Zein Subunit Homology Revealed Through Amino-Terminal Sequence Analysis

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ABSTRACT

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Zein, the prolamine fraction of maize endosperm protein, consists of more than 15 polypeptides differing in isoelectric point and distributed mainly in two molecular weight groups (21,000 and 23,000 daltons). N-Terminal amino acid sequences of mixtures of zein polypeptides from normal and near-isogenic high-lysine opaque-2 (o_2) maize were determined to establsih structural and genetic relationships. Reduced and alkylated zein polypeptides were subjected to automated sequential Edman degradation, and the resulting phenylthiohydantoin derivatives were identified by chromatographic analyses. Sequencing of zein from a normal corn hybrid was successful for 33 residues. Only one or two major amino acids were present at each position, which indicates that most zein polypeptides are homologous in structure. The observed amino acid sequence was H_2N-(Thr+Phe)-Ile-(Phe+Ile)-Pro-Gln-Cys-Ser-(Gln+Leu)-Ala-Pro-Ile-Ala-Ile-Leu-Leu-Gln-(Phe+Pro)-Tyr-(Leu+Phe)-Pro-Val-Ala-(Val+Ile)-(Met+Ala)-(Gly+Phe)-(Val+Tyr)-Gln-(Glx+Pro)-(Asn+Leu)-Ala-Val-Leu-Ala-. The two normal corn hybrid zeins sequenced were very similar, but two significant differences occurred: The second zein lacked Phe at position 1 and had a Pro/Gln substitution at position 16. Six minor differences in sequence were noted between normal zein and its near-isogenic o_2 counterpart, but otherwise the protein fractions were identical. These data support the concept that the genes that control zein synthesis arose through duplication and mutation from a single ancestral gene. Furthermore, they suggest that zein polypeptide chains of 21,000 and 23,000 daltons have similar amino-terminal sequences and that the 23,000-dalton chains have internal insertions or C-terminal extensions of about 20 amino acid residues.

Prolamines probably are the most abundant and certainly are one of the most interesting classes of cereal endosperm proteins. They may occur as monomers or small oligomers or may be incorporated with other proteins into polymeric glutelins. Their only known physiologic role in the plant is as a source of nourishment for the developing embryo. Widespread feed and food use of cereals also makes them important in mammalian nutrition. Prolamines have unusual amino acid compositions: Their high glutamine, proline, and hydrophobic amino acid contents give them unique solubility (insoluble in water or aqueous salt solutions, but soluble in aqueous alcohol), and their deficiency in the essential amino acids, lysine, and tryptophan, gives them poor nutritional quality.

Zein, the prolamine fraction of maize endosperm protein (Wall and Paulis 1977), is very heterogeneous; polyacrylamide gel electrophoresis (PAGE) in the presence of denaturing and reducing agents (Alexandrescu et al 1976) or isoelectric focusing (IEF) (Gentinetta et al 1975) reveals up to 25 individual polypeptides. PAGE in 5% gels in the presence of the detergent sodium dodecyl sulfate (SDS), which separates proteins on the basis of molecular size, reveals that most zein subunits were in a broad, heterogeneous band of molecular weight approximately 21,300 (Paulis et al 1975). In 10% gels, zein's 21,300 mol wt band resolves into two bands with mol wt approximately 21,000 and 23,000 (Gianazza et al 1976).

Bread wheat, *Triticum aestivum*, also contains a very heterogeneous prolamine fraction, gliadin; two-dimensional electrophoresis reveals that gliadin has about 46 individual components (Mecham et al 1978, Wrigley and Shepherd 1973). Bietz et al (1977) showed by amino-terminal sequence analysis that these gliadins can be divided into two groups of homologous proteins with very similar primary structures; these two groups are also, to a lesser extent, related to each other. A recent study (Autran et al 1977) suggested that prolamines of rye and diploid *Triticum* and *Aegilops* species also exist as homologous groups and are closely related in amino acid sequence to gliadins.

Bietz et al (1977) and Kasarda et al (1976) postulated that cereal prolamine homology and heterogeneity resulted from gene duplication, followed by mutations. In wheat, complexity is further increased due to polyploidy. When such extensive homology exists, mixtures of proteins can give essentially the same sequence results as individual proteins.

