Compound (VIII B) was obtained similarly after additional purification by column chromatography [with chloroform–ethanol (25:1) as eluent] with a yield of 76%, $[\alpha]_{546}$ +4° (c 1.0; $CHCl_3$), R_f 0.49 (system 3); ν_{max}^{KBr}, cm^{-1}: 3415-3275 (OH, NH), 2905, 2835 (CH_2), 1745 (C=O), 1660, 1545 (amide), and so was compound (VIII C), with a yield of 74%, $[\alpha]_{546}$ −2° (c 1.0; $CHCl_3$), R_f 0.40 (system 3); ν_{max}^{KBr}, cm^{-1}: 3405-3275 (OH, NH), 2905, 2835 (CH_2), 1735 (C=O), 1660, 1545 (amide).

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HYDROPHOBIC PROPERTIES OF MAIZE ZEIN

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The proteins of the zein complex from maize endosperm have been studied with the aid of hydrophobic chromatography. Their best separation was achieved on a column with TSK gel HW-65f. By comparing the results of fractionation by hydrophobic chromatography with those of electrophoresis, it was found that electrophoresis in PAAG under denaturing conditions separates the zein protein into groups according to their surface hydrophobicity. The most hydrophobic is the high-molecular-mass group of zein polypeptides.

It is known that prolamines, the main reserve proteins of cereal grain, are hydrophobic compounds. They can exist in dissolved form only in the presence of polar solvents (ethanol, propanol, isopropanol, dimethyl sulfoxide, dimethylformamide) or in a buffer containing detergents (Na dodecyl sulfate, cetyltrimethylammonium bromide, Na deoxycholate, Triton X-100), urea, or guanidine-HCl. Their hydrophobicity is due, above all, to their peculiar amino acid composition: large amounts of isoleucine, leucine, proline, alanine, and amidated amino acids [1].

Using the principles of hydrophobic interaction, the inhomogeneity of individual groups of wheat gliadin has been demonstrated [2]. The separation of wheat gliadins and glutelins by hydrophobic chromatography on phenyl-Sepharose CL-4B showed that the unreduced aggregated polypeptides of these proteins were present in several fractions, which indicated their different surface hydrophobicities [3]. The surface hydrophobicities of α-, β-, and γ-gliadins depend on the presence of aromatic and aliphatic amino acid residues, while that of the ω-gliadins depends mainly on aromatic side chains [4]. This type of hydrophobicity (surface hydrophobicity) has been well studied mainly on wheat gliadins, and therefore an investigation of the zein proteins from maize endosperm with the aid of hydrophobic chromatography

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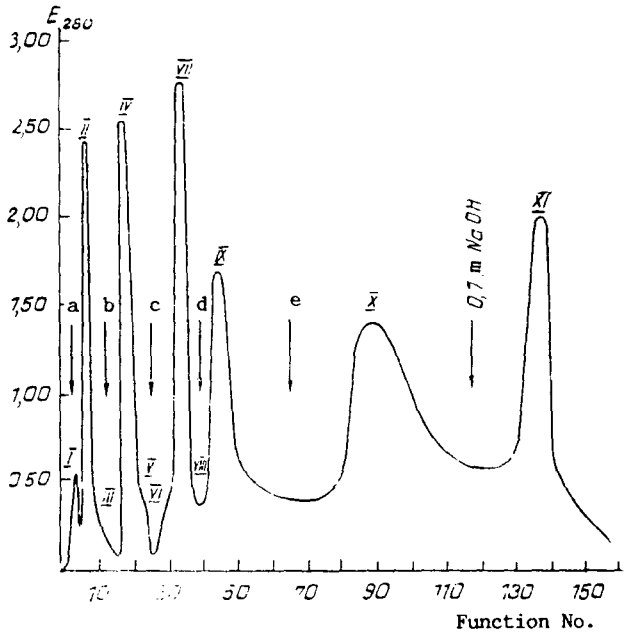

Fig. 1. Chromatographic separation of zein from maize endosperm on a column (1 × 20 cm) filled with TSK gel HW-75f by stepwise elution with increasing concentrations of isopropanol: I-XI) peaks subjected to electrophoretic analysis, a) 20%; b) 30%; c) 40%; d) 50%; e) 60% isopropanol.

