

## Fractionation of Zein by Ion-Exchange Chromatography on Phosphocellulose

To the Editor:

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Zein (maize prolamine) is the major protein fraction in the corn kernel, constituting 50-60% of the total protein. Although this protein fraction was discovered by Gorham in 1822, it has defied thorough characterization, mainly because it is insoluble in the aqueous buffer systems commonly used in protein purifications. Isolation and purification of zein have been the subjects of numerous investigations. Earlier purification work used precipitation as a fractionation method with stepwise addition of water to solutions of zein in ethanol (Watson et al 1936). Craine et al (1961) reported the fractionation of zein by column chromatography on Amberlite IRC-50, a weak cation exchanger, equilibrated with 70% ethanol and developed with a linear or step gradient of NaCl. The gradient elution yielded one broad peak and two or more poorly defined peaks. Zein also was fractionated by gel filtration on Sephadex G-200 in the presence of 8M urea, which yielded six to eight fractions (Landry 1965). Fraij and Melcher (1978) fractionated zein on hydroxylapatite columns in the presence of sodium dodecyl sulfate (SDS). This method gave a broad and irregular elution profile. Zein fractions obtained from the above-mentioned studies were not homogeneous on the basis of electrophoretic criteria. Fraij and Melcher (1978) reported that selected fractions from hydroxylapatite chromatography gave single bands when subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE), one fraction including zein B (mol wt 19,600),<sup>1</sup> the other including zein A (mol wt 21,600).<sup>1</sup> However, comparison of peptide maps for zeins A and B with those of mixtures of A and B suggested that the zein A and B fractions were heterogeneous.

Recently I have found that zein can be chromatographed on a column of phosphocellulose under nondissociating conditions, without using urea or detergents. Separation on phosphocellulose results in an elution profile with one major peak having a shoulder on both sides and four well-defined minor peaks.

Endosperm meal was obtained from a hybrid corn, 6-10-X08, by grinding it in a Wiley mill to pass a 60-mesh screen. Zein was extracted from the meal with 60% isopropanol by stirring for 24 hr. The extraction was repeated, but for the second extraction, stirring was followed by heating in a waterbath at 60° for 30 min. The solvent/meal ratio was 50:1 (v/w). After centrifugation, the two supernatants containing zein were pooled, dialyzed against water, and freeze-dried. The resulting powder was dissolved in starting buffer at a final concentration of 5 mg/ml and loaded onto the column. Phosphocellulose (medium mesh) was purchased from Sigma Chemical Co., precycled, and then equilibrated with the starting buffer before packing. The chromatography column was a Pharmacia product, SR25/100, suitable for use with organic solvents. Two different buffers, each containing isopropanol at a final concentration of 60%, were tried: 1) 25 mM Na-acetate (pH 4.5) and 2) 10 mM Na-lactate (pH 3.8). The column bed length was either 36 or 65 cm. Columns were eluted with a linear gradient of either 0-0.5M or 0-1M NaCl. All chromatographic separations were performed at room temperature.

All of the protein applied was bound to the exchanger; the "pass-through" fraction included only pigments that absorb at 280 nm. The development of the column with 0-1M salt gradient yielded one major and two minor peaks with 25 mM acetate buffer (pH 4.5). However, the three peaks eluted so closely that the trailing of components from one peak to the next was evident. When the column was developed with a 0-0.5M salt gradient, resolution of the three peaks considerably improved. SDS-PAGE of pooled tube contents under each peak showed the presence of two major zein components (zein A and B) in all three fractions. However, some minor zein components were concentrated under separate peaks. The best resolution was obtained when chromatography was performed using 10 mM Na-lactate buffer (pH 3.8) and eluting the column with a 0-0.5M salt gradient. In this case, increasing the column length from 36 to 65 cm improved resolution further. Under these conditions, the elution profile showed one major peak with shoulders on both sides and four minor peaks (Fig. 1). The salt molarity required to elute the last peak in the profile was 0.18M. When pooled fractions from each peak and the shoulders were subjected to SDS-PAGE (Fig. 2), the first subfraction contained the smaller (zein B, mol wt 22,800) of the two major zein components. Both the zein A (mol wt 23,900) and the zein B eluted under the major zein peak. The second subfraction (not shown in Fig. 2) contained the third major zein component (mol wt 16,700) in addition to zein A and B. The remaining fractions included essentially all of the components that appear in the profile of the whole zein, but the minor zein components were concentrated under different peaks. For example, the last fraction contained the smallest zein polypeptide (mol wt 15,600) as its predominant protein. The appearance of polypeptides with the same molecular weights under widely separated peaks can not be explained with certainty. Conceivably, zein A and B polypeptides form aggregates with each other and with other polypeptides through intermolecular disulfide linkages or through hydrophobic and other types of protein to protein interactions, resulting in zein forms with