We therefore chose to examine by amino-terminal sequence analysis the total prolamine fraction of maize, zein, with the following aims: (a) to determine whether and to what extent homology exists among zein subunits, (b) to relate zein's unusual solubility and other properties to its sequence, (c) to begin comparing zein's amino acid sequence to those of prolamines of other grains, and (d) to determine whether the opaque-2 (o_2) gene has an apparent effect on zein's amino-terminal sequence.

[1]Mention of firm names or trade products does not imply endorsement or recommendation by the USDA over other firms or similar products not mentioned.

MATERIALS AND METHODS

Protein Isolation

The normal corn hybrid (B37TMS × H84) (Oh43RF × A619), subsequently referred to as A(N), was obtained from G. E. Shove (University of Illinois); the grain was dry milled (Paulis and Wall 1971), and zein was isolated from the low-oil meal as described by Paulis et al (1975). Zeins were also prepared as described by Paulis et al (1975) from the normal hybrid corn, P-A-G SX52, and its near-isogenic o_2 hybrid derivative, P-A-G 50001, subsequently referred to as B(N) and B(o_2), respectively.

Protein Derivatization

Protein disulfide bonds were reduced with β-mercaptoethanol and converted to their S-pyridylethyl (PE) derivatives as described by Friedman et al (1970) using the modifications previously described (Bietz et al 1977).

Electrophoretic Methods

Proteins were examined by SDS-PAGE in pH 8.9 Tris-borate buffer (Bietz and Wall 1972, Koenig et al 1970), except that gel concentration was increased to 10% to permit separation of zein's 21,000 and 23,000 mol wt polypetides. Double staining was used routinely (Bietz et al 1975).

PAGE in 0.0085*M* aluminum lactate buffer, pH 3.2, was performed as described by Mecham et al (1978), except that the buffer contained 8*M* (deionized) urea. Aliquots of approximately 50 μl of each sample solution (4 mg/ml) were applied to gel slots, and electrophoresis proceeded for about 8 hr with a voltage drop of about 20 V/cm in the gel (V = 415; current, 50 mA). Under these conditions, the fastest major PE-zein polypeptide migrated approximately 10.0 cm.