TABLE 1. Ratio of the Subfractions and Groups of Zein Polypeptides after Hydrophobic Chromatography on a Column of TSK Gel HW-65f (% on the sum of the fractions)

Sample	Unreduced								Reduced	
	EP in PAAG with urea				EP in PAAG in DDS-Na, kDa					
	A	B	C	D	100—400	46—63	22—24	19—20	22—24	19—20
Initial preparation	55,6	26,1	11,5	2,5	15,5	14,3	25,0	35,0	32,7	67,3
40%*	Tr.	17,3	71,3	11,5	Tr.	Tr.	27,7	72,3	18.0	82,0
50%*	25,7	40,0	29,9	4,5	13,9	25,4	32,5	28,2	21,7	78,3
60%*	34,9	52,8	12,2	Tr.	28,9	35,5	29,1	6,5	50,6	49,4
60% with 1 M preparation	100,0	Tr.	Tr.	Tr.	70,9	20,1	9,0	Tr.	33,3	66,7

*Fractions eluted from the column by isopropanol of the corresponding concentration.

on soft (of the octyl-Sepharose type) and semirigid (of the TSK gel type) supports has been undertaken.

The elution profile of the zein proteins on stepwise fractionation on a column of TSK gel HW-75f is shown in Fig. 1. The proteins of each of the main and minor peaks were analyzed with the aid of electrophoresis in polyacrylamide gel (PAAG) in the presence of Na dodecyl sulfate (DDS-Na). It was found that the proteins of lowest molecular mass (12-16 kDa) were scarcely retained on the column and were eluted with 20% isopropanol. The following fractions contained mainly polypeptides with molecular masses of 19-24 kda and other molecules of higher molecular mass. However, it must be mentioned that no clear separation into groups was achieved on this support. The use of TSK gel of another type, HW-65f, permitted a sharper separation of the zeins according to degrees of hydrophobicity. Thus, 40% isopropanol eluted mainly polypeptides with molecular masses of 19-24 kDa, and 50% isopropanol polypeptides of higher molecular mass (46-63 kDa). When a 60% concentration of the alcohol was used, the proportion of medium- and high-molecular-mass polypeptides

exceeded 50%, while 60% isopropanol containing 1 M formic acid eluted mainly polypeptides with molecular masses of 100-400 kDa. It must be mentioned that after reduction of the disulfide bonds with 2-mercaptoethanol, in the samples collected all the fractions contained polypeptides with molecular masses of 19-24 kDa present in various proportions (with the exception of the first peak, which included low-molecular-mass polypeptides (Table 1). The results of the present investigation indicate the existence of a different type of polymeric complexes consisting of zein polypeptides with molecular masses of 19-24 kDa bound by disulfide bonds and agrees with results obtained previously using two-dimensional electrophoresis [1].

The use of electrophoresis in PAAG containing acetic acid with 8 M urea showed that the first peak consisted mainly of the proteins of the C-subfraction with a small amount of the D and E components, and the second of C and B, the proportion of D having decreased to a minimum; the third peak consisted mainly of the B-subfraction although the amount of proteins of the A-zone had risen considerably, and, finally, the last peak (eluted with 60% isopropanol containing formic acid) completely included the components of the A-subfraction of zein (Table 1). The bulk of the components electrophoretically more mobile than the D- and E-subfractions were not retained on the column and were eluted by the starting 20% isopropanol solution.

The separation of the zein proteins on a column with octyl-Sepharose CL-4b gave similar results: 40% isopropanol eluted mainly polypeptides with molecular masses of 19-24 kDa, and 50-60% isopropanol polypeptides with molecular masses of 46-63 kDa, while the final eluate from the column contained the highest-molecular-mass group of polypeptides (100-400 kDa). The low-molecular-mass polypeptides (12-16 kDa) were not bound to the gel matrix in 20% isopropanol.

To this must be added the fact that investigations performed previously had established that only the most hydrophobic subfractions (A, B, and C) contained true prolamines, while the other two (D and E) were proteins of the glutelin type for which a lowered mean hydrophobicity and a smaller amount of nonpolar amino acid side chains of characteristic [1]. According to the results of DDS-Na-binding capacity, the B- and C-subfractions of the zein possessed the highest total hydrophobicity [5].

