

Characterization of Zeins Fractionated by Reversed-Phase High-Performance Liquid Chromatography¹

J. W. PAULIS² and J. A. BIETZ²

ABSTRACT

Cereal Chem. 65(3):215-222

Zein, the major alcohol-soluble protein of corn endosperm, was extracted with 55% 2-propanol. It was then fractionated by preparative reversed-phase (C₁₈) high-performance liquid chromatography (RP-HPLC) with an acetonitrile-trifluoroacetic acid gradient. From 15 to 17 peaks resulted for native or reduced and alkylated (R-A) zeins. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed early eluting HPLC fractions of native zein to be primarily monomers; later-eluting fractions increasingly tend to be or to form oligomers. SDS-

PAGE showed that for R-A zeins, all RP-HPLC fractions are monomers. Acidic urea PAGE and isoelectric focusing showed native zeins in each RP-HPLC fraction to contain 3-10 components, compared to 1-5 for R-A zein. Proteins differing in hydrophobicity frequently had similar electrophoretic mobilities, emphasizing zein's heterogeneity. Amino acid compositions of all RP-HPLC fractions were similar, suggesting marked homology of zein polypeptides. Preparative RP-HPLC was also useful for isolating zeins for further studies.

Zein, the major storage protein in corn endosperm, is usually extracted with aqueous ethanol or isopropanol. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) without β -mercaptoethanol (β -ME), as well as ultracentrifugation, have shown zein to consist of almost equal amounts of dimers and monomers, with lesser amounts of higher molecular weight (mol wt) oligomers. The monomeric polypeptides have molecular weights of 19,000-25,000 (referred to here as 22,000 and 24,000 mol wt) (Turner et al 1965, Ganchev et al 1976, Tsai 1980, Paulis 1981, Landry and Sallantin 1983, Abe et al 1985). Upon SDS-PAGE in the presence of β -ME, zein appears as only 22,000 and 24,000 mol wt subunits.

Although virtually insoluble in water, zein's conformation is characteristic of more conventional globular proteins (Danzer et al 1975). It contains 45% α -helix in 70% ethanol (Wu et al 1983). Osmotic studies in dimethylformamide and formamide (Danzer and Rees 1976) show zein to have a number average mol wt of 21,000-24,000. Some zein (B-zein) exists as an aggregate, which dissociates to monomers in formamide, but reaggregates at higher protein concentrations. In contrast, sulfhydryl analysis (Abe et al 1985) showed that almost all zein cysteine residues form intermolecular disulfide bonds in dimers, polymers, and in protein bodies (Abe et al 1981).

Recently, reversed-phase high-performance liquid chromatography (RP-HPLC) was used to identify corn varieties (Bietz 1985, Smith 1986, Paulis and Bietz 1986, Paulis et al 1986, Smith and Smith 1986). To further understand the aggregated structure of zein, we used preparative RP-HPLC to fractionate native and reduced and alkylated (R-A) zeins, both solubilized in 8M urea. Resulting fractions were characterized by isoelectric focusing (IEF), acidic urea (AU) PAGE, SDS-PAGE, and amino acid analysis to determine whether oligomer formation is random or ordered, or results from disulfide interchange or association during isolation. Differences in zein surface hydrophobicities revealed by RP-HPLC show that subunit structure and conformation direct oligomer formation. "Three-dimensional" characterization of zein in terms of surface hydrophobicity, molecular weight, and charge heterogeneity, using RP-HPLC, SDS-PAGE, and IEF + AU-PAGE, respectively, may be superior to two-dimensional separations based on SDS-PAGE and IEF or on AU-PAGE + IEF

(Hagen and Rubenstein 1980, Wilson et al 1981, Wall et al 1984, Wilson 1986).

MATERIALS AND METHODS

Protein Preparation

Zein was isolated from maize inbred W64A (Landry et al 1983). Samples of native zein for RP-HPLC, IEF, and AU-PAGE were dissolved in 8M urea. Zeins were also reduced in 8M urea with 1% β -ME (16 hr at room temperature), alkylated for 1 hr with acrylonitrile, and acidified with aluminum lactate buffer to form R-A zein (Paulis and Wall 1977). RP-HPLC fractions of native zein were examined both unreduced and after reduction and alkylation.

RP-HPLC

A 250 \times 10 mm SynChropak RP-P column (C₁₈) having 300 Å pores was used for preparative RP-HPLC. The column was eluted at room temperature first for 10 min with 42.3% aqueous acetonitrile (CH₃CN) (+ 0.1% trifluoroacetic acid) and then with an 80-min linear gradient from 42.3 to 57.3% CH₃CN (Paulis and Bietz 1986). The column effluent was monitored at 280 nm (absorbance range = 0.4). Protein was injected as two 1.5-ml aliquots (10 mg/ml 8M urea).

Collected peak fractions (Fig. 1) were dried in 1.2 \times 15 cm test tubes at room temperature in a Buchler Vortex-Evaporator, and stored at 4°C until analyzed. Dried fractions were dissolved in 0.5-2.0 ml of 70% (v/v) aqueous ethanol at 600-1,500 μ g/ml. Protein concentrations of fractions were estimated by integration after injection of 30 mg R-A or native zein. Aliquots for SDS-PAGE, AU-PAGE, and IEF were dried in 4 cm³ disposable culture tubes by a stream of nitrogen. Corresponding fractions from replicate zein RP-HPLC fractionations were collected and lyophilized for amino acid analyses.

Analytical Methods

Lyophilized RP-HPLC fractions (equivalent to 1-10 mg of protein) were hydrolyzed by refluxing with 6M HCl (2-10 ml/mg of protein) for 24 hr. Liberated amino acids were determined on a Dionex D-300 amino acid analyzer using a physiological fluids analysis column with lithium buffers. Reported levels of methionine and cysteine include methionine sulfone and cysteine acid. Tryptophan and carboxyethylcysteine (for R-A fractions) were not determined.