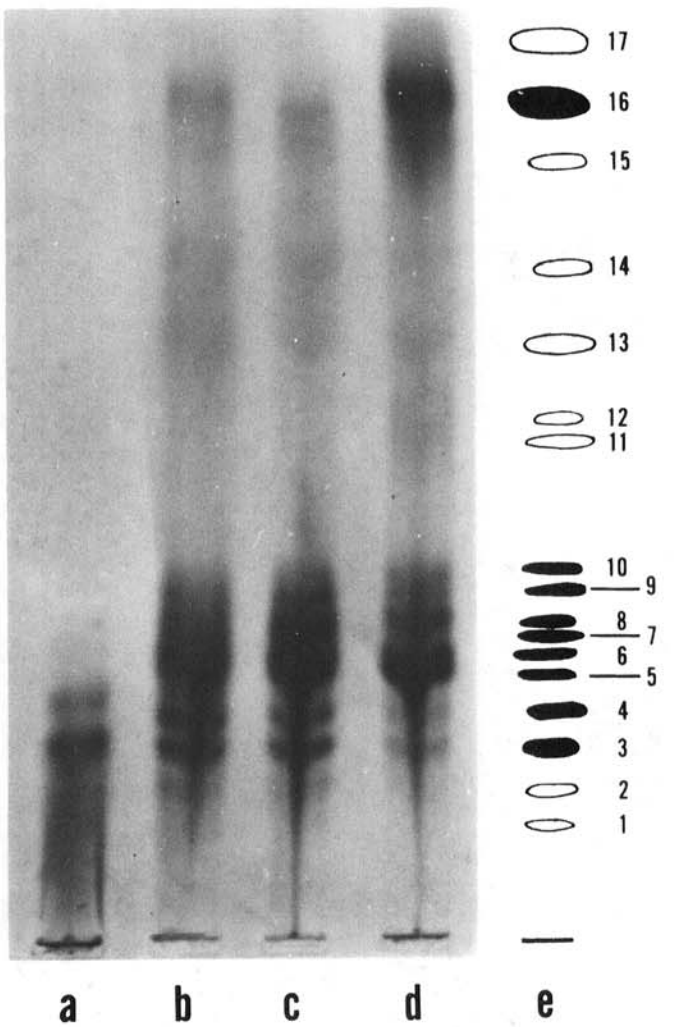

Fig. 1. 8*M* urea-aluminum lactate polyacrylamide gel electrophoresis, pH 3.2, of: **(a)** normal native zein, **(b)** pyridylethyl (PE)-zein of normal hybrid A(N), **(c)** PE-zein of normal hybrid B(N), **(d)** PE-zein of *opaque*-2 hybrid B(o_2), and **(e)** diagram of PE-zein bands typical of the three hybrids studied, with most intense bands shaded.

Sequence Analysis

Automated Edman degradation was performed on a Beckman Model 890C sequencer using Quadrol program 122974, with a delay step after heptafluorobutyric acid (HFBA) delivery (Beckman Instruments, Inc. 1976). Sequanal™ grade chemicals (Pierce) were used throughout. Repetitive yields of 94–96% were routinely obtained for the standard protein apomyoglobin (Beckman). Protein samples (14–18 mg) were dissolved in HFBA (sequencer reagent 3) and dried by nitrogen and vacuum (sample application subroutine 02772) for each run. Each sample was examined two or three times. Other methodology used has been described (Bietz et al 1977).

Analysis of Sequencer Fractions

Most methods used for characterizing sequencer fractions have been described (Bietz et al 1977). Two-dimensional thin-layer chromatography (TLC) was performed on 5 × 5-cm Cheng-Chin polyamide layer plates (Accurate Chemical and Scientific Corporation) (Summers et al 1973). Gas chromatography (GC) (Pisano 1975) was done using a Bendix 2600 instrument with flame ionization detection; 4 ft × 2-mm coiled glass columns were packed with 10% DC 560 on Chromosorb W-HP 100/120 (Alltech Associates). Adequate column conditioning required 60 hr at 290°. Phenylthiohydantoin (PTH) derivatives of arginine, histidine, and PE-cysteine were determined by two-dimensional TLC on 5 × 5-cm polyamide plates (Hopp 1976), a method far superior to those used previously (Bietz et al 1977). Fractions from three sequencer runs also were hydrolyzed to free amino acids in sealed ampules for 24 hr at 130° in constant boiling HCl-0.05% β-mercaptoethanol (Beckman Instruments, Inc. 1973) and were characterized by amino acid analysis (Benson and Patterson 1965) on a Beckman Model 121 amino acid analyzer (Bietz et al 1977). Amino acid analysis especially was useful for differentiating leucine and isoleucine and for quantitating glutamic acid plus glutamine and aspartic acid plus asparagine.

Methods for identifying cleaved amino acids in sequencer fractions using the various chromatographic data were described previously (Bietz et al 1977). Protein degradations were terminated when the yield of cleaved product decreased to less than 10% of the original value, and unambiguous assignments could no longer be made.

RESULTS

Heterogeneity of Zein Subunits

Zeins from corn hybrids A(N), B(N), and B(o_2) were examined as their PE derivatives by pH 3.2 aluminum lactate 8*M* PAGE (Fig. 1b–d). A diagram of bands in PE-zein is also included (Fig. 1e) as is a pattern of zein before reduction and alkylation (Fig. 1a).

PE-zein contains numerous subunits: 17 electrophoretic bands usually are present, and additional faint ones occasionally are apparent. It is very unlikely that this heterogeneity is due to incomplete cystine reduction or alkylation of resulting cysteine residues, since no disulfide bonds can be detected by amperometric titration in alkylated-reduced zien (J. W. Paulis, unpublished). In contrast, native zein exhibits only two to four slow-moving bands (Fig. 1a). This significant increase in band number suggests that disulfide cleavage and stabilization of the resulting sulfhydryls through alkylation, converts all zein species to monomers, thereby amplifying existing charge differences or differences in cysteine content by adding positively charged PE groups to all cysteines.

Electrophoretic patterns of PE-zein polypeptides from the two normal corn hybrids (Fig. 1b and c) appear identical qualitatively and quantitatively. Zeins from B(N) and B(o_2) Fig. 1c, d) have definite quantitative differences, however, although the two samples are qualitatively very similar. Specifically, the o_2 hybrid is deficient in bands 1–4 and has considerably more of band 16 than

its near-isogenic normal hybrid. These apparent quantitative differences confirm similar observations by Paulis et al (1969) using starch gel electrophoresis (SGE) and by Soave et al (1976) using IEF.

