trate was evaporated at 15 mm Hg. To the ZAsnOSu obtained in 8 ml of DMFA, was added 450 mg ($0.51 \cdot 10^{-3}$ mole) of (XIV), and 0.1 ml of Et_3N, and the mixture was kept at room temperature for 50 h. Then 100 ml of $CHCl_3$ was added and it was washed with H_2O (3 × 20 ml), after which the organic solution was dried with anhydrous Na_2SO_4 and the $CHCl_3$ was evaporated off at 15 mm Hg; the residue was treated with 150 ml of ether and the resulting precipitate was filtered off, washed with ether (2 × 25 ml) and dried at 40°C/0.1 mm Hg. This gave 220 mg ($2.0 \cdot 10^{-4}$ mole) of (I) (Table 1).

SUMMARY

Two schemes for the synthesis of a heptapeptide having sequence 17-23 of human calcitonin with the minimum protection of the lateral functions of the amino acids have been proposed.

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A STUDY OF THE WATER-SOLUBLE PROTEINS OF THE SEEDS OF THE COTTON PLANTS Gossypium hirsutum AND G. Barbadense

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It has been shown by electrophoresis in polyacrylamide gel that different enzymes with similar electrophoretic mobilities may be localized in one and the same zone of water-soluble cottonseed proteins. Some zones not stained by the usual protein dyes also possess enzymatic activity. It has been established that the majority of the electrophoretic fractions consist of a series of polypeptides with different molecular weights, mainly of low-molecular-weight nature. The results are given of an investigation of the peroxidase, α-amylase, lipase, NADH-DCPIP oxidoreductase, and NADPH-DCPIP oxidoreductase activities of the water soluble fraction of the proteins of the seeds of the species mentioned.

The water soluble proteins of cotton seeds are separated on electrophoresis in polyacrylamide gel (PAAG) in the alkaline pH range into several main and a number of minor components. One of the main components of the proteins from the seeds of *Gossypium hirsutum* L. and *G. barbadense* L. is distinguished by its electrophoretic mobility and is inherited as a monofactorial trait [1]. In a study of the water-soluble proteins of cotton seeds by electrophoresis in PAAG in the presence of sodium dodecyl sulfate (NaDDS) more than 20 components were obtained and no differences were detected between the seeds of *G. hirsutum* and *G. barbadense* [2].

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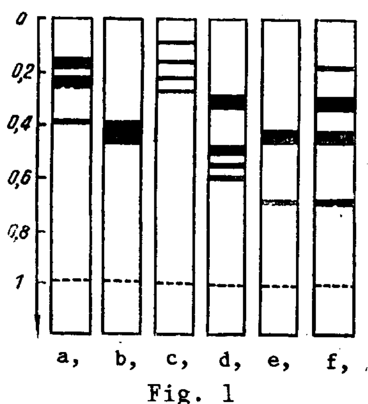

Fig. 1

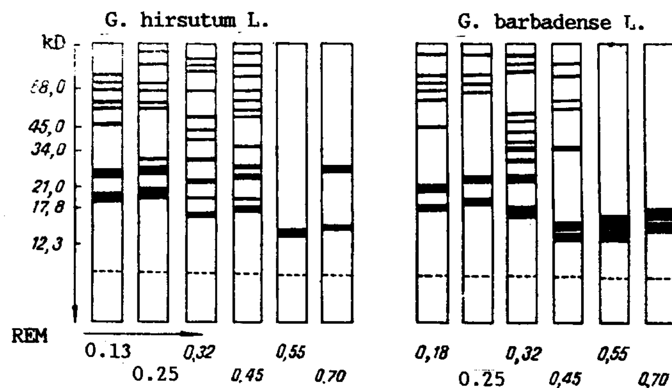

Fig. 2

Fig. 1. Sketches of zymograms of the water-soluble fractions of the proteins from defatted cottonseed flour: a) peroxidase; b) catalase; c) α-amylase; d) lipase; e) NADH diaphorase; f) NADPH diaphorase.

Fig. 2. Sketches of electrophoretograms of individual fractions of the water-soluble proteins from cotton seeds in the presence of NaDDS.