Lyophilized native or R-A zein and their RP-HPLC fractions (40-50 μ g/5-10 μ l sample) were analyzed by SDS-PAGE (Laemmli 1970) using a 10-15% gradient gel (Landry et al 1983). To analyze native zein, no β -ME was used in samples or gel. β -ME was added to reduce samples of native zein and to R-A zein fractions.

Native and R-A zein RP-HPLC fractions were also analyzed by

¹Presented at the 189th ACS National Meeting, Div. Agricultural and Food Chemistry, Miami, Florida, May 1985.

²Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

IEF (pH 6–8) (Wall et al 1984) and AU-PAGE (Landry et al 1983). Native fractions were reduced and alkylated for IEF as described by Paulis and Wall (1977). Gels were fixed overnight in 30% methanol containing 10% trichloroacetic acid before silver staining (Bio-Rad Laboratories 1982).

RESULTS AND DISCUSSION

Isolation and RP-HPLC

Zein was extracted with 55% isopropanol from defatted W64A corn endosperm following salt extraction, and purified after extraction with petroleum ether, ethanol, and 55% *tert*-butanol by LH-20 chromatography (Landry 1979). RP-HPLC separated native zein (Fig. 1A) into about 17 overlapping peaks; 27 fractions were collected for subsequent electrophoresis and amino acid analysis. Gel filtration chromatography separates similar unreduced zein preparations into peaks corresponding to about 40% monomers, 40% dimers, and 20% higher molecular weight oligomers (Ivanov et al 1976, Rewa et al 1978, Rewa and Bruckner 1979, Paulis 1981, Landry and Sallantin 1983, Landry and Guyon 1984, Landry et al 1986).

R-A zein also yields 15–17 peaks upon preparative RP-HPLC (Fig. 1B). The chromatogram contains more early-eluting protein than does that of native zein. Eighteen R-A zein RP-HPLC fractions were collected for subsequent analysis.

SDS-PAGE

Figure 2A shows SDS-PAGE patterns, under nonreducing conditions, of RP-HPLC fractions of native zein. Initial fractions contained mainly 22,000 mol wt monomers plus some 44,000 mol wt dimers. Later fractions become more enriched in 24,000 mol wt monomers, and dimers of 46,000 and 48,000 mol wt become evident. In late fractions (19–27), monomers diminish, dimers predominate, and trimers, tetramers, and higher molecular weight oligomers appear.

SDS-PAGE under reducing conditions of native zein RP-HPLC fractions (Fig. 2B) shows, as does nonreducing SDS-PAGE (Fig. 2A), that initial fractions contain primarily 22,000 mol wt monomers. Bands corresponding to 24,000 mol wt appear at about fraction 5. These polypeptides are subunits of 46,000 and 48,000 dimers, and of later-eluting higher molecular weight oligomers that contain both 22,000 and 24,000 mol wt subunits.

SDS-PAGE under reducing conditions of RP-HPLC fractions of R-A zein (Fig. 2C) shows that initial fractions (1–4) contain primarily 22,000 mol wt polypeptides, corresponding in elution volume to native 22,000 mol wt zein monomers (Fig. 2B). Later-eluting RP-HPLC fractions (13–18) of R-A zein are also enriched in 22,000 mol wt polypeptides. Polypeptides of 24,000 mol wt occur predominately in fractions 7–12, which correspond to those RP-HPLC fractions of native zein enriched in dimers (Fig. 2A).

