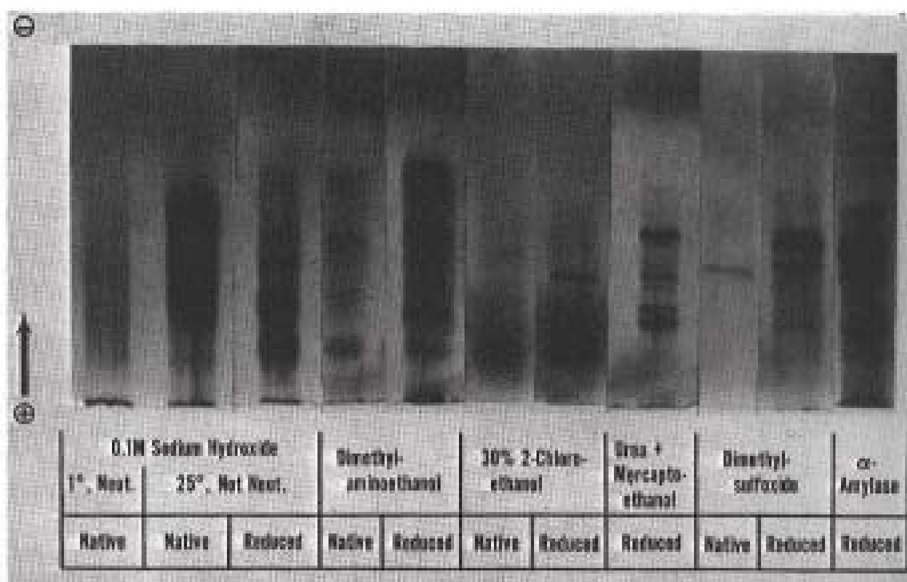

Fig. 3. Electrophoretic patterns of glutelin obtained by various procedures in starch gels containing 8M urea-0.52M lactic acid-0.008M aluminum lactate, pH 3.1.

Apparently dimethylaminoethanol is not alkaline enough to extract glutelin efficiently; however, it degrades the small amount of glutelin it extracts.

The major feature in the starch-gel electrophoretic pattern of the protein extracted with 30% 2-chloroethanol (Fig. 3) is a slow-moving band or pair of bands characteristic of zein (compare with Fig. 2). When the protein is reduced and alkylated, a characteristic glutelin band appears. The gel-electrophoretic pattern of the 30% 2-chloroethanol extract, plus its amino acid composition, indicates extraction of both residual zein not previously separated by neutral 70% ethanol and some glutelin apparently not bound into a three-dimensional disulfide cross-linked structure.

The small amount of protein extracted by 8M urea at pH 8 was reduced, alkylated, and then analyzed by moving-boundary electrophoresis (Fig. 1). It had two peaks with mobilities of 2 and 5 Tiselius units. In addition, there was a large amount of material at the initial boundary attributable to starch, which was also extracted by 8M urea under these conditions.

The protein extracted by 8M urea plus 0.075M mercaptoethanol followed by alkylation has a moving-boundary pattern (Fig. 1) identical with that of the reduced-alkylated protein extracted by 8M urea alone. The starch-gel electrophoretic pattern of the material (Fig. 3) is characterized by four sharp bands. The fastest band apparently corresponds to the peak at 5 Tiselius units in the moving-boundary pattern and the slowest pair of bands apparently corresponds to the peak at 2 Tiselius units in the moving-boundary pattern.

When glutelin is prepared by extracting starch from the glutelin-containing residue with 90% DMSO, the product is virtually insoluble in the urea-containing electrophoresis buffer. The gel-electrophoretic pattern of the portion that is soluble (Fig. 3) shows only one band. The corresponding band is also seen in glutelins prepared by other mild procedures. When the disulfide bonds of this glutelin preparation are cleaved by reduction and alkylation, it has a gel-electrophoretic pattern with a number of bands (Fig. 3). This pattern is considered characteristic of the peptide subunits of glutelin.

Glutelin obtained by removal of starch from the glutelin-containing residue with alpha-amylase was also insoluble in the urea-containing electrophoresis solvents. Therefore, its disulfide bonds were cleaved by reduction and alkylation before electrophoresis. The moving-boundary pattern of this material (Fig. 1) is characterized by a wide distribution of components plus nonmigrating material attributable to partially digested starch. The presence of such starch is also indicated by the low nitrogen content of alpha-amylase-prepared glutelin. The gel-electrophoretic pattern of the reduced-alkylated material (Figs. 2 and 3) is quite similar to that of reduced-alkylated glutelin obtained by extracting starch from the glutelin-containing residue with 90% DMSO.

GENERAL DISCUSSION

Glutelin cannot be extracted from corn endosperm in good yield without disulfide bond cleavage, whereas other corn-endosperm proteins are readily extractable with appropriate solvents. Also, glutelin prepared by digesting starch away from the glutelin-containing matrix with alpha-amylase is not soluble even in strong protein solvents, such as 8M urea and 90% DMSO. This is strong evidence

that glutelin is present in corn endosperm as a three-dimensional network cross-linked by disulfide bonds. The large amount of cystine we found in glutelin is quite adequate to form a disulfide cross-linked network in three dimensions. Other workers (1,5,6,20) have reported lesser amounts of cystine in glutelin because analyses were made on NaOH-treated samples and different methods for determining cystine were used.