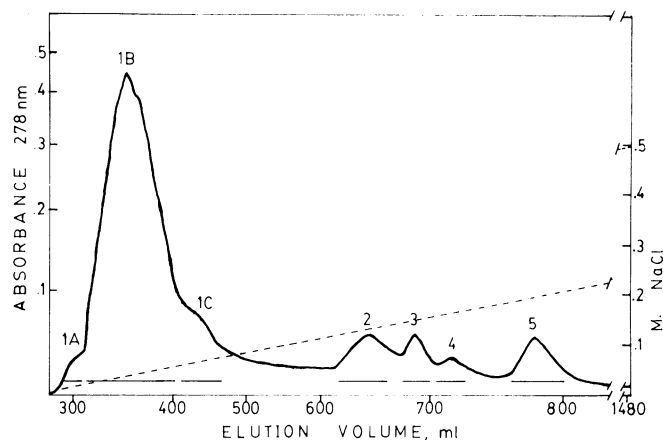
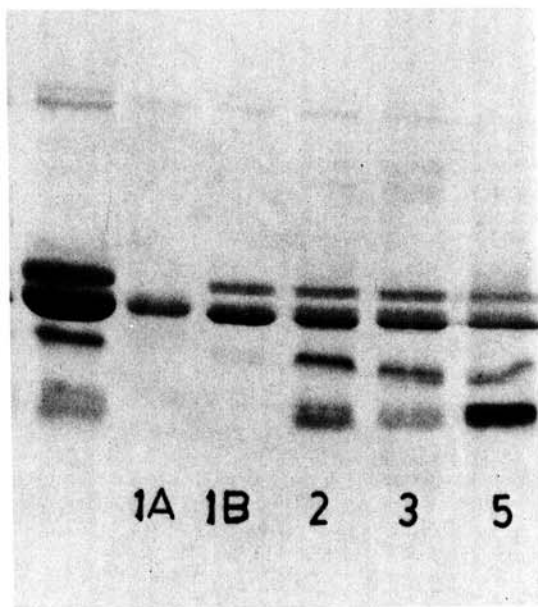


Fig. 1. Elution profile of zein chromatographed on phosphocellulose. Column, 2.5 × 65 cm; temperature, 25° C; buffer, 10 mM sodium-lactate in 60% (v/v) isopropanol, pH 3.8. -- = salt gradient. Straight lines along the abscissa under the peaks indicate the position of tubes with pooled contents. 1A, subfraction containing zein B; 1B, major peak containing zeins A and B; 1C, subfraction containing zeins A and B and third component; 2-5, minor peaks.

<sup>1</sup> Both components have mobilities that fall between chymotrypsinogen A (mol wt 25,000) and soybean trypsin inhibitor (mol wt 21,000). In our laboratory, estimated molecular weights for zein A and B were 23,900 and 22,800, respectively.



**Fig. 2.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10–15% gradient gel) patterns of zein fractions 1A, 1B, 2, 3, and 5 from chromatography of zein on phosphocellulose (Fig. 1). The first lane (unlabeled) on the left shows the profile of whole zein loaded onto the column. The profile of fractions 1C and 4 (not shown) were similar to those of 1B and 3, respectively.

different tenacities of binding to the exchanger. Alternatively, zein is very likely a mixture of many polypeptides displaying limited size but extensive charge heterogeneity. Gentinetta et al (1975) showed that zein contained more than 20 components that could be resolved by gel isoelectric focusing. This may account for the elution of zein components with the same molecular sizes under widely separated peaks during chromatography.

The results communicated in this report indicate that column chromatography on phosphocellulose with buffers containing traditional zein solvents (alcohols) offers considerable promise for purification and characterization of zein polypeptides.

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