ISOLATION AND PARTIAL CHARACTERIZATION OF THE ALKALINE PROTEIN FROM THE ZEIN COMPLEX

A. N. Vinnichenko, O. A. Livenskaya,
L. V. Shupranova, and M. V. Sysoeva

UDC 547.96.7+633.15

Information on individual polypeptides of the prolamine complex of cereals is sparse. We have previously reported on the isolation from maize seeds by the method of isoelectric focussing (IEF) in Ultrodex of a zein polypeptide with pI 6.1 [2]. In the present paper we consider the isolation of a zein polypeptide from the alkaline pH region of preparative IEF in polyacrylamide gel (PAAG).

The extraction and purification of a zein were carried out by a method described previously [3]. Since the separation of zein in the alkaline pH region by the IEF method in Ultrodex according to [2] proved to be difficult, for fractionating the polypeptides IEF was performed in 3.5% PAAG containing 6.5 M urea and 2% of ampholines on a 12 × 26 cm horizontal plate in a Multiphor instrument (Sweden) at pH 5-8 by a modified procedure [4]. The zein was dissolved in 8 M urea with 2% of mercaptoethanol at a protein concentration of 10 mg/ml. The samples were deposited on the horizontal surface of the gel with the aid of pieces of filter paper with dimensions of 4.5 × 1 cm (8 pieces) impregnated with 1-1.5 ml of the solution of the sample. Focussing was conducted for 14 h at a constant power of the electric current of 8 W and a temperature of 10°C along the gel. After the end of the process, the gel was placed in 7% trichloracetic acid until dense bands had appeared. The component with pI 8.1 was homogeneous according to the results of gradient SDS electrophoresis in 12.5-17.5% PAAG in Laemmli's system [5] in the presence of 2-mercaptoethanol, and also from the determination of the N-terminal amino acid by the dansyl methyl [6]. The molecular mass of the polypeptide was 20,600. A proline residue was identified as the N-teminal amino acid residue. The CD spectrum of the polypeptide in 0.5% sodium dodecyl sulfate was characterized by two negative bands in the 212-222 nm region. The amino acid composition of the protein was characterized by a high lysine content (three residues per molecule).

LITERATURE CITED

1. J. W. Dieckert and M. C. Dieckert, in: New Protein Foods (A. M. Altschull and H. L. Wilcke (eds.), Academic Press, Orlando, Vol. 5 (1985), p. 1.
2. A. N. Vinnichenko, L. V. Shupranova, V. S. Fedenko, N. I. Shtemenko, O. A. Livenskaya, and I. N. Plakhotnii, Khim. Prir. Soedin., 612 (1988).
3. A. N. Vinnichenko, V. S. Fedenko, L. B. Shupranova, N. I. Shtemenko, and M. V. Sysoeva, Fiz. Biokhim. Kul'tern. Rast., 21, No. 4, 338 (1989).
4. A. Vitale, C. Soave, and E. Galante, Plant Sci. Lett., 18, No. 1, 57 (1980).
5. U. K. Laemmli, Nature, 227, No. 15, 680 (1970).
6. W. R. Gray, Methods Enzymol., 25, 121 (1972).

Scientific-Research Institute of Biology, Dnepropetrovk State University. Translated from Khimiya Prirodnykh Soedinenii, No. 6, p. 844, November-December, 1990. Original article submitted February 19, 1990; revision submitted July 3, 1990.

0009-3130/90/2606-0725$12.50