PE-zeins were also examined by SDS-PAGE (Fig. 2). All three hybrids contained two major subunit bands with calculated molecular weight of 22,300 and 25,400, plus less intense bands corresponding to polypeptides or higher and lower molecular weight. Molecular weights calculated for PE-zein polypeptides from these data, shown in Fig. 2, are similar to those reported previously (Paulis et al 1975, Soave et al 1976); we do not consider the numerical differences to be significant. Zeins of the two normal hybrids (Fig. 2 b and c) are similar qualitatively and quantitatively. B(o_2) hybrid zein (Fig. 2d) differs from B(N) zein (Fig. 2c) in apparently having less of the larger polypeptide class (our calculated mol wt 25,400) and in having increased amounts of 12,600 and 32,700 mol wt polypeptides. These quantitative effects of the o_2 gene are similar to those noted by Soave et al (1976) and Jones et al (1977), who showed that the o_2 gene inhibited synthesis of zein's large major polypeptides more than synthesis of smaller ones.

Sequence of Zein Polypeptides

Automated Edman degradations were performed on A(N), B(N), and B(o_2) zein polypeptides for 40 cycles. Amino-terminal sequences were very similar for each sample; the following material presents the sequence of A(N) zein and the analytical data that support it.

Table I summarizes the GC, TLC, and amino acid analysis (AAA) data for each degradative cycle from A(N) zein and presents the sequence assignments made from these data. For simplicity, the quantitative AAA and GC data are expressed as percentage of the yield of amino acid at cycle 1; this convention facilitates comparison of sequencer runs. Because the major amino acid at cycle 1, threonine, is difficult to quantitate accurately, the initial yield was extrapolated from the yield of isoleucine at cycle 2.

Yields at cycle 1 averaged 19–23 nmol/mg of each sample. This is slightly more than half of that expected for a purified polypeptide of mol wt 25,000 (40 nmol/mg), similar to molecular weight of zein polypeptides. Initial yields of this magnitude are not uncommonly low, however; 68% yields were obtained with gliadins (Bietz et al 1977). Indeed, "it is rare to obtain more than 50–70% of the yield of the amino-terminal residue expected from the weight of the protein being degraded" (Niall 1973), due to blocked N-termini, the presence of impurities and water, mechanical losses, or other reasons.

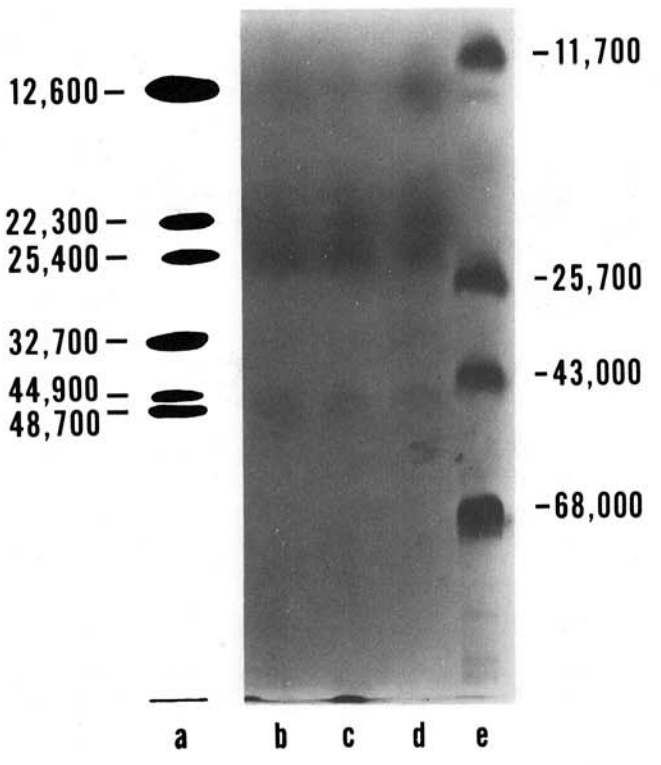

Fig 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of pyridylethyl (PE)-zein preparations: (**a**) diagram of subunits, (**b**) PE-zein of normal hybrid A(N), (**c**) PE-zein of normal hybrid B(N), (**d**) PE-zein of *opaque*-2 hybrid B(o_2), and (**e**) protein standards: cytochrome c, chymotrypsinogen A, ovalbumin, and serum albumin. The approximate molecular weights shown with pattern **a** were calculated from the linear, least squares fit of the mobility of the protein standards with the log of their molecular weight ($r = -0.997$).