EXPERIMENTAL

Isolation of the Zein. The germ was removed from maize grain (the self-pollinated line Grushevskaya 380), and the endosperm was milled to a fine powder. The protein was extracted with 70% ethanol containing 2 M urea at 60°C for 1 h [6]. After the separation of the solid matter by centrifugation, the supernatant liquid was dialyzed against 20% isopropanol and was used for chromatographic separation.

Chromatography. Hydrophobic chromatography was performed in a column with dimensions of 1 × 20 cm (Bio-Rad) filled with octyl-Sepharose CL-4B or with TSK gel of type HW-75f or HW-65f and equilibrated with 20% isopropanol. After the addition of 20-25 mg of protein to the column, elution was performed with the starting buffer at the rate of 30 ml/h until the UV absorption in samples had fallen considerably (5-ml samples were collected). Then the column was eluted successively with isopropanols of increasing concentration: 30, 40, 50, and 60%; and then with 60% isopropanol containing 1 M formic acid (for the TSK gels) or 3 M urea (for the octyl-Sepharose). The columns were regenerated with a 0.1 M solution of NaOH. The fractions collected were combined, dried under a hot-air blower, and used for electrophoretic separation.

Electrophoresis. Electrophoresis in the acetic acid system was performed in 10% PAAG containing 8 M urea in a Khiiu Kalur apparatus using plane gels 1 mm thick [1, 5], and in an alkaline system in 12.5% PAAG containing 0.1% DDS-Na [7] using gels of the same thickness [1].

To determine the molecular masses of the zein polypeptides we used a Serva set of marker proteins with known molecular masses. After the end of electrophoresis, the gels were stained with a 0.001% solution of Coomassie R-250 and were scanned in a laser densitometer [1]. The areas of the peaks obtained were calculated and, taking their sums as 100%, the relative amounts of the groups of polypeptides of subfractions were determined.

Protein in solutions was determined by its binding with the dye Amido Black 10B [8].

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IMMUNOCHEMICAL CHARACTERIZATION OF A PROTEOLYTIC ENZYME — PROTEASE A FROM COTTON SEEDS

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It has been established by double immunodiffusion in agar that protease A (a proteolytic enzyme from dormant cotton seeds hydrolyzing the native reserve proteins) is present for the first 3-4 days during the germination of the seeds. An immunological affinity between trypsin and protease A has been revealed which indicates the presence of common structural elements in them.

In the germination of plant seeds, degradation of the reserve proteins takes place under the action of proteolytic enzymes. In the initial phase of this complex multistage process a modification of the reserve proteins takes place as a result of which they become accessible to the action of other proteases [1]. Which of the proteinases is the initiator of proteolysis (that which is present in the dormant seed or that which is synthesized anew in the process of growth) it has not yet been possible to determine [2, 3]. It is known that a criterion of the participation of a protease in the breakdown of a reserve protein is not only its capacity for hydrolyzing the latter but also its presence in the germinating seeds [4]. It is unlikely that a protease disappearing during germination can play a fundamental role in the hydrolysis of reserve proteins.

Proteases A, B, and C have been isolated from dormant cotton seeds in the homogeneous state and have been characterized completely. Protease A [5] cleaves native reserve proteins, while proteases B and C [6] act only on modified reserved proteins. To reveal protease A in extracts of cotton seeds in various stages of germination we used the method of immunochemical analysis — double diffusion in gel. The performance of this reaction presupposes the presence of antigens and antibodies. If in two organisms there are similar or identical antigenic determinants, precipitation bands are formed, while when there are no similar determinants the reaction does not take place.

Rabbit antiserum to protease A was obtained. Its specificity was determined by double immunodiffusion in a gel. The results of the immunodiffusion of the extracts obtained from dormant and swollen cotton seeds and those that had germinated for 1-3 days showed that protease A from the dormant seeds and those that had germinated for 3 days possessed antigenic identity, i.e., protease A is present during the first 3 days of germination (Fig. 1), and, as we had determined previously by thin-layer chromatography [5], hydrolyzes the native reserved proteins, modifying them for deeper hydrolysis by the other proteases. The maximum proteolytic activity in the shoots was observed on the 3rd-4th and also on the 8th-9th days of germination in the total fractions. The results of immunochemical analysis

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