At the present time, for the genetic investigation of plant proteins use is made mainly of methods of one-dimensional electrophoresis in polyacrylamide gel or other supports. The application for these purposes of the high-resolution method of two-dimensional electrophoresis combining electrofocusing with electrophoresis in PAAG in the presence of NaDDS is impossible because of the low productivity of this method [3]. It is therefore very important to have information on the component composition of the individual electrophoretic fractions separated in ordinary electrophoresis. We have studied the localization of some enzymes on electrophoretograms of the water-soluble fraction of cottonseed proteins and the polypeptide compositions of the individual electrophoretic fractions and also of fractions obtained in the separation of an aqueous extract of cotton seeds on Sephadex G-100.

The main electrophoretic components of the water-soluble fraction of the seeds of the cotton plant G. hirsutum are proteins with R_f 0.13, 0.25, 0.32, 0.45, 0.55, and 0.70. The seeds of the G. barbadense have the same set of proteins except that the component with R_f 0.13 is replaced by a protein with R_f 0.18 [4].

The detection of different enzymes in one electrophoretic zone may indicate a heterogeneity of this protein band. Figure 1 shows the results of a determination of the localization of a number of enzymes in the electrophoretic separation of the proteins of the seeds of the cotton plant of variety 108-F (G. hirsutum).

Peroxidase activity was detected in three zones with electrophilic mobilities of 0.18 (the main zone) and 0.25 and 0.4 (minor zones), and catalase activity was present in one zone with a mobility of 0.24, α-amylase activity in four zones with mobilities of 0.12, 0.19, 0.25, and 0.28, lipase activity in four zones: 0.31 – the main one, identical with a lipase from the porcine pancreas; and 0.51, 0.56, and 0.61 – minor zones; reduced nicotinamide adenine dinucleotide (NADH)–2,6-dichlorophenolindophenol (DCPIP) oxidoreductase activity was present in zones with mobilities of 0.44 (main) and 0.70 (minor); and reduced nicotinamide adenine dinucleotide phosphate (NADPH)-DCPIP oxidoreductase activity in zones with mobilities of 0.32 and 0.45 (main) and 0.18 and 0.70 (minor).

In all these cases, a weak band appeared in the zone of the electrophoretogram with a mobility of 1. In the unstained electrophoretograms, this zone was usually colored yellow. Some zones of the electrophoretograms possessing enzymatic acitivity were not revealed by the usual staining with a protein dye. This relates to a zone with a mobility of 0.18 in the case of the proteins of G. hirsutum, where the main band of peroxidase and α-amylase activities was localized. The zones with mobilities of 0.18 and 0.25 were, moreover, heterogeneous, since various activities were detected in them. The diaphorase activity in the zones of the electrophoretograms with mobilities of 0.44 and 0.70 apeared with the use both of NADH and of NADPH.

To study the composition of the polypeptides of the main electrophoretic components, the protein zones cut out after electrophoresis in PAAG at pH 8.3 were subjected to NaDDS-

PAAG electrophoresis. The results of electrophoresis performed both in the absence and in the presence of 2-mercaptoethanol showed that the electrophoretic fractions with mobilities of 0.13, 0.25, 0.32, and 0.45 in the case of the seeds of the cotton plant of variety 108-F and with 0.18, 0.25, 0.32, and 0.45 in the case of 5904-I (G. barbadense) were heterogeneous in composition and contained a number of minor polypeptides with molecular weights of from 20,000 to 100,000 (Fig. 2).

The main polypeptides, quantitatively, in all the fractions had low molecular weights. The fractions with mobilities of 0.45 and 0.55 from the seeds of the cotton plant of variety 108-F each consisted mainly of a single polypeptide the molecular weights of these polypeptides being close, and in the variety 5904-I each was represented by two polypeptides: a main one and a minor one. Electrophoretic analysis of the water-soluble proteins of the seeds of the cotton plant of variety 108-F after separation by gel filtration on Sephadex G-100 showed that the majority of the electrophoretic components consisted of several molecular forms. Below we give the electrophoretic mobilities of individual fractions of the proteins obtained by the separation of an extract of the seeds of the cotton plant of variety 108-F on a column of Sephadex G-100:

Molecular weight, kD	Relative electrophoretic mobility
140	0,13
85	0,25
59	0,25; 0,45
40,7	0,31
35	0,13; 0,25
58,9	0,45; 0,52
23,2	0,71
15,9	0,45; 0,52

The results obtained showed that the majority of electrophoretic fractions separated under nondenaturing conditions were oligomeric proteins consisting of dimeric and tetrameric molecules.