TABLE I
Characterization of Sequencer Fractions of Pyridylethyl-Zein Subunits from Normal Corn Hybrid A(N)[a]

Cycle	GC[b]	TLC[c]	AAA[d]	Sequence Assignment
1	T(60), F(30)	T, F	F(28)	T, F
2	L/I(75)		I(95)	I
3	F(73), L/I(39)	F	F(73), I(34)	F, I
4	P(74)	P	P(48)	P
5	Q(39, E(24)	E, Q	E(77)	Q
6		C		C
7	S(37)	S		S
8	E(30), Q(20), L(16)	E, Q	E(41), L(21)	Q, L
9	A(59)	A	A(51)	A
10	P(30)	P	P(19)	P
11	L/I(25)		I(26)	I
12	A(21)	A	A(27)	A
13	L/I(7)		I(7)	I
14	L/I(14)		L(14)	L
15	L/I(21)		L(17)	L
16	E(6), Q(9)	E, Q	E(10)	Q
17	F(7), P(5)	F, P	F(9), P(7)	F, P
18	Y(17)	Y	Y(12)	Y
19	L/I(15), F(7)	F	L(9), F(3)	L, F
20	P(12)	P	P(4)	P
21	V(10)	V	V(7)	V
22	A(5)		A(5)	A
23	V(5), L/I(8)		V(3), I(3)	V, I
24	M(6)	A	M(6), A(3)	M, A
25	G(5)		G(6), F(2)	G, F
26	V(7)	V, Y	V(6), Y(3)	V, Y
27		E, Q	E(8)	Q
28	P(3)	E, Q, P	E(5), P(3)	Z, P
29	L/I(3)	N	D(6), L(3)	N, L
30		A	A(4)	A
31	V(7)		V(5)	V
32	L/I(9)		L(3)	L
33	A(3)	A		A

[a] For each test, identified amino acids or their derivatives are listed in order of decreasing abundance or relative spot intensity. Amino acids present in very minor amounts or at background levels are omitted. Yields (in percentage of yield at cycle 1, rounded to the nearest integer) are shown in parentheses for the GC and AAA data. All yields are corrected for background and carryover from the preceding cycle and generally represent the best results obtained from normal zeins for that cycle. Single-letter abbreviations of amino acids are: A, alanine; C, cysteine (identified as PE-cysteine); E, glutamic acid; F, phenylalanine; G, glycine; I, isoleucine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; S, serine; T, threonine; V, valine; Y, tyrosine; and Z, glutamic acid or glutamine.

[b] Includes results of characterization of amino acids in each fraction by gas chromatography (GC) both as their phenylthiohydantoin (PTH) derivatives and (after silylation) as their trimethylsilyl derivatives. Leucine and isoleucine are not differentiated.

[c] Includes thin-layer chromatographic (TLC) analysis of PTH-amino acids in both ethyl acetate and aqueous phases obtained by extraction of each converted sequencer fraction. Leucine and isoleucine co-chromatograph with PTH-norleucine, the internal standard, and are not included. The most prominent or increased amino acids are indicated; faint spots are not listed.

[d] Amino acid analysis.

The sequence assignments for each residue in Table I are generally based on agreement between at least two GC, TLC, or AAA runs. For certain amino acids, identifications were not as certain, due to sample lability or weakness in characterization techniques; their identifications represent, in part, a lack of positive data for other amino acids. For example, cysteine (as PE-cysteine) and histidine were identified only by TLC. PTH-serine is labile, gives weak identifications both by TLC and GC, and is destroyed by acid hydrolysis. PTH-threonine, PTH-proline, and PTH-glycine generally give low recoveries by GC and AAA.

