NOTE

Electrophoretic Analyses of Various Commercial and Laboratory-Prepared Zeins[1]

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Zein, the major storage protein isolated from corn endosperm, has been isolated and characterized in numerous experiments (Wall and Paulis 1978, Shewry and Miflin 1985, Wilson 1987). Such knowledge is important for industrial utilization of zein, as well as to provide basic information, because zein makes up much of the protein in corn gluten meal, one of the by-products of starch production (Watson 1984). Zein has also found some industrial applications (Reiners et al 1973) and is still produced.

Craine et al (1961) and Boundy et al (1967) reported on properties of commercial zeins, but recent analyses are rare. Augustine and Baianu (1986) characterized commercial Colorcon zein by nuclear magnetic resonance. Commercial zeins from corn gluten meal have also been subjected to sulfur dioxide or other reducing agents for long periods at high temperature (Watson 1984); such treatments could cause degradation.

Because of the lack of recent information concerning the properties of commercial zeins, I used two sensitive methods of protein analysis to characterize such materials from several sources. Results reveal that appreciable portions of several commercial zein samples are indeed very much like zein isolated in the laboratory under mild conditions.

MATERIALS AND METHODS

Zein Preparations

The following samples were used as received: 1) zein, U.S. Biochemical Corp., control no. 12784, purchased in 1983; 2) zein, Colorcon, Inc., lot 704-25 (possibly prepared according to patent 3,535,305), provided by I. Baianu, Univ. of Illinois (this product is no longer marketed); 3) zein, regular grade, lot 4000519C, provided (1985) by Freeman Industries, Tuckahoe, NY 10707; 4) zein, special grade, lot F5000520C, Freeman Industries (Freeman regular grade zein differs from the special grade, according to the literature accompanying the sample, as being lower in color). It is probable that all of these samples were derived from hybrid corn. Reiners et al (1973) described two procedures that are used commercially for isolation of zein, but procedures used to prepare the samples described here could not be determined. Zeins were dissolved at 12 mg/ml in 55% isopropanol with 2% 2-mercaptoethanol. Cloudy solutions were treated in an ultrasonic bath and then centrifuged. Protein was determined by a modification of the Read and Northcote (1981) Coomassie Blue G reagent 2 system, using a zein that dissolved completely in the solvent as the standard, and reading the color change within 3 min. All clarified samples contained at least 10.6 mg/ml of protein (88% of the original concentration).

Fresh laboratory samples of zein (from inbreds B84, N28, and WF9) for isoelectric focusing (IEF) were prepared as previously noted (Wilson 1985b) by direct extraction from the endosperm with isopropanol-mercaptoethanol solvent. Isolated zeins (B57, N28, and Oh43) were prepared as previously noted (Wilson et al 1981), and were then dissolved as were the commercial zein samples. The isolated inbred zeins had been stored three years at room temperature. IEF in agarose was performed as reported (Wilson 1985a).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with tris-borate buffers, with a gel that gives good separations of zeins, and with a sensitive Coomassie Blue R staining procedure (Wilson 1986). Samples for SDS-PAGE were prepared by adding the alcohol extracts directly to SDS sample buffer. Approximately 5 or 12 μg was applied to the gels.

RESULTS AND DISCUSSION

Agarose IEF patterns of zein preparations are shown in Figure 1. The commercial zeins (lanes 3–6) had discrete banding patterns, similar to those in the inbreds N28 and WF9 (lanes 1 and 2). The commercial zeins' patterns were not matched to standards, but appeared to have band 10 (the upper band in WF9, faint in lanes 3–6) and several other bands common in corn belt inbreds and seen in N28 (Wilson 1985a). Some staining occurred at the anode for some commercial zeins (not shown, at the bottom of the gel), which could indicate that deamidation had occurred. No estimates were made of the amount of protein that moved in the opposite direction to normal zein. Visual comparisons with fresh extracts at equal protein loads suggest that perhaps half the protein in commercial samples migrated upon IEF to positions typical of normal zein bands. These results indicate long-term stability of zein, its resistance to deamidation, and confirm the value of IEF in