These results show that 22,000 and 24,000 mol wt zein

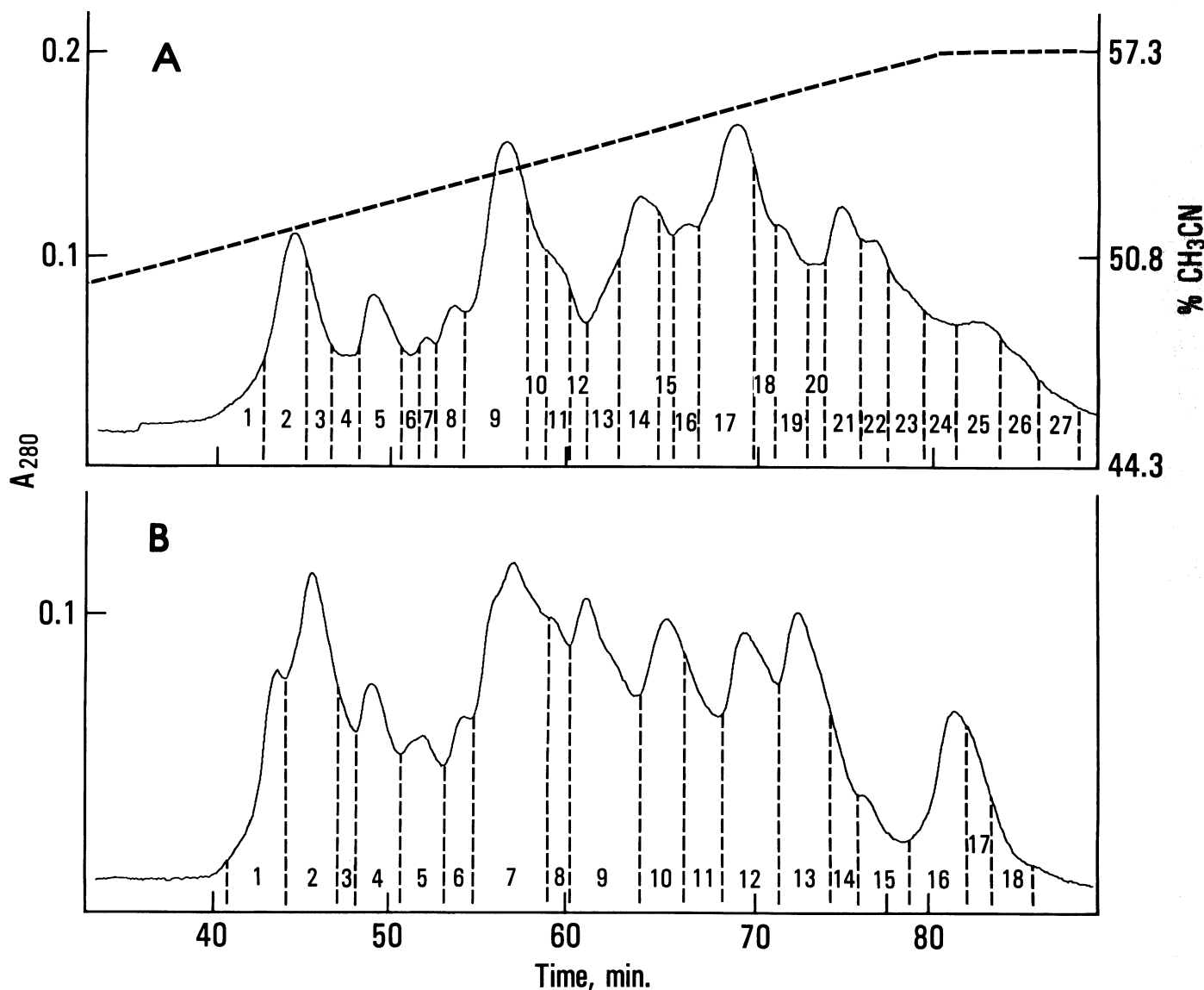


Fig. 1. Reversed-phase high-performance liquid chromatography of (A) native and (B) reduced and alkylated zein, dissolved in 8M urea, on SynChropak RP-P (C₁₈) using a linear gradient from 42.3 to 57.3% CH₃CN (+ 0.1% CF₃COOH).

polypeptides, in spite of sequence homology (Marks and Larkins 1982), differ significantly in their tendencies to form oligomers. In addition, differences in elution characteristics of native and R-A polypeptides indicate differing surface hydrophobicities. Thus, differences in zein primary structures, though conservative, appear sufficient to significantly alter surface hydrophobicities, and change RP-HPLC elution characteristics of native and R-A polypeptides.

Because some 22,000 mol wt zein monomers have RP-HPLC elution characteristics equivalent to some 22,000 mol wt R-A zeins, while other R-A 22,000 mol wt zeins elute later upon RP-HPLC, intramolecular disulfide bonding may give native 22,000 mol wt zeins conformations having relatively polar surfaces.

Zein polypeptides of 24,000 mol wt, however, differ significantly

in reactivity from 22,000 mol wt polypeptides. The 24,000 mol wt polypeptides are primarily dimers and higher molecular weight oligomers, rather than monomers, suggesting that stable conformations are not easily achieved through intramolecular disulfide bonds. Alternatively, surface reactivity that promotes association is significantly greater for 24,000 than for 22,000 mol wt zein polypeptides.

SDS-PAGE reveals that native zein oligomers are more hydrophobic than monomers, and that apparent hydrophobicity increases as molecular size and extent of association increase. Oligomeric zeins are probably stabilized by intermolecular disulfide bonds, because reduction and alkylation changes elution order of polypeptides. Also, oligomeric forms exist in the presence of SDS in native zein (Fig. 2A) but appear as monomers in the