The quantitative data from Table I are presented graphically in Fig. 3 as a plot of log recovery vs. fraction number. Although repetitive yields of 94–96% were routinely obtained for protein standards, maximum amino acid recoveries suggested that repetitive yields of 90–91% were achieved for zein (Fig. 3). Such lower yields may be due to increased zein hydrophobicity as compared with apomyoglobin, leading to increased extractive losses during each cycle. Partial blockage of amino-terminal residues during a run could also reduce repetitive yields, eg, newly exposed glutamines at positions 5, 8, 16, and 27 could cyclize to pyroglutamic acid in the prescence of HFBA after cleavage of the preceding residue.

It is apparent from Table I and Fig. 3 that most PE-zein subunits, with the possible exception of those present in very small amounts, must have the same N-terminal sequence. At 11 of 33 assigned residues, a second amino acid was present. In early cycles, however, the minor amino acid was only one-third to one-half as prevalent as the major one. Also, most residues at which two amino acids were identified were relatively far into the sequencer runs, where assignments become slightly less certain. The total determined N-terminal sequence is presented in Fig. 4. Thus, although zein polypeptides are very numerous (Fig. 1) and also are of more than one molecular size (Fig. 2), most are homologous, ie, they have such a significant degree of N-terminal sequence identity that they have a common origin.

Zein from B(N) was successfully sequenced for 31 residues. Zeins from A(N) and B(N) had identical sequences except at positions 1, 13, and 16. At position 1, zein B(N) contained 7–8% phenylalanine, compared with 29% A(N). At position 13, cysteine (as PE-cysteine) was tentatively identified in place of isoleucine; at position 16, glutamine was absent and proline occurred (7% yield). A proline/glutamine substitution may result from a single nucleotide change in the m-RNA codons for these amino acids.

Zein from B(o_2) was sequenced for 29 residues, but no identifications were possible at positions 20, 27, and 28. Several minor differences in sequence were noted between the zeins from normal hybrid B(N) and its near-isogenic o_2 counterpart, B(o_2). At position 1, no phenylalanine was found in B(o_2) compared with 7–8% in B(N). At position 2 of B(o_2), histidine was identified by TLC; however, isoleucine recovery at this position was as high as in normal zein, which indicated that histidine is a minor constituent. At position 4 of B(o_2), glycine (15.3%) apparently replaced some proline—reduced from 74% in B(N) to approximately 49% in B(o_2). This mutation could not result from a single base change and may relate to the different relative distribution of polypeptides in the normal and o_2 near-isogenic zeins. At position 13 of B(o_2), phenylalanine was present (about 6%), and little isoleucine was noted; this probably resulted from a single base change. At position 21, asparagine was identified in B(o_2) but not in B(N); and at residue 25, no glycine was found in B(o_2) as it was in B(N). Recoveries at positions 21 and 25 were variable in B(N) and B(o_2), however, so no conclusion was made concerning the importance of these changes.