The main components of the polypeptides of the proteins with electrophoetic mobilities of 0.13 and 0.18 from the seeds of varieties 108-F and 5904-I did not differ with respect to their molecular weights. As is well known, these proteins are denoted by the symbols H-0,13 and B-0,18, respectively [1].

On NaDDS—PAAG electrophoresis in the absence of 2-mercaptoethanol, one main band with a molecular weight of 35,000 and a minor band with a molecular weight of 21,000 were detected in the H-0,13 and B-0,18 fractions [5]. The polypeptide with a molecular weight of 21,000 corresponds in its mobility to the heavy polypeptides of the H-0,13 and B-0,18 proteins. Thus, the majority of the electrophoretically mobile proteins observed on separation under nondenaturing conditions are heterogeneous in component and polypeptide composition. However, quantitatively, the main components of these zones are polypeptides of low molecular weights.

EXPERIMENTAL

Seeds of the cotton plant G. hirsutum (variety 108-F) and of G. barbadense (variety 5904-I) were investigated.

Before the isolation of the proteins, the seeds were freed from hulls and were defatted with acetone and diethyl ether in a Soxhlet apparatus. The defatted flour was extracted with distilled water in a ratio of 1:10 for 30 min. The extract was decolorized at 5000 rpm on a TsLR-1 centrifuge, and the centrifuged material was used without further purification for gel filtration on Sephadex G-100 and electrophoresis in PAAG according to B. J. Davis [6]. The upper gel was not used. To each tube with dimensions of 0.7 × 7.0 cm was added 0.1-0.2 ml of the centrifuged material. To prevent convection of the protein solution in the tube after the sample had been added, dry Sephadex G-100 powder was added. After electrophoresis, the gels were stained with a 1% solution of Amido Black 10B in 7% CH_3COOH for 5 min and after washing with 7% CH_3COOH the bands were cut out with a blade, washed in distilled water for 2 min, and placed in ethanol for 10 min to decrease the volume of the gel, and then the lumps of gel with the protein bands were placed in test tubes to each of which was added 0.2 ml of a solvent with the following composition: 0.125 M Tris-HCl, pH 6.7, 2% of NaDDS, 1% of 2-mercaptoethanol, 5% of glycerol, and 0.1% of Bromophenol Blue, and the mixtures were heated in the water bath for 3 min. The contents of the tubes were

then transferred to tubes for NaDDS-PAAG electrophoresis, which was carried out as described by Laemmli [10].

The peroxidase, catalase, and α-amylase activities on the electrophoretograms were determined by the method of Safonov and Safonova [7], lipase activity as described by Maurer [8], and NAD(P)H diaphorase activity by a procedure that we have described previously [9].

For the gel filtration of the cottonseed extract a 1.5 × 94 cm Sephadex G-100 column (Pharmacia) was used. Elution was performed with water. Fractions with a volume of 3.5 ml were collected and subjected to spectrophotometry at 280 ml in a SF-16 spectrophotometer. All the fractions obtained were subjected to electrophoretic analysis as described in [6]. The calibration curve for determining the apparent molecular weights of the proteins was plotted with the aid of standard proteins: bovine serum albumin, 67,000; ovalbumin, 45,000; chymotrypsinogen, 25,000; myoglobin, 17,800; and cytochrome C, 12,000.

SUMMARY

1. It has been shown that different enzymes with similar electrophoretic mobilities may be located in one and the same electrophoretic zone.

2. Several zones not stained by the usual protein dyes exhibit enzymatic activity.

3. It has been established that the majority of electrophoretic fractions consist of a number of polypeptides with different molecular weights, but the quantitatively main polypeptide has a relatively low molecular weight.

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