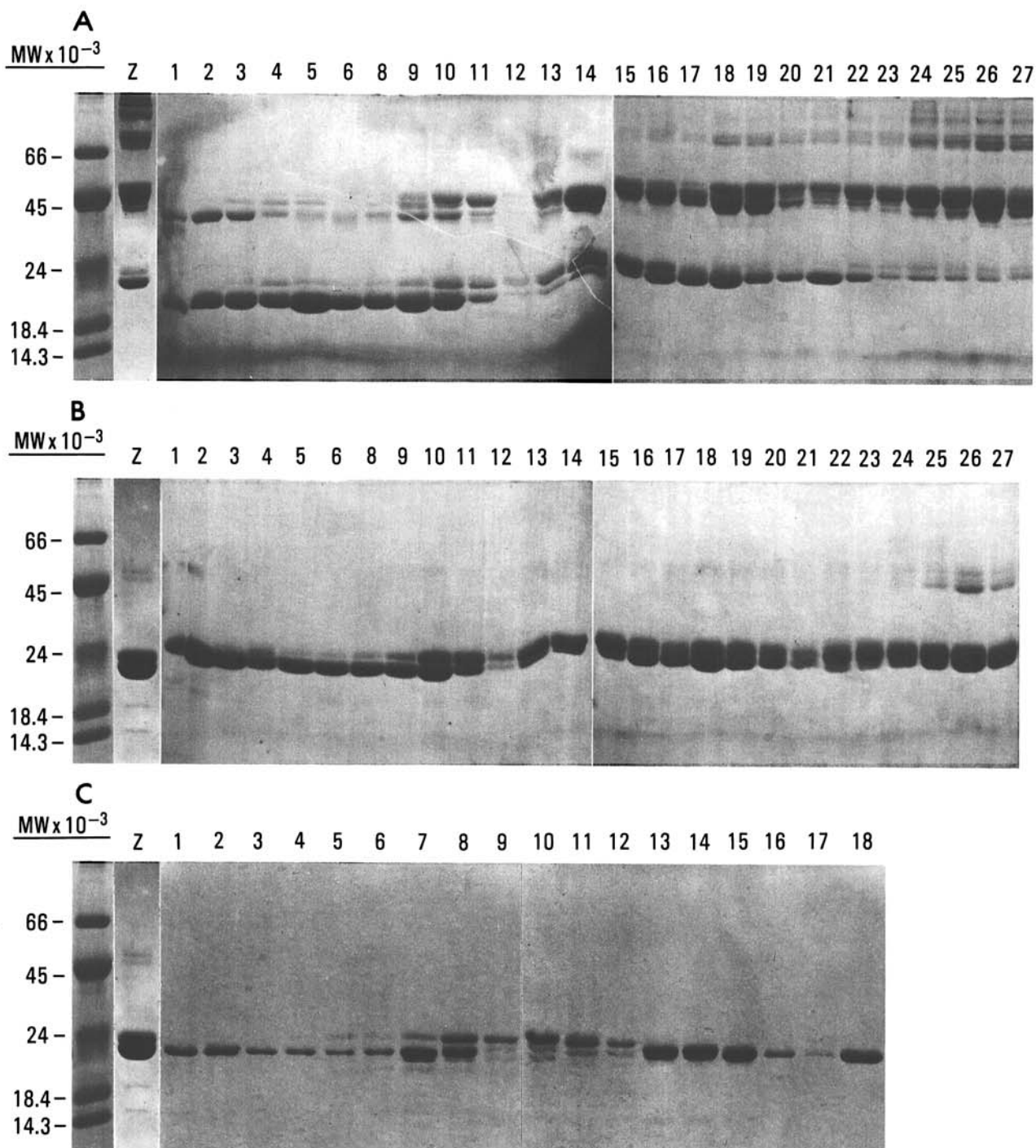


Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of (A) reversed-phase high-performance liquid chromatography (RP-HPLC) fractions of native zein (Fig. 1A), run under nonreducing conditions; (B) RP-HPLC fractions of native zein, run under reducing conditions; and (C) reduced and alkylated zein (Fig. 1B). Whole zein (Z) was treated the same as fractions for SDS-PAGE.

presence of SDS after reduction (Fig. 2B) or reduction and alkylation (Fig. 2C).

Our results do not totally rule out the possibility, however, that most zeins are monomers in a solvent such as CH₃CN, and that oligomers may form in solutions containing SDS or urea through noncovalent interactions. Studies of zein association in the presence both of denaturants and detergents would help resolve this point. Thus, it is conceivable that zeins could associate through noncovalent (e.g., hydrogen) bonds that could remain stable in the presence of SDS and acetonitrile (Fig. 2A). Further studies of zein molecular weight in additional solvents, including those containing acetonitrile, are needed.

These results also demonstrate that zein oligomers do not form randomly, but rather by specific combinations of 22,000 and

24,000 mol wt monomers of different reactivities. Oligomeric zeins adsorb more strongly to the C₁₈ column than do monomers, suggesting that disulfide bonds involved in oligomer formation are in relatively hydrophilic regions of the molecule. Resulting oligomers thus have higher surface hydrophobicities. If such association were noncovalent (e.g., through hydrogen bonds), similar masking of ionic character could increase surface hydrophobicity of oligomers.

AU-PAGE

PAGE under acidic denaturing conditions was also used to characterize zeins fractionated by RP-HPLC (Fig. 3). Differences are readily apparent among RP-HPLC fractions of native zein (Fig. 3A). Here, late-eluting fractions (15–27), which contain