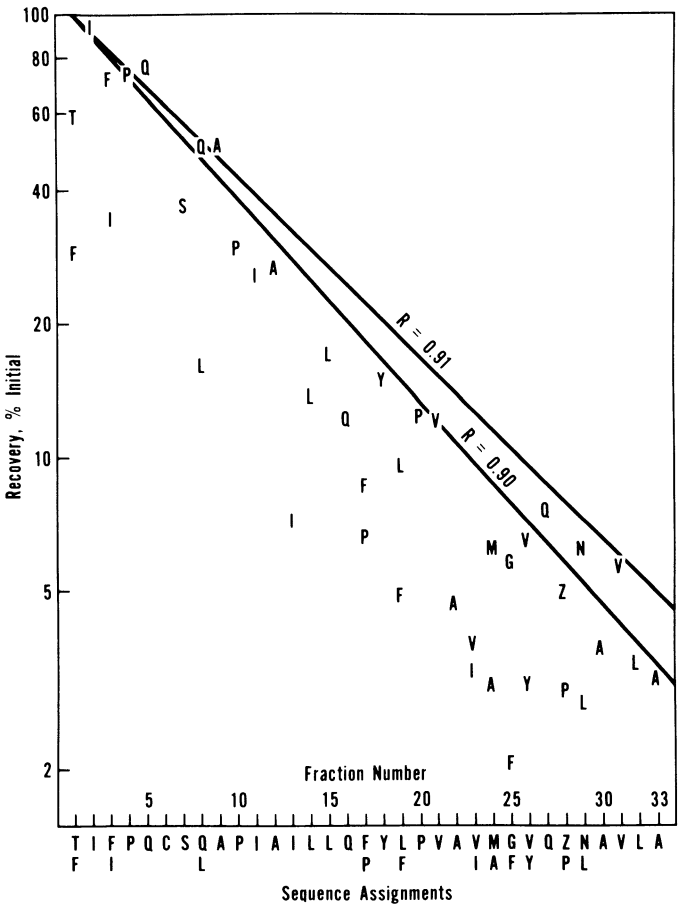

Fig. 3. Semi-logarithmic plot of the recovery of amino acid at each cycle, expressed as percentage of initial yield vs cycle number. Each data point is identified by the single-letter abbreviation (see Table I) of the amino acid it represents, and the final sequence assignments for each cycle are indicated by the same abbreviations along the abscissa. Also shown are plots of the maximum expected recoveries at each cycle for repetitive yields (R) of 0.91 and 0.90.

DISCUSSION

Amino-Terminal Sequence of Zein Subunits

The N-terminal sequence of zein (Fig. 4) is the first data on the primary structure of intact zein molecules. Because more than 80% of zein consists of polypeptides with 21,000–23,000 mol wt (Soave et al 1976), this sequence is representative of these molecules but probably not of minor zein polypeptides of higher and lower molecular weight. Indeed, sequence analyses routinely yielded very small amounts of other amino acids, which may represent other zein polypeptides. Other investigators have previously studied zein's N-terminal amino acids (Ganchev et al 1972, Waldschmidt-Leitz and Metzner 1962). Our results generally agree with the study by

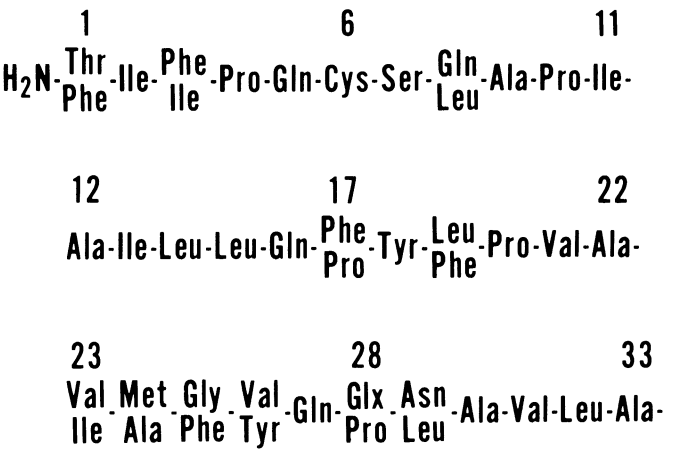

Fig. 4. Amino-terminal amino acid sequence of whole pyridylethyl-zein isolated from normal corn endosperm A(N). Where two amino acids occur in one position, the upper predominates, and the minor component is present at generally one-third to one-half the concentration of the major one. Standard three-letter abbreviations of amino acids are used.

Ganchev et al (1972), which found phenylalanine as the major N-terminal amino acid and also detected small amounts of threonine. Our results also agree with the observation of Waldschmidt-Leitz and Metzner (1962) that threonine is a major N-terminal amino acid of zein, although we did not detect N-terminal serine as they did. Differences between these results and our own may further indicate a tendency toward N-terminal amino acid variability between varieties, as we noted among the two normal and one near-isogenic o_2 hybrid examined. Some bitter zein peptides isolated from an enzymatic hydrolysate were previously sequenced (Wieser and Belitz 1975). One of these peptides (Ala-Ile-Ala) may correspond to positions 22–24 of our determined sequence.

Zein's N-terminal sequence is very hydrophobic, which is consistent with its solubility. Of the major amino acids identified in the first 33 positions, 20 (60.6%) are hydrophobic (alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, and methionine). By comparison, whole zein contains 47.3% hydrophobic residues (Paulis and Wall 1971). These data indicate a somewhat uneven distribution of amino acids in zein and suggest that other portions of its sequence may be relatively hydrophilic.