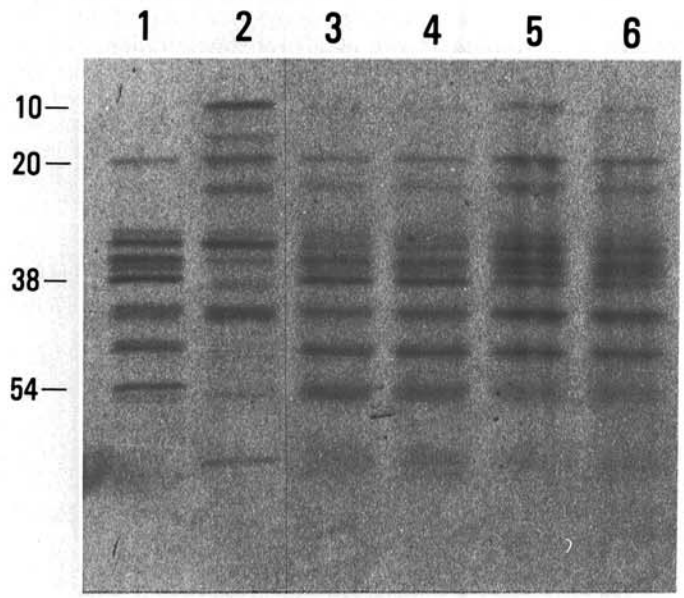

Fig. 1. Isoelectric focusing of zeins in agarose (cathode at top). **1,** Inbred N28, fresh extract; **2,** inbred WF9, fresh extract; **3,** U.S. Biochemical Corp. zein; **4,** Colorcon zein; **5,** Freeman regular zein; **6,** Freeman special zein. Band identification numbers are from Wilson (1985a,b).

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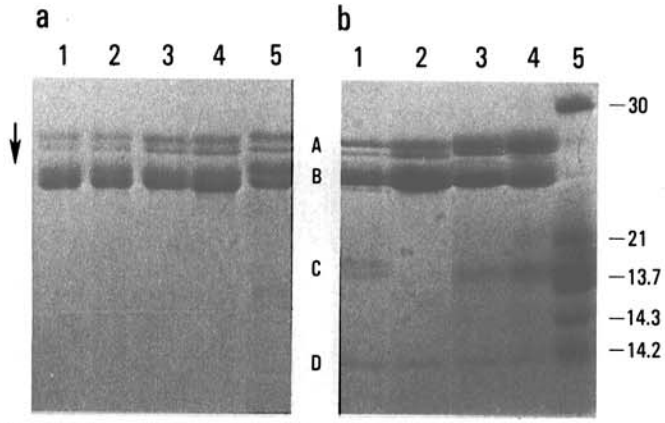

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of zeins. **a,** Approximately 5 μg of zein was applied per slot. **1,** U.S. Biochemical Corp. zein; **2,** Colorcon zein; **3,** Freeman regular zein; **4,** Freeman special zein; **5,** Zein isolated from N28. **b,** Approximately 12 μg of zein was applied per slot. **1,** Fresh zein extract, B84; **2,** Freeman special zein; **3,** Zein isolated from Oh43; **4,** Zein isolated from B57; **5,** Molecular mass standards: carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 21 kDa; pancreatic ribonuclease, 13.7 kDa; lysozyme, 14.3 kDa; and α-lactalbumin, 14.2 kDa. A-, B-, C-, and D-zeins are identified in the text and in Wilson (1985b).

revealing phenotypic polypeptide variability (Righetti et al 1977).

Figure 2 shows SDS-PAGE banding patterns of zeins. The A- and B-zeins are the major maize prolamins easily extracted by aqueous alcohol, whereas C- and D-zeins are minor high-sulfur proteins of lower molecular mass extracted by aqueous alcohol plus reducing agent (Wilson 1985b, 1986, 1987). The A-, B-, C-, and D-zeins are commonly referred to as 22, 19, 15, and 10 kDa zeins, respectively. However, true molecular masses calculated from cDNA sequence analyses are 26–27, 23–24, and 17–18 kDa for the first three zeins (Shewry and Miflin 1985). Again, the four commercial zeins (Fig. 2a, lanes 1–4) were similar to zeins isolated from a corn belt inbred (lane 5), although patterns were less sharp.

With heavier sample loads (Fig. 2b), minor low molecular mass C- and D-zeins (Wilson 1985b) were visible in a fresh zein extract (lane 1) and in three-year-old isolated laboratory zeins (lanes 3 and 4). The Freeman special zein contained a D-zein but no C-zein (lane 2). Note that SDS-PAGE does not give accurate molecular mass estimates for smaller zeins, as noted by variability for low M_r standard proteins (lane 5) (Wilson 1986).

The appearance of zein at the anode after IEF suggests that some molecules had been at least partially deamidated. However, a large fraction of zein molecules remained unchanged. Deamidation of zein increases its solubility; some commercial zeins are deamidated (McKinney and Johnsen 1957). This may improve zein's properties for some purposes but could be deleterious for others. Such "partially deamidated" zeins could be a mixture of highly deamidated and native molecules, or all molecules could be partially deamidated. IEF could distinguish between these alternatives and could be used to monitor extent of deamidation, leading to commercial zeins having reproducible properties. Such reproducibility in performance might improve the ability of zein to compete as a raw material for various industrial and food uses.

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[Received December 22, 1986. Revision received September 28, 1987. Accepted September 28, 1987.]