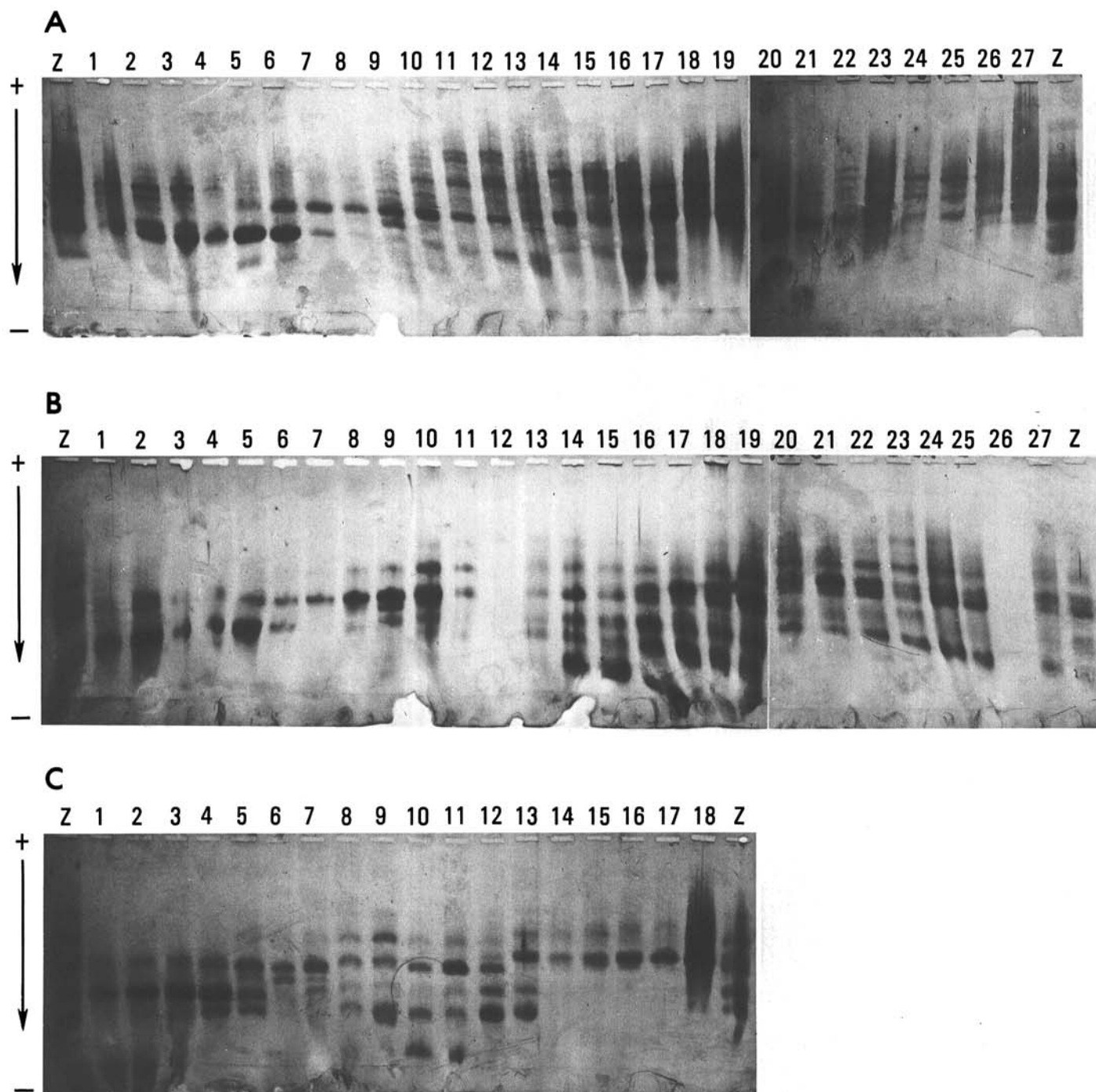


Fig. 3. Acidic urea-polyacrylamide gel electrophoresis (AU-PAGE) of (A) reversed-phase high-performance liquid chromatography (RP-HPLC) fractions of native zein, run under nonreducing conditions; (B) RP-HPLC fractions of native zein, run under reducing conditions; and (C) reduced and alkylated zein. Whole zein (Z) was treated the same as fractions for AU-PAGE. Fractions were those of Fig. 1.

higher molecular weight oligomers (Fig. 2A), appear more heterogeneous than earlier-eluting fractions, which are also heterogeneous and which are enriched in 22,000 mol wt zein monomers (Fig. 2A). Later-eluting fractions also appear more streaked than early fractions.

After reduction and alkylation of RP-HPLC fractions of native zein, AU-PAGE shows less streaking and more distinct bands in later fractions (Fig. 3B). This appears to be due to lower amounts of high molecular weight oligomers in these fractions (see also Fig. 2B). Fractions 1-8 each contain one or two major bands (differing

between fractions); later fractions are more heterogeneous, showing charge heterogeneity among proteins of similar hydrophobicity.

Similarly, AU-PAGE of R-A zeins separated by RP-HPLC (Fig. 3C) reveals that zeins of comparable hydrophobicity may differ significantly in distribution of charged amino acids. These results also show that polypeptides with similar charge or mass characteristics may have markedly different surface hydrophobicities. These results confirm the heterogeneity of the zein family, for which more than 100 structural genes exist (Soave and