The extremely hydrophobic nature of zein's amino-terminal region, plus the observation of Burr et al (1978) that zein synthesized in vitro is 1100–2000 mol wt heavier than native zein, prompted us to compare zein to several preproteins that have hydrophobic amino-terminal extensions of 15–29 residues (for examples see Burstein and Schechter 1977, Chan et al 1976, Gaye et al 1977, Strauss et al 1977). No homology was noted between zein and any preprotein, however; zein does not have N-terminal methionine, nor does it contain three or more consecutive leucine residues, characteristics of many preproteins. Also, zein synthesized in vivo would be expected to have such an amino-terminal sequence cleaved after synthesis. The zein sequence we determined, therefore, is probably not of a preprotein form (Burr et al 1978), even though its hydrophobic nature suggests such a possibility.

Perhaps the most interesting residue in the zein subunit sequence is cysteine at position 6. Although cysteine was detected only by TLC, it was consistently identified; because no other amino acids could be identified at position 6, we are quite certain of the assignment. Various published amino acid compositions of zein suggest that there may be three (Paulis and Wall 1971), two (Lee et al 1976), one (Sodek and Wilson 1971), or zero (Righetti et al 1977) cysteine residues per zein subunit. Our results definitely locate one cysteine residue.

Homology of Zein Subunits

Our amino-terminal sequence results indicate significant homology, or sequence identity, among most zein subunits. The great extent to which this homology occurred was somewhat surprising, considering the heterogeneity of zein subunits in charge, as demonstrated by IEF (Gentinetta et al 1975, Soave et al 1976) or PAGE (Alexandrescu et al 1976; Fig. 1), and in size, as demonstrated by SDS-PAGE (Paulis et al 1975, Soave et al 1976; Fig. 2).

The large number of bands in IEF patterns of zeins, the similarities and differences of zein amino acid compositions, and zein's fairly simple molecular size distribution led Righetti et al (1977) to consider reasons for zein's observed charge heterogeneity. They concluded that spot mutations in genes for zein synthesis, deriving from a common ancestral gene, were primarily responsible for zein's observed heterogeneity but that some in vivo cellular deamidation is also possible. An almost total lack of selective pressure against diversification of genes controlling zein synthesis must exist similar to the mutations to which are attributed the heterogeneity and homology of wheat's prolamine fraction, gliadin (Bietz et al 1977, Kasarda et al 1976). Our results, which demonstrate homology among zein subunits, support the concept that genes that conrol zein synthesis have arisen through duplication and mutation from a single ancestral gene. In addition, they extend this concept by showing that the two major zein subunit classes, differing in molecular weight by approximately 2,000 (Gianazza et al 1976), must also be homologous. Thus, the larger polypeptides are similar in sequence to the smaller ones, except that the larger ones must have an internal insertion or C-terminal extension of approximately 20 amino acid residues.

Corn's genetic relationship to other cereals for which prolamine amino acid sequence information is available is quite distant, but it is still of interest to compare their determined sequences. No homology seems to exist between amino-terminal sequences of zein and those of prolamines from wheat (Bietz et al 1977), rye (Autran et al 1977), or several diploid *Triticum* and *Aegilops* species (Autran et al 1977). It should be of considerably more interest to compare zein to prolamines from more closely related cereals, such as teosinte (*Zea mexicana*), *Tripsacum*, and *Sorghum*.

Differences in Zein Subunit Sequences Between Hybrids

Our results show that zein polypeptides from two normal corn hybrids have very similar amino-terminal sequences; yet, at least two definite differences were noted, one qualitative and one quantitative. Thus, additional slight zein polypeptide sequence heterogeneity may be expected in corn hybrids differing in genetic background.

Only relatively minor differences in sequence were noted between zeins from the near-isogenic normal and o_2 hybrids. This agrees with demonstrations by IEF (Soave et al 1976), SDS-PAGE (Jones et al 1977, Soave et al 1976; Fig. 2) SGE (Paulis et al 1969), and PAGE (Fig. 1) that zein polypeptides from normal hybrids and their near-isogenic o_2 mutants are very similar, but that some qualitative and quantitative differences are attributable to the o_2 gene. The minor sequence differences we noted may represent repression of synthesis of some of zein's more alkaline (Gianazza et al 1976) or larger (Jones et al 1977, Soave et al 1976) subunits by the o_2 gene.

ACKNOWLEDGMENTS

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