Disulfide cleavage of glutelin yields a distinct group of subunit proteins whose mobilities are between those of albumins and globulins and those of zein (Fig. 2). The complex electrophoretic patterns of glutelin extracted with dilute NaOH or dimethylaminoethanol result from degradation.

Cytological studies also demonstrate that glutelin is a distinct protein system in mature corn endosperm (21). The tissue contains protein bodies in a protein matrix. The protein bodies are soluble in 70% ethanol, hence are zein; whereas the matrix is not ethanol-soluble. The protein matrix was isolated from immature corn endosperm by means of density gradient sedimentation (22) and has electrophoretic properties, solubility properties, and an amino acid composition quite suggestive of glutelin.

In our work the best methods for isolating glutelin from mature corn endosperm were those which involved removing starch from glutelin either by dissolving with 90% DMSO or by digesting with alpha-amylase. The DMSO procedure did not completely remove starch, and the solvent probably also removed the small portion of glutelin not in the form of a three-dimensional disulfide network plus some residual zein. The alpha-amylase procedure also had certain drawbacks. Heating the glutelin residue to gel the starch may alter the protein, and the alpha-amylase used may have had some protease activity. Work is currently under way to refine this method of preparing glutelin, as well as to develop other procedures for isolating glutelin from corn.

Acknowledgment

Discussions concerning amino acid analyses with James Cavins are gratefully acknowledged.

Literature Cited

1. BOUNDY, JOYCE A., WOYCHIK, J. H., DIMLER, R. J., and WALL, J. S. Protein composition of dent, waxy, and high-amylose corns. Cereal Chem. 44: 160 (1967).
2. DONOVAN, J. W. Spectrophotometric observation of the alkaline hydrolysis of protein disulfide bonds. Biochem. Biophys. Res. Commun. 29: 734 (1967).
3. PARKER, A. J., and KHARASCH, N. The scission of the sulfur-sulfur bond. Chem. Rev. 59: 583 (1959).
4. MERTZ, E. T., LLOYD, N. E., and BRESSANI, R. Studies on corn proteins. II. Electrophoretic analysis of germ and endosperm extracts. Cereal Chem. 35: 146 (1958).
5. LLOYD, N. E., and MERTZ, E. T. Studies on corn proteins. III. The glutelins of corn. Cereal Chem. 35: 156 (1958).
6. CONCON, J. M. The proteins of opaque-2 maize. In: Proceedings High-Lysine Corn Conf., ed. by E. T. Mertz and O. E. Nelson, pp. 67–73. Corn Industries Research Foundation: Washington, D.C. (1966).
7. INAMINE, E. S., NOBLE, ELAINE G., and MECHAM, D. K. Solubilization and fractionation of wheat flour proteins insoluble in dilute acetic acid. Cereal Chem. 44: 143 (1967).
8. TSCHOEGL, N. W. Chloroethanol as a cereal protein dispersant. Cereal Chem. 38: 516 (1961).

9. LEACH, H. W., and SCHOCH, T. J. Structure of the starch granule. III. Solubilities of granular starches in dimethyl sulfoxide. Cereal Chem. 39: 318 (1962).
10. BENSON, J. V., Jr., and PATTERSON, J. A. Accelerated automatic chromatographic analysis of amino acid on a spherical resin. Anal. Chem. 37: 1108 (1965).
11. CAVINS, J. F., and FRIEDMAN, M. Automatic integration and computation of amino acid analyses. Cereal Chem. 45: 172 (1968).
12. MOORE, S. On the determination of cystine as cysteic acid. J. Biol. Chem. 238: 235 (1963).
13. JONES, R. W., TAYLOR, N. W., and SENTI, F. R. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. 84: 363 (1959).
14. PAULIS, J. W., and WALL, J. S. Albumins and globulins in extracts of corn grain parts. Cereal Chem. 46: 263 (1969).
15. 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).
16. CAVINS, J. F., and FRIEDMAN, M. Specific modification of protein sulfhydryl groups with α,β-unsaturated compounds. J. Biol. Chem. 243: 3357 (1968).
17. TKACHUK, R. Nitrogen-to-protein conversion factors for cereals and oilseed meals. Cereal Chem. 46: 419 (1969).
18. BENESCH, RUTH E., LARDY, H. A., and BENESCH, R. The sulfhydryl groups of crystalline proteins. I. Some albumins, enzymes, and hemoglobins. J. Biol. Chem. 216: 663 (1955).
19. ROTHFUS, J. A. Improved apparatus for the amperometric titration of sulfhydryl groups. Anal. Biochem. 16: 167 (1966).
20. WALL, J. S. Cereal proteins. In: Symposium on foods: Proteins and their reactions, ed. by H. W. Schultz and A. F. Anglemier, pp. 315–341. AVI Pub. Co.: Westport, Conn. (1961).
21. WOLF, M. J., KHOO, U., and SECKINGER, H. L. Subcellular structure of endosperm protein in high-lysine and normal corn. Science 157: 556 (1967).
22. CHRISTIANSON, D. D., NIELSEN, H. C., KHOO, U., WOLF, M. J., and WALL, J. S. Isolation and chemical composition of protein bodies and matrix proteins in corn endosperm. Cereal Chem. 46: 372 (1969).

[Received February 12, 1969. Accepted February 2, 1970]