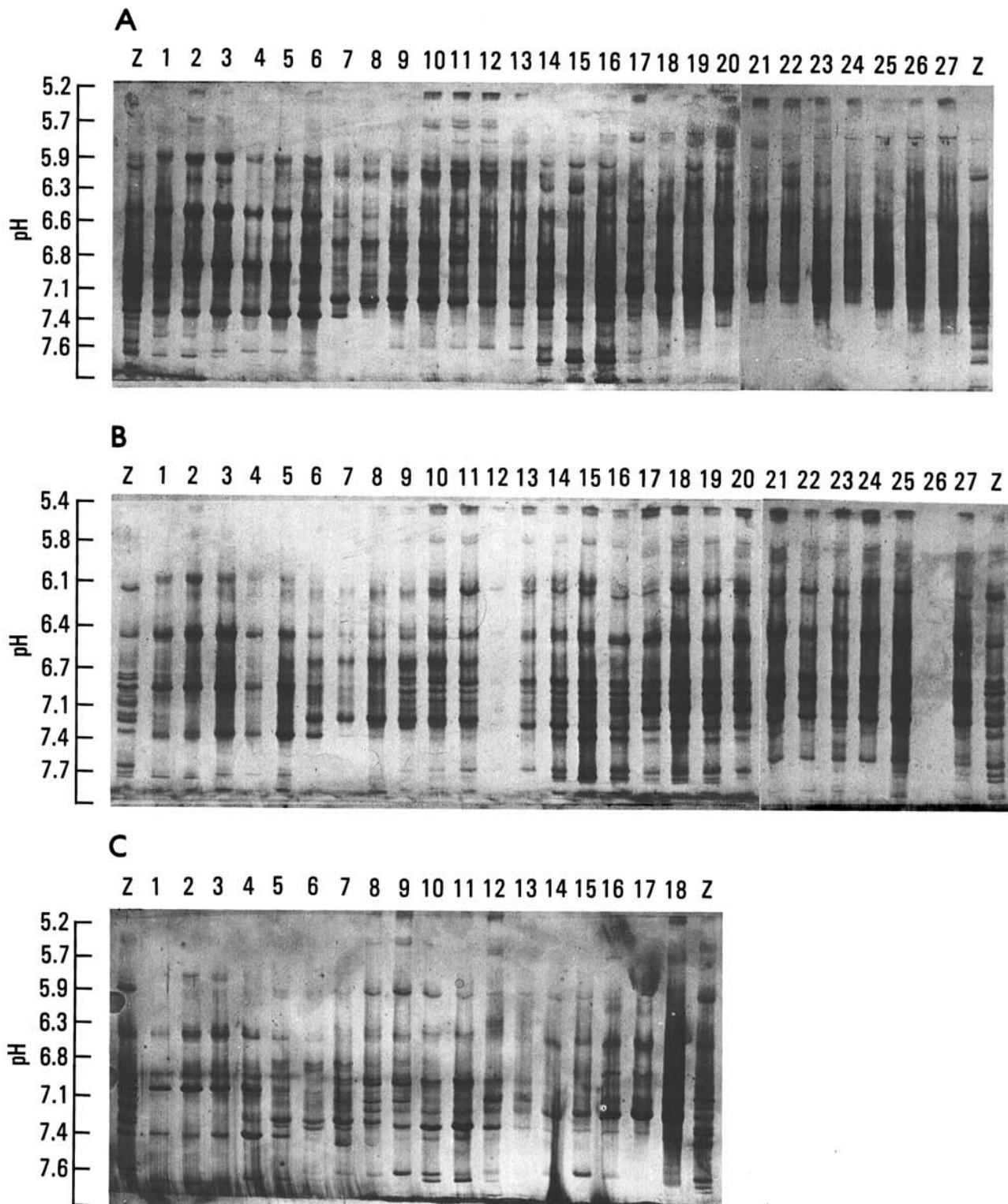


Fig. 4. Isoelectric focusing (IEF; pH 6-8) of (A) reversed-phase high-performance liquid chromatography (RP-HPLC) fractions of native zein, run under nonreducing conditions; (B) RP-HPLC fractions of native zein, run under reducing conditions; and (C) reduced and alkylated zein. Whole zein (Z) was treated the same as fractions for IEF. Fractions were those of Fig. 1.

Salamini, 1982). Our results therefore emphasize the complementary nature of AU-PAGE (based on charge and mass) and RP-HPLC (based on surface hydrophobicity). AU-PAGE also reveals (Fig. 3C) that several RP-HPLC fractions (14-17) of R-A zein have only one major band.

AU-PAGE (Fig. 3A), like SDS-PAGE, reveals unique differences between monomeric (early eluting; Fig. 2A) and oligomeric (late-eluting) zeins. These results support our above interpretation of SDS-PAGE data that zein oligomer formation is nonrandom; different polypeptides have distinctly different reactivities and tendencies to associate. AU-PAGE of reduced and alkylated native zein RP-HPLC fractions also shows that oligomers contain several components, while late-eluting RP-HPLC fractions of R-A zein are much less heterogeneous (Fig. 3C). Thus, oligomers differ in composition from late-eluting monomers.

IEF

IEF (pH 6-8) was also used to characterize RP-HPLC fractions of native zeins, without (Fig. 4A) or with (Fig. 4B) subsequent reduction and alkylation. IEF was also used to characterize RP-HPLC fractions of R-A zein (Fig. 4C). IEF of native zein fractions (Fig. 4A) revealed more bands than did AU-PAGE (Fig. 3A). Again, many bands eluted in a narrow range of surface hydrophobicity, while many bands similar in isoelectric points varied widely in hydrophobicity. Later-eluting fractions (Fig. 4A) are more streaked than earlier fractions because of the presence of oligomers.

After reduction and alkylation, IEF patterns (Fig. 4B) of native zein RP-HPLC fractions generally resemble those of unreduced native zein fractions (Fig. 4A). Later-eluting oligomer fractions, however, have more components than are apparent upon IEF of fractions of unreduced native zein (Fig. 4A), further showing that 22,000 and 24,000 mol wt subunits differ both in hydrophobicity and charge.

IEF patterns of RP-HPLC fractions of R-A zein are shown in Figure 4C. Fractions 1-4, shown by SDS-PAGE (Fig. 2C) to contain 22,000 mol wt polypeptides, appear similar to IEF patterns of native zein fractions 1-4 that were reduced and alkylated (Fig. 4B). Later fractions (13-18) differ in IEF pattern (Fig. 4C) from R-A native zein fractions of similar elution volumes (Fig. 4B), showing that 22,000 and 24,000 mol wt components of oligomers

differ from 22,000 mol wt monomers. As observed by SDS-PAGE (Fig. 2) and AU-PAGE (Fig. 3), IEF (Fig. 4) shows that some RP-HPLC fractions are enriched in specific bands. Components of similar isoelectric point frequently occur in several fractions of differing hydrophobicity, and polypeptides having similar surface hydrophobicities may vary widely in amount and distribution of ionizable amino acids. IEF combined with RP-HPLC (Fig. 4C) may reveal more heterogeneity than readily observed by two-dimensional electrophoresis combining IEF and AU-PAGE (Wall et al 1984), since RP-HPLC, based on surface hydrophobicity, has good resolution and separates proteins by characteristics different from those of IEF, AU-PAGE, and SDS-PAGE (size and charge).

Amino Acid Analysis

Amino acid compositions of various RP-HPLC fractions of native and of R-A zein are presented in Tables I and II, respectively. Fractions 2 and 5 of native zein, which contain mainly 22,000 mol wt monomers, differ slightly from fractions 2 and 4 of R-A zein, which contain mainly 22,000 mol wt monomers. Later-eluting fractions of native and R-A zeins of similar hydrophobicities also vary somewhat in composition. Thus, surface hydrophobicity may not relate closely to total hydrophobicity. These results also support the hypothesis that 22,000 and 24,000 mol wt monomers differ from subunits of native zein oligomers. This also agrees with different SDS-PAGE, AU-PAGE, and IEF patterns for different compositions for R-A and native zeins of similar elution characteristics.

Total contents of nonpolar amino acids (Tables I and II) are similar among native and R-A zein fractions, irrespective of RP-HPLC elution volumes. Thus, conservative sequence differences may significantly change surface hydrophobicity, as revealed by the RP-HPLC separations. Resulting tendencies to form oligomers also differ, as shown by electrophoresis (Figs. 2-4) of resulting fractions.

CONCLUSION

RP-HPLC, based on differences in protein surface hydrophobicity, reveals considerably more heterogeneity in zein than is readily apparent by separations based on size or charge. RP-HPLC thus essentially adds a third possible dimension to protein separations. In combination with SDS-PAGE, AU-

TABLE I
Amino Acid Composition of Reversed-Phase High-Performance Liquid Chromatographic (RP-HPLC) Fractions of Native Zein Residues/1,000 Residues

Acid	RP-HPLC Fractions ^a										
	2	5	9	14	16	17	19	21	22	23	25
Asparagine											
+ aspartic acid	54	50	48	51	53	53	46	50	53	52	51
Threonine	28	25	23	27	28	30	27	27	29	32	27
Serine	58	65	59	63	67	62	58	56	59	65	59
Glutamine											
+ glutamic acid	207	206	220	229	226	209	199	251	241	210	214
Proline	100	102	69	65	102	61	122	72	87	85	120
Glycine	25	23	22	18	24	13	12	15	15	20	16
Alanine	145	137	135	149	146	155	143	139	137	161	134
Cysteine	3	2	3	2	...	3	3	2	2	2	3
Valine	38	28	40	48	50	38	37	48	51	53	37
Methionine	tr ^b	tr	4	5	tr	6	7	9	6	8	tr
Isoleucine	27	36	42	39	34	43	43	40	42	37	45
Leucine	200	209	221	205	191	210	206	204	187	166	208
Tyrosine	43	36	38	32	34	37	34	30	30	36	32
Phenylalanine	58	58	55	44	45	55	48	39	42	48	48
Lysine	0	0	0	0	0	0	0	0	0	2	0
Histidine	16	15	11	11	tr	6	7	8	8	10	7
Arginine	tr	10	10	12	tr	13	10	9	11	13	tr
Nonpolar ^c (sum)	568	568	566	555	570	568	563	551	554	558	592

^a Fractions collected from Fig. 1A. Note that similar elution positions in Fig. 1A and 1B do not correspond to the same numbers.

^b tr = Trace.

^c Nonpolar amino acids include proline, alanine, valine, methionine, isoleucine, leucine, and phenylalanine.

TABLE II
Amino Acid Composition of Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) Fractions
of Reduced-Alkylated Zein Residues/1,000 Residues

Amino Acid	RP-HPLC Fractions ^a											
	2	4	6	7	8	9	10	11	12	13	15	16
Asparagine												
+ aspartic acid	54	54	53	49	53	56	53	52	56	54	51	56
Threonine	28	27	27	23	30	28	30	30	33	31	42	32
Serine	62	72	72	65	54	73	65	60	58	69	77	78
Glutamine												
+ glutamic acid	235	213	193	205	224	218	208	224	233	212	194	204
Proline	89	115	118	121	91	96	94	88	96	102	90	88
Glycine	31	29	47	26	23	34	21	19	14	14	26	12
Alanine	157	140	117	126	131	134	139	151	126	138	130	156
Valine	32	35	26	37	55	60	55	65	47	34	40	28
Methionine	10	tr ^b	tr	tr	7	tr	9	1	tr	tr	4	tr
Isoleucine	37	37	38	34	44	30	38	33	39	42	39	59
Leucine	179	202	188	198	183	175	189	190	182	197	185	177
Tyrosine	33	38	37	39	35	32	33	33	35	37	37	37
Phenylalanine	53	58	56	57	48	38	41	38	56	54	56	56
Lysine	0	0	4	0	0	0	0	0	5	0	5	0
Histidine	10	14	12	11	12	13	12	11	5	tr	7	4
Arginine	tr	tr	13	9	9	14	14	6	15	16	17	14
Nonpolar ^c												
(sum)	558	552	548	573	559	533	565	566	546	567	544	564

^aFractions collected from Fig. 1B. Note that similar elution positions in Fig. 1A and 1B do not correspond to the same numbers.

^btr = Trace.

^cNonpolar amino acids include proline, alanine, valine, methionine, isoleucine, leucine, and phenylalanine.

PAGE, and IEF, RP-HPLC reveals major differences in abilities of 22,000 and 24,000 mol wt zeins to form oligomers.

Our studies also suggest specific compositions for zein oligomers. Specific monomers tend to form intermolecular, rather than solely intramolecular bonds. This tendency relates to protein surfaces. RP-HPLC is a highly sensitive probe for revealing such different surface reactivities among zeins.

Differences between RP-HPLC fractionations of native and R-A zeins, and analyses of resulting fractions under reducing and nonreducing conditions, suggest that disulfide bonds cause much oligomer formation in zein. The possibility that extensive noncovalent (e.g., hydrogen) bonding facilitates bonding of monomers into oligomers cannot be totally eliminated, however. While SDS affects primarily hydrophobic bonds and urea disrupts hydrogen bonds, the dissociating-denaturing characteristics of RP-HPLC solvents (i.e., acidic aqueous acetonitrile solutions) are largely unknown. Further studies are required to explain fully the relative contributions of covalent and noncovalent bonds to zein oligomer formation.

The maize genome may contain more than 100 zein genes, considerably more than the number of proteins resolved by electrophoresis. Not all of these genes may be expressed. Nevertheless, our results show that RP-HPLC, in combination with electrophoresis, may reveal greater zein heterogeneity than can paired electrophoresis methods. Proteins of similar molecular weight and charge may differ significantly in hydrophobic amino acid content and distribution, giving them different conformations and surface hydrophobicities. RP-HPLC also reveals major differences in associative tendencies within each zein class. Clearly, RP-HPLC is a valuable tool for isolation and characterization of zein polypeptides.

ACKNOWLEDGMENTS

We thank G. M. Frankenbach and L. Cooker for technical assistance and M. Young and J. Cavins for amino acid analyses.

LITERATURE CITED

ABE, M., ARAI, S., KATO, H., and FUJIMAKI, M. 1981. Electrophoretic analysis of zein and isolation of its components. *Agric. Biol. Chem.* 45:1467.
 ABE, M., MIYASAKA, S., and ARAI, S. 1985. Analyses of sulfhydryl groups in zein. *Agric. Biol. Chem.* 49:1415.
 BIETZ, J. A. 1983. Separation of cereal proteins by reversed-phase high-

performance liquid chromatography. *J. Chromatogr.* 255:219.
 BIETZ, J. A. 1985. Identification of maize inbreds and hybrids by reversed-phase high-performance liquid chromatography. *Cereal Foods World* 30:562.
 BIO-RAD LABORATORIES. 1982. The Bio-Rad Silver Stain Bulletin 1089. Bio-Rad Laboratories: Richmond, CA.
 DANZER, L. A., ADES, H., and REES, E. D. 1975. The helical content of zein, a water insoluble protein, in non-aqueous solvents. *Biochim. Biophys. Acta* 386:26.
 DANZER, L. A., and REES, E. D. 1976. Molecular weight of an extremely hydrophobic protein, zein, in dimethylformamide and in formamide. *Can. J. Biochem.* 54:196.
 GANCHEV, K. D., IVANOV, Ch. P., and DRENSKA, A. I. 1976. Studies on the heterogeneity of zein and its fractions by disk electrophoresis. *C. R. Acad. Bulg. Sci.* 29:689.
 HAGEN, G., and RUBENSTEIN, I. 1980. Two-dimensional gel analyses of the zein proteins in maize. *Plant Sci. Lett.* 19:217.
 IVANOV, Ch. P., GANCHEV, K. D., and DRENSKA, A. I. 1976. Zein fractions A and B—Aggregates with disulfide cross-links of fraction C. *C. R. Acad. Bulg. Sci.* 29:571.
 LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680.
 LANDRY, J. 1979. La zeine du grain de maïs. Preparation et characterization. *Biochimie* 61:549.
 LANDRY, J., and GUYON, P. 1984. Zein of maize grain: I. Isolation by gel filtration and characterization of monomeric and dimeric species. *Biochimie* 66:451.
 LANDRY, J., and SALLANTIN, M. 1983. Polymorphism of native zein as detected by gel filtration and electrophoresis in the presence and absence of sodium dodecylsulfate. *Cereal Chem.* 60:243.
 LANDRY, J., PAULIS, J. W., and FEY, D. A. 1983. Relationship between alcohol-soluble proteins extracted from maize endosperm by different methods. *J. Agric. Food Chem.* 31:1317.
 LANDRY, J., PAULIS, J. W., and WALL, J. S. 1986. Chromatographic and electrophoretic analysis of zein heterogeneity. *J. Cereal Sci.* 5:51.
 MARKS, D. M., and LARKINS, B. A. 1982. Analysis of sequence microheterogeneity among zein messenger RNAs. *J. Biol. Chem.* 257:9976.
 PAULIS, J. W. 1981. Disulfide structures of zein proteins from corn endosperm. *Cereal Chem.* 58:542.
 PAULIS, J. W., and BIETZ, J. A. 1986. Separation of alcohol-soluble maize proteins by reversed-phase high-performance liquid chromatography. *J. Cereal Sci.* 4:205-216.
 PAULIS, J. W., and WALL, J. S. 1977. Comparison of the protein compositions of selected corns and their wild relatives, teosinte and *Tripsacum*. *J. Agric. Food Chem.* 25:265.
 PAULIS, J. W., BIETZ, J. A., BOGYO, T. P., DARRAH, L. L., and ZUBER, M. S. 1986. Analysis of alcohol-soluble endosperm proteins in

- maize single and double mutants by reversed-phase high-performance liquid chromatography. (Abstr. 204) 10th Int. Symp. on Column Liquid Chromatography.
- REWA, W. A., and BRUCKNER, C. 1979. Contribution of hydrophobic interaction and intermolecular disulfide bonds to the heterogeneity of zein. *Biochem. Physiol. Pflanz.* 174:451.
- REWA, W. A., KLIMENKO, V. G., and BRYUKNER, K. K. 1978. Electrophoretic and chromatographic investigation of zein. *Biokhimiya* 43:904.
- SMITH, J. S. C. 1986. Biochemical fingerprints of cultivars using reversed-phase high performance liquid chromatography and isozyme electrophoresis: A review. *Seed Sci. Technol.* 14:753.
- SMITH, J. S. C., and SMITH, O. S. 1986. Environmental effects on zein chromatograms of maize inbred lines revealed by reversed-phase high-performance liquid chromatography. *Theor. Appl. Genet.* 71:607.
- SOAVE, C., and SALAMINI, F. 1982. Zein proteins: A mutigene family from maize endosperm. *Qual. Plant. Plant Foods Hum. Nutr.* 31:191.
- TSAI, C. Y. 1980. Note on the effect of reducing agent on zein preparation. *Cereal Chem.* 57:288.
- TURNER, J. E., BOUNDY, J. A., and DIMLER, R. J. 1965. Zein: A heterogeneous protein containing disulfide-linked aggregates. *Cereal Chem.* 42:452.
- WALL, J. S., FEY, D. A., PAULIS, J. W., and LANDRY, J. 1984. Improved two-dimensional electrophoretic separation of zein proteins: Application to study of zein inheritance in corn genotypes. *Cereal Chem.* 61:141.
- WILSON, C. M. SHEWRY, P. R., and MIFLIN, B. J. 1981. Maize endosperm proteins compared by sodium dodecyl sulfate gel electrophoresis and isoelectric focusing. *Cereal Chem.* 58:275.
- WILSON, C. M. 1986. Serial analysis of zein by isoelectric focusing and sodium dodecyl sulfate gel electrophoresis. *Plant Physiol.* 82:196.
- WU, Y. V., PAULIS, J. W., SEXSON, K. R., and WALL, J. S. 1983. Conformation of corn zein and glutelin fractions with unusual amino acid sequence. *Cereal Chem.* 60:342.

[Received July 13, 1987. Accepted December 18